Molecular Genetics of Rust Resistance in Poplars (*Melampsora larici-populina* Kleb/*Populus sp.*) by Bulked Segregant Analysis in a 2 × 2 Factorial Mating Design

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ABSTRACT

With random amplified polymorphic DNA (RAPD) markers, we have tagged a genomic region in *Populus sp.* involved in qualitative resistance to *Melampsora larici-populina.* Our approach was based on three steps: use of RAPD markers that can be quickly and efficiently researched; application of "bulked segregant analysis" technique on individuals of one interspecific family *P. trichocarpa* × *P. deltoides* to search for RAPD markers linked to resistance; and validation of these markers in two other families linked with the first one in a 2 × 2 factorial mating design. Of five detected markers, only one marker M03/04_480 was polymorphic in the three segregating families, involving 89 individuals and four different parents. We have estimated the recombination value of 1 cM with 1 cM sampling error.

THE genus *Populus* (poplars, cottonwoods and aspens) is one of the most widespread and economically important wood resources in the world. Its culture mainly consists of monoclonal plots with generally a limited number of cultivars. One of the consequences of this monoculture is that many pests and diseases are encountered on these forest trees, such as foliar fungi, stem cankers (fungal or bacterial), viruses and insects. Among fungi, *Melampsora* leaf rust is probably the most widely distributed and serious foliar disease of the *Aigeiros* and *Tacamahaca* poplars and their hybrids (THIELGES 1985). Poplar leaf rusts are mainly caused by four *Melampsora* species: *M. larici-populina* KLEB., *M. allii-populina* KLEB., *M. medusae* THUEM. and *M. occidentalis* JACKS. (PINON 1992a). Severe economic losses have been attributed to these pathogens, including growth reduction or even death of the plants (reviewed in HSIANG *et al.* 1993). In *M. larici-populina*, seven virulences have been identified in Europe based on clone-race compatibility reaction (PINON *et al.* 1987; PINON and PEULON 1989; PINON and LEFÈVRE 1994; STEENACKERS *et al.* 1994).

Two kinds of interactions can be found between *Populus sp.* and *Melampsora sp.* that LEFÈVRE *et al.* (1994) have described as "qualitative" (*i.e.,* complete incompatibility) or "quantitative" (*i.e.,* different forms of partial resistance). We present the identification of a single genomic region involved in qualitative resistance to *M. larici-populina* using bulked segregant analysis on progenies of four families linked in an intra- and interspecific factorial mating design.

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This work is part of a broader study at INRA on the *Populus/Melampsora* system, both on the host and the pathogen side (PINON 1992a; PICHOT and TEISSIER DU CROS 1993a,b; LEFÈVRE *et al.* 1994).

MATERIALS AND METHODS

***Populus* mating design:** Experiments have been carried out on two interspecific families, family TD (*Populus trichocarpa* × *P. deltoides)* and family DT (*P. deltoides* × *P. trichocarpa*) and two intraspecific families, family TT (*P. trichocarpa* × *P. trichocarpa)* and family DD (*P. deltoides* × *P. deltoides*) linked in a 2 × 2 factorial mating design (Figure 1). The two *P. deltoides* parents originate from Illinois, Union county for D7 (INRA code L123 011) and McLean county for D8 (INRA code L155 079). The two *P. trichocarpa* parents originate from Oregon, Clackamas county for T2 (INRA code 36-134) and Linn county for T3 (INRA code 19-77). The number of individuals per family is listed in Figure 1.

Rust resistance assay: The four families and their parents have been tested for resistance to *M. larici-populina.* These data have been obtained through observations in nursery trials and stool beds in 1991, 1992 and 1993 (LEFÈVRE *et al.* 1994) and with *in vitro* inoculations with *M. larici-populina* race E1 in summer 1993 on foliar disks. Leaf disk were inoculated with 5000–10,000 spores/ml (four disks per clone, two to six replications). Disks were kept at 18° for 15 days before scoring. We report here only qualitative data (presence or absence of uredia after natural or artificial inoculations), and we scored a clone as sensitive regardless of the degree of infection.

RAPD assays and bulked segregant analysis: DNA was extracted from young leaves of 129 F_1 trees and the four parents according to the procedure described in BRADSHAW and STETTLER (1993). Bulked DNA samples (MICHELMORE *et al.* 1991) were made from individuals of the interspecific family TD. The two bulks "resistant" and "sensitive" were screened with 760 primers and 120 pairwise combinations of primers (Operon Technologies Inc., Alameda, CA). The reaction conditions were as described by WILLIAMS *et al.* (1990) and BRAD-

F \ M	T3	D7
T2	Family TT n = 40 R = 0 S = 40	Family TD n = 29 R = 16 S = 13
D8	Family DT n = 30 R = 10 S = 20	Family DD n = 30 R = 21 S = 9

FIGURE 1.—Four families used in these experiments as plant material and score of resistance of *Melampsora larici-populina*. F, female parent; M, male parent; T, *Populus trichocarpa*; D, *P. deltoides*; n, number of individuals per family; S, number of individuals that are sensitive to *Melampsora larici-populina*; R, number of individuals that are resistant to *M. larici-populina*.

SHAW *et al.* (1994). Reactions were carried out in MJ Research PT-100 thermocyclers programmed for 35 cycles of 94° × 1 min, 36° × 1 min and 72° × 2 min. The number of detectable bands was counted for each reaction, and the results were averaged over the number of tested primers or primer combination. Segregating markers were identified by the manufacturer primer code corresponding to a particular 10-base sequence, followed by a number indicating the fragment size in base pairs, *e.g.*, AG18_470 is a 470-bp fragment generated by Operon primer AG18.

Another series of bulks were made with six resistant and 15 sensitive progenies of the second interspecific family DT to test the primers selected in the bulked segregant analysis of family TD. Genotypes of individual F_1s were then determined on 29 clones of family TD, 30 clones of family DT and 30 clones of family DD.

Linkage analysis: A combined estimate of the recombination values over the three families was obtained following ALLARD (1956).

RESULTS

Segregations for rust resistance in the four families: Qualitative data, *i.e.*, the presence or absence of uredia on leaves after natural and artificial infection are presented in Figure 1. Two phenotypes, resistant and susceptible, were found in the progenies of the two interspecific families TD and DT and the intraspecific family DD. *P. trichocarpa* parents and progenies of the intraspecific *P. trichocarpa* × *P. trichocarpa* family TT were all susceptible. Segregation for the compatibility was not significantly different from a 1:1 ratio in the two interspecific families (χ^2 = 0.31 for family TD and 3.33 for family DT), but it rather fitted a 1:3 ratio for the DD family (χ^2 = 0.15). For families TT, TD and DT, *in vitro* experiments with *M. larici-populina* race E1 were perfectly reproducible and consistent with nursery observations after natural infections in 1991, 1992 and 1993. For family DD, clone-race compatibility was more difficult to assess since the *P. deltoides* × *P. deltoides* clones also have high levels of quantitative resistance and since a clear response was never obtained after *in vitro* inoculations with race E1 for the clones that were consistently diseased in the nursery. Laboratory tests have demonstrated that this race was not E1, since different virulences were detected (PINON and LEFÈVRE 1994). Therefore, segregation in the DD family does not necessarily reflect resistance to the same virulences.

Heterozygosity of parental species: Heterozygosity of the *P. trichocarpa* (T2) and *P. deltoides* (D7) parents of family TD was estimated by testing for segregation of RAPD bands in six F_1 trees at, respectively, 309 and 339 presumptive loci. The number of detectable bands was 3.6 bands/primer for the *P. deltoides* parent (mean of 92 primers) and 3.4 bands/primer for the *P. trichocarpa* parent (mean of 88 primers). The average level of marker heterozygosity for the two parents of the family TD is 29% for the *P. trichocarpa* female parent and 28% for the *P. deltoides* male parent.

Each RAPD primer used singly amplified an average of 5.5 fragments (mean of 187 primers), whereas pairs of primers amplified 5.6 fragments (mean of 109 primer pairs). However, novel amplified bands are observed in the paired primers, thus permitting more information to be obtained from an already available collection of primers.

Bulked segregant analysis on the interspecific family TD: From the 880 primer/primer pairs screened, 87% were able to reveal amplification products in the two bulks. Of these 775 primers, five primer/primer pairs (M03/04, AG18, K20, AD04, AF06) generated fragments that were present in one bulk but not in the other (*e.g.*, M03/04 in Figure 2). The polymorphism was confirmed by a repeated amplification and compared with the two parents and 29 F_1 individuals of family TD (*e.g.*, M03/04_480 in Figure 3). Except for marker AF06_600, presence of band was correlated with the rust resistant phenotype and was inherited from the *P. deltoides* parent. The genotypes of each F_1 tree within family TD is listed in Table 1 for each of the five RAPD markers. Eight recombinants between the marker and the putative resistance locus were observed for markers K20_720, AD04_780 and AF06_600. Two recombinants were observed for marker G18_470 and one for marker M03/04_480.

Screening of the five primers on parents and F_1 individuals of family DT: Two bulks were also created with the other interspecific family (family DT), whose parents are not related to those of family TD. Of the markers linked to rust resistance in family TD, only M03/04_480 differentiated the resistant and susceptible bulks of the family DT. Genotypes of individual F_1s in the family DT with M03/04_480 revealed no recombinants: the 10 resistant individuals inherited the band while the 20 sensitive F_1 did not (results not shown). Therefore, from the two bulked segregant analyses, both *P. deltoides* parents were heterozygous for rust resis-

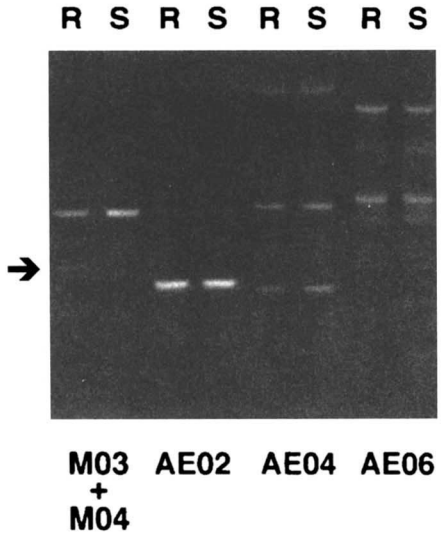

FIGURE 2.—RAPD markers detecting polymorphisms between the two bulks of family TD. Each set of two lanes results from PCR amplification with a different primer/primer pair: M03/04, AE02, AE04 and AE06. The first lane contains bulked DNA from the resistant individuals (Bulk R) and the second lane contains bulked DNA from the sensitive individuals (Bulk S). Only M03/04 allows to distinguish polymorphism between the two bulks (arrow).

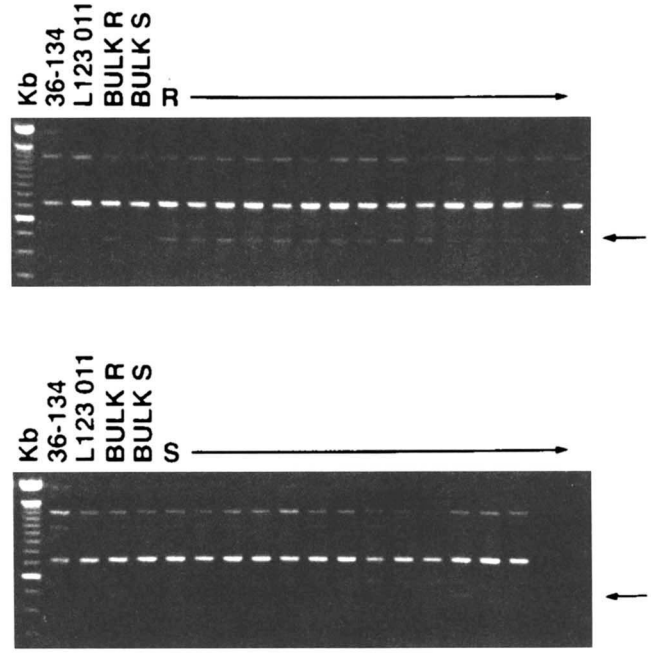

FIGURE 3.—DNA polymorphisms of the parents of the resistant and the sensitive progenies of family TD with primer pair M03/04 (illustration of 28 clones). The first lane is the 100-bp ladder (Kb). The second lane contains DNA from the female parent T2 (36-134). The third lane contains DNA from the male parent D7 (123 011). The fourth lane contains bulked DNA from the resistant individuals (Bulk R). The fifth lane contains bulked DNA from the sensitive individuals (Bulk S). At the top of the gel, the 15 other lanes contain DNA from the 15 resistant individuals (R→). At the bottom of the gel, the 13 other lanes contain DNA from the 13 sensitive individuals (S→).

tance and for M03/04_480, with the "band-present" allele linked in coupling to rust resistance, while both pure *P. trichocarpa* parents were homozygous for the null ("band-absent") allele.

Screening of M03/04_480 on progenies of the intraspecific family DD: RAPD assays were performed on the 30 F_1 individuals of family DD. This family results from the intraspecific cross of the female parent of the family DT and the male parent of the family TD. As already shown, the two parents are heterozygous at the M03/04_480 marker. Genotypes of F_1s revealed no recombination between rust resistance and M03/04_480; the nine susceptible F_1s are all homozygous for the null allele of M03/04_480 (Figure 4).

Case of the progenies of the intraspecific family TT: As previously shown, the two *P. trichocarpa* parents lack the marker M03/04_480. Thus, no segregation was expected in their progeny for that marker.

Linkage analysis: According to chi-square test, the five RAPD markers, identified by bulked segregant analysis in family TD, were linked with the genomic region (χ^2 = 6.01 for markers K20_720, AD04_780 and AF06_600).

A combined estimate was computed from data of the three segregating families (89 clones of the families TD, DT and DD). This has only been possible with M03/04_480, as this marker is the only one to cosegregate with the genomic region involved in the qualitative rust resistance in all four families of the 2 × 2 factorial mating design. Considering that only one individual is a recombinant (clone 33 of family TD), we have estimated the recombination value of 1 cM with 1 cM sampling error.

DISCUSSION

These results represent the first starting point in the molecular analysis of rust resistance in poplars. These results have been obtained through the study of a unique plant material, combining intraspecific variation and interspecific hybridization.

Genetics of resistance: If we consider that the two phenotypes "compatible" and "incompatible" as a single trait whatever the rust isolate, then the phenotypic segregation data of the four families (TD, DT and DD) fit a single gene model for resistance to *M. larici-populina*. This hypothesis is not obvious, since usually in host-pathogen interactions, compatibility to different isolates are usually considered as different traits. However, in our case, a single marker M03/04_480 makes a link between compatibilities to different virulences and leads to the conclusion of monogenic inheritance of that particular resistance. Rather than strictly monogenic, we can conclude that resistance is due to a single genomic region, composed of a cluster of tightly linked genes, as is frequently observed in host-pathogen sys-

TABLE 1

Genotypes of the F_1s within the family TD at the RAPD loci

Clone number	Rust test	RAPD markers									
		M03/04	480	AG18	470	K20	720	AD04	780	AF06	600
T	S	−		−		−		−		+	
D	R	+		+		+		+		−	
6	S	−		−		−		−		+	
13	S	−		−		−		−		+	
14	S	−		−		−		−		+	
15	S	−		−		−		−		+	
18	S	−		−		−		−		+	
21	S	−		+		−		−		+	
22	S	−		−		−		−		+	
24	S	−		−		+		+		−	
28	S	−		−		−		−		+	
32	S	−		−		−		−		+	
33	S	+		+		+		+		−	
34	S	−		−		−		−		+	
37	S	−		−		+		+		−	
1	R	+		+		−		−		+	
3	R	+		+		−		−		+	
5	R	+		+		+		+		−	
7	R	+		+		+		+		−	
8	R	+		+		+		+		−	
9	R	+		+		+		+		−	
10	R	+		+		+		+		−	
11	R	+		+		−		−		+	
12	R	+		+		+		+		−	
16	R	+		+		−		−		+	
20	R	+		+		+		+		−	
23	R	+		+		+		+		−	
26	R	+		+		+		+		−	
31	R	+		+		−		−		+	
35	R	+		+		+		+		−	
36	R	+		+		+		+		−	

The two first lanes are the parental clones: T, clone 36-134 of *Populus trichocarpa;* D, clone L123 011 of *P. deltoides;* S, for clones sensitive to *Melampsora larici-populina* race E1; R, clones resistant to *Melampsora larici-populina* race E1; +, band-present RAPD allele; −, band-absent (null) RAPD allele. Family TD had 29 clones.

tems (review in LEFÈBVRE and CHÈVRE 1995) or a single gene with pleiotropic action, since different rust races could share some of their virulences (PINON 1992b).

Furthermore, these results point out the perfect complement between classical and molecular genetics: without this marker approach, the different resistances found in the TD, DT and DD families would not have been related *a priori.*

Efficiency of bulked segregant analysis (BSA): We have identified a single marker very tightly linked to resistance to *M. larici-populina.* On 89 F_1s of three segregating families, we have detected one recombination (clone TD-33), but as this clone proved to be recombinant for these five markers, we suspect an error in the labeling of this clone.

The efficiency of the BSA can be measured by the frequency at which linked markers were identified. Under our conditions, an average of 3.5 bands were amplified per arbitrary primers and the average level of heterozygosity of the two parents was 28.5%. We have screened in our study 773 polymorphic loci (775 primers × 3.5 loci × 28.5%). If we consider that RAPD markers are distributed uniformly throughout the genome, that the length of the *Populus* genome is of 2600 cM (BRADSHAW *et al.* 1994) and that we are looking for a marker within a window of 10% recombination either side of the target locus (MICHELMORE *et al.* 1991), we expect ~0.75% of the polymorphic loci to be linked to the target gene. Five of the 773 polymorphic loci (0.65%) were detected, therefore the efficiency of the method fits or is slightly below expectations.

The identification of this marker has been possible through the use of BSA, a method appropriate for forest trees due to the lack of refined classical genetic tools such as near isogenic lines for mapping monogenic traits (due to the long generation). In conifers, such an approach has been realized in sugar pine (*Pinus lambertiana*) for resistance to blister rust, *Cronartium ribi-*

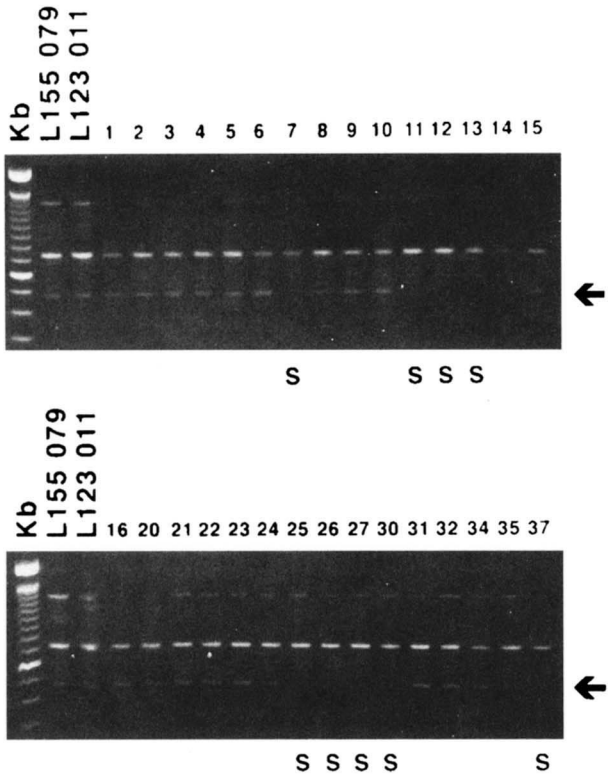

FIGURE 4.—DNA polymorphisms of the parents of the resistant and the sensitive progenies of family DD with primer pair M03/04. The first lane is the 100-bp ladder (Kb). The second lane of the top and the bottom of the gel contains DNA from the female parent D8 (L155 079). The third lane of the top and the bottom of the gel contains DNA from the male parent D7 (L123 011).The other lanes are the 30 progenies of the family (15 on the top and 15 at the bottom). The nine sensitive progenies are indicated by an S.

cola (DEVEY *et al.* 1995), and is progressing in the model fusiform rust/loblolly pine (WILCOX 1995). Genetic linkage maps of both host and pathogen are applied in the model fusiform rust/slash pine (NANCE *et al.* 1994). In fruit trees, such BSA approach has been developed on resistance to citrus tristeza virus (CHENG *et al.* 1994). Molecular genetics of other diseases and pests such as fungi (MICHELMORE *et al.* 1991), virus (CHENG *et al.* 1994), and insects (MOHAN *et al.* 1994) have been studied through BSA.

Multiparental approach: *Populus trichocarpa* as testcross parent for study of resistance in *P. deltoides*: The M03/04_480 marker mapped in the family TD is also polymorphic in family DT involving different parents. A potential drawback of RAPD markers is that the band of the same apparent size in family TD and DT could be derived from an entirely different locus in each. This could be tested in part by cloning, sequencing and comparing the two RAPD products. We had additional evidence in our study since the two *P. deltoides* parents were crossed in the factorial mating design and this locus was also found segregating within the DD intraspecific family. Thus, linkage between the marker and the resistance-gene locus was not affected by genetic background, since consistent results were obtained with the different genotypes of the four parents. The use of several crosses linked in a factorial mating design has allowed a better estimation of the distance between the marker and the resistance gene. Further studies will focus on other segregating families involving eight other *P. deltoides* parents of a 9 × 9 factorial mating design, from which this 2 × 2 design comes.

The novelty of this approach relies on the plant material and especially of the use of interspecific crosses involving *P. trichocarpa* as parents in the study of resistance genes carried by the *P. deltoides* genome. Intraspecific family TT reveals no segregation for resistance, as *P. trichocarpa* genotypes in our nursery (several hundreds of clones) have never shown any incompatibility against *M. larici-populina* isolates (F. LEFÈVRE, unpublished results). Intraspecific family DD reveals segregation, but only in the nursery and at a very low degree of infection. Interspecific families are particularly interesting for such studies, since they show a bimodal distribution between highly susceptible and highly resistant progenies (LEFÈVRE *et al.* 1994). Thus, the *P. trichocarpa* parents involved in that study has served as tester to determine *P. deltoides* genotypes. Evaluation of the *P. deltoides* germ plasm through the use of interspecific combination with *P. trichocarpa* is in progress, as this 2 × 2 is part of a 9 × 9 factorial mating design involving eight other *P. deltoides* parents.

The use of this marker in breeding for resistance to rusts: Dominant resistance (incompatibility) was provided by the *P. deltoides* parent. This molecular analysis confirms the genetic study developed on a factorial mating design of *P. deltoides* and *P. trichocarpa* with 17 intraspecific and 15 interspecific progenies (part of the 9 × 9 mating design previously mentioned), where genes controlling incompatibility were contributed only by the *P. deltoides* parent (LEFÈVRE *et al.* 1994).

This marker will be of great use for the study of clone-race incompatibility as this marker is linked to qualitative resistance. But from a breeding point of view, qualitative resistance is no more desirable, as breakdown of qualitative resistance has been observed three times on interspecific F_1 hybrids previously selected for immunity (PINON *et al.* 1987; PINON and PEULON 1989; STEENACKERS *et al.* 1994). Thus, we have to determine breeding strategy for durable resistance in poplars and to take into account quantitative components of resistance, which is assumed to be under polygenic control. The dissection of quantitative components of resistance is in progress, involving number and size of uredia, latent period in laboratory tests, date of first infection in nursery and tolerance (LEFÈVRE *et al.* 1995).

This *Populus*/*M. larici-populina* system will serve as a model for testing linkage or pleiotropic effects between genes controlling incompatibility and quantitative components of resistance. Such link has been pointed out

in maize by FREYMARK *et al.* (1993) who have demonstrated that loci in the vicinity of three monogenic resistance genes to *Exserohilum turcicum* had minor effects on two quantitative resistances to this pathogen. Three other pathogens are under study on the progenies of this factorial mating design, such as the fungi *Marssonina brunnea*, the bacterial canker *Xanthomonas populi* and the leaf beetle *Chrysomela populi*. These data will allow us to identify the possible genomic regions involved in these resistances with the research of clusters of resistance genes as multiallelic series at a locus or as multiple linked loci (such as reported in lettuce in KESSELI *et al.* 1993; MAISONNEUVE *et al.* 1994).

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LITERATURE CITED

ALLARD, R. W., 1956 Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia **24:** 235–278.

BRADSHAW, H. D., and R. F. STETTLER, 1993 Molecular genetics of growth and development in *Populus*. I. Triploidy in hybrid poplars. Theor. Appl. Genet. **86:** 301–307.

BRADSHAW, H. D., M. VILLAR, B. D. WATSON, K. OTTO and R. F. STETTLER, 1994 Molecular genetics of growth and development in *Populus*. III. A genetic map composed of RFLP, STS and RAPD markers. Theor. Appl. Genet. **89:** 167–178.

CHENG, F. S., M. L. ROOSE, C. T. FEDERICI and R. S. KUPPER, 1994 A detailed genetic linkage map including a citrus tristeza virus resistance gene derived from a cross between two intergeneric *Citrus* × *Poncirus* hybrids. Plant Genome II; January 24–27 1994, San Diego, CA, Poster P32.

DEVEY, M. E., A. DELFINO-MIX, B. B. KINLOCH and D. B. NEALE, 1995 Random amplified polymorphic DNA markers tightly linked to a gene for resistance to white pine blister rust in suger pine. Proc. Natl. Acad. Sci. USA **92:** 2066–2070.

FREYMARK, P. J., M. LEE, W. L. WODMAN and C. A. MARTINSON, 1993 Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). Theor. Appl. Genet. **87:** 537–544.

HSIANG, T., G. A. CHASTAGNER, J. M. DUNLAP and R. F. STETTLER, 1993 Genetic variation and productivity of *Populus trichocarpa* and its hybrids. VI. Field susceptibility of seedlings to *Melampsora occidentalis* leaf rust. Can. J. For. Res. **23:** 436–441.

KESSELI, R., H. WITSENBOER, M. STANGHELLINI, G. VANDERMARK and R. W. MICHELMORE, 1993 Recessive resistance to *Plasmopora lactucae-radicis* maps by bulked segregant analysis to a cluster of dominant disease resistance genes in Lettuce. Mol. Plant-Microbe Interact. **6:** 722–728.

LEFÈBVRE, V., and A. M. CHÈVRE, 1995 Tools for marking plant disease and pest resistance genes: a review. Agronomie **15:** 3–19

LEFÈVRE, F., C. PICHOT and J. PINON, 1994 Intra- and interspecific inheritance of some components of the resistance to leaf rust (*Melampsora larici-populina* Kleb.) in poplars. Theor. Appl. Genet. **88:** 501–507.

LEFÈVRE, F., S. CHARTIER, P. FAIVRE-RAMPANT, O. FORESTIER, M. C. GOUÉ *et al.*, 1995 Composantes de la résistance aux rouilles chez les peupliers: utilisation en sélection. C. R. Acad. Agric. Fr. **81:** 111–121.

MAISSONEUVE, B., Y. BELLEC, P. ANDERSON and R. W. MICHELMORE, 1994 Rapid mapping of two genes for resistance to downy mildew from *Lactuca serriola* to existing clusters of resistance genes. Theor. Appl. Genet. **89:** 96–104.

MICHELMORE, R. W., I. PARAN and R. V. KESSELI, 1991 Identification of markers linked to disease-resistant genes by bulked segregant analysis: a rapid method to detect markers in specific genome regions by using segregating populations. Proc. Natl. Acad. Sci. USA **88:** 9828–9832.

MOHAN, M., S. N. NAIR, J. S. BENTUR, U. P. RAO and J. BENETT, 1994 RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of gall midge (*Orseolia oryzae*). Theor. Appl. Genet. **87:** 782–788.

NANCE, W. L., C. D. NELSON and R. L. DOUDRICK, 1994 Genetic characterization of the slash pine: fusiform rust interaction. A molecular marker aided approach. Plant Genome II; January 24–27 1994, San Diego, CA, Poster F13.

PICHOT, C., and E. TEISSIER DU CROS, 1993a Susceptibility of *Populus deltoides* BARTR. to *Melampsora larici-populina* and *M. alii-populina*. I. Qualitative analysis of a 6 × 6 factorial mating design. Silvae Genet. **42:** 179–188.

PICHOT, C., and E. TEISSIER DU CROS, 1993b Susceptibility of *Populus deltoides* BARTR. to *Melampsora larici-populina* and *M. alii-populina*. II. Quantitative analysis of a 6 × 6 factorial mating design. Silvae Genet. **42:** 188–199.

PINON, J., 1992a Variability in the genus *Populus* in sensitivity to rusts. Silvae Genet. **41:** 25–34.

PINON, J., 1992b Poplar rusts: variability and populations in France. Proceedings of the 19th International Poplar Commission, working group of diseases, Zaragossa, Spain, Vol. 1, pp. 211–222.

PINON, J., and F. LEFÈVRE, 1994 A new virulence found among isolates of *Melampsora larici-populina*. Proceedings of the International Poplar Commission and FAO, working group of diseases. Ismit, Turkey, October 1994.

PINON, J., and V. PEULON, 1989 Mise en évidence dèune troisième race physiologique de *Melampsora larici-populina* Klebahn en Europe. Cryptogam. Mycol. **10:** 95–106.

PINON, J., B. C. VAN DAM, I. GENETET and M. DE KAM, 1987 Two pathogenic races of *Melampsora larici-populina* in north-western Europe. Eur. J. For. Path. **17:** 47–53.

STEENACKERS, M., STEENACKERS, V. and T. DELPORTE, 1994 A new physiological race of *Melampsora larici-populina* in Belgium. Proceedings of the International Poplar Commission and FAO, working group of diseases. Ismit, Turkey, October 1994.

THIELGES, B. A., 1985 *Breeding Poplars for Disease Resistance*. FAO, Rome, pp. 66.

WILCOX, P. L., 1995 Genetic dissection of fusiform rust resistance in loblolly pine. Ph.D. Thesis, North Carolina State University, Raleigh.

WILLIAMS, J. G. K., A. R. KUBELIK, K. J. LIVAK, J. A. RAFALSKI and S. V. TINGEY, 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:** 6531–6535.

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