

Breeding Poplars With Durable Resistance to *Melampsora larici-populina* Leaf Rust: A Multidisciplinary Approach to Understand and Delay Pathogen Adaptation

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Introduction

During the last decades, European poplar breeders learned the hard way that *Melampsora larici-populina* (commonly abbreviated as *Mlp*, fig. 1) has an impressive adaptive potential (McDonald and Linde 2002). This fungal pathogen defeated all the deployed cultivars carrying qualitative (i.e., complete) resistances inherited from the American eastern black cottonwood *Populus deltoides* Bartram ex Marsh. in less time than needed to grow a poplar tree.



Figure 1—Sporulating *Melampsora larici-populina* uredinia on the abaxial side of a *Populus deltoides* x *P. nigra* cv ‘Robusta’ poplar leaf. (Photo by A. Dowkiw)

Populus deltoides is a key species for poplar breeding and growing in Europe. As an illustration, 90 percent of the cultivars sold by nurseries to growers in 2009 in France were interspecific hybrids of two types: *P. deltoides* x *P. trichocarpa* (i.e., interamerican hybrids) or *P. deltoides* x *P. nigra* (i.e., Euramerican hybrids, *P. nigra* being a European species) (French Ministry of Agriculture – DGPATT – 2009 national statistics of forest reproductive material sales). Of the three parental species involved, *P. deltoides* is the only one where we ever found qualitative resistances to *Mlp*. Moreover, hybrid vigor (i.e., positive heterosis for growth traits) is generally high in these cultivars. Unfortunately, most of the hybrid material deployed over the last 30 years exhibited high rust susceptibility once their one or two major resistance genes inherited from *P. deltoides* were defeated.

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The present situation is as follows: (i) all of the 44 clones found in the French registry of poplar cultivars, except two cultivars involving the non-host species *P. alba*, are susceptible to *Mlp*, and (ii) from 1990 to 2004, the proportion of immune clones in the *P. deltoides* breeding material grown in the nursery of INRA Orléans decreased from 45 percent to 2 percent (fig. 2).

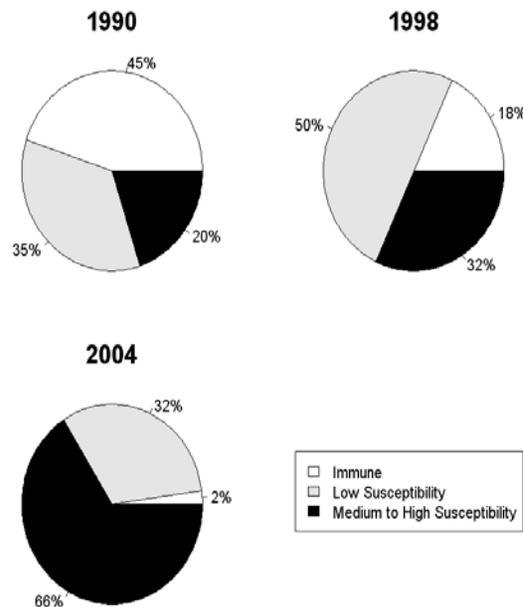


Figure 2—Evolution of *Mlp* rust susceptibility from 1990 to 2004 in a breeding collection of 545 *P. deltoides* clones evaluated under natural rust infection at the Institut National de la Recherche Agronomique in Orléans, France.

Breeding for quantitative resistance (QR) was considered a reasonable option as it is often described as an a priori for a more durable strategy. Although significant genetic variability was found for slow rusting traits in laboratory inoculation experiments and for field resistance under natural rust pressure in the three considered poplar species and their hybrids (Dowkiw et al. 2003, Lefèvre et al. 1994, Lefèvre et al. 1998, Pinon 1992, Pichot and Teissier du Cros 1993), three major results raised doubts on this optimistic point of view. First, elucidating the genetic determinism of QR in *P. deltoides* × *P. trichocarpa* hybrid progenies essentially yielded a few loci with major effects (Dowkiw and Bastien 2004) while small effect QTLs were either rare or hard to detect and often exhibited strain specificity (Jorge et al. 2005, fig. 3a). Second, quantitative and qualitative resistances did not appear to be completely independent as most identified defeated qualitative resistances inherited from *P. deltoides* happened to have statistical (possibly residual) effects on QR against virulent strains of the pathogen (Dowkiw and Bastien 2007, fig. 4). Third, *Mlp* strains able to completely defeat a major QR factor inherited from *P. richocarpa* have been identified before any commercial deployment of this resistance factor (Dowkiw et al. 2010).

Based on these considerations, poplar breeders and pathologists developed a broad holistic approach to understand the factors governing the durability of resistance, from genes to landscape, in order to conceive new breeding and deployment strategies.

Functional Genomics of the Poplar-*Mlp* Interaction

Following Eenink (1976), we believe that “the stability (i.e., the durability) of resistance is determined by the genetics of host-parasite relationships and not by the genetics of resistance. Quantity as well as quality of resistance and pathogenicity genes may be important. Monogenic and polygenic resistances can be stable or unstable.” Consequently, many efforts are made to elucidate the

functions of both qualitative and quantitative resistances and plant and rust geneticists try to bring the knowledge about the host-pathogen interaction at the same level for both protagonists.

Because genes with strong effects are the easiest ones to study and to follow among pedigrees, an integrated approach combining quantitative genetics, transcriptomics, and proteomics was conducted on three major loci: R_1 and Mer , two qualitative resistances inherited from *P. deltooides* defeated by virulences 1 and 7 of the pathogen, respectively, and R_{US} , a major QR factor inherited from *P. trichocarpa* with strong effect on uredinia size. R_{US} and R_1 were identified at INRA (Dowkiw and Bastien 2004) whereas Mer was identified by a Belgian team (Cervera et al. 1996). All outputs from this combined approach led to the same conclusion of similarities between qualitative and quantitative resistances.

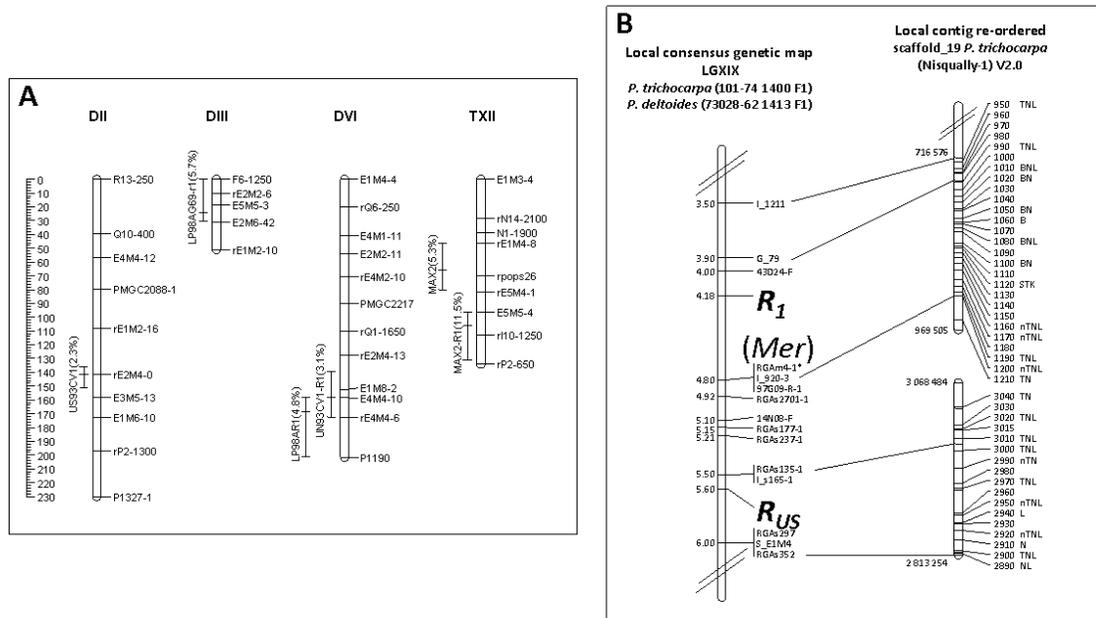


Figure 3— Mlp resistance loci described in the litterature (Bresson et al. 2011, Cervera et al. 1996, Cervera et al. 2001, Jorge et al. 2005, Lescot et al. 2004): **A**: Minor QTLs described in Jorge et al. (2005) and detected in *P. deltooides* (D) and *P. trichocarpa* (T) after inoculation in controlled conditions with 7 Mlp strains followed by QR assessments (UN: uredinia size; LP: latent period; UN: uredinia number) or after natural rust infection in the field (MAX). Numbers in parentheses represent the percentage of clonal variance explained by each QTL. See Jorge et al. (2005) for further details. **B**: Localization of three major resistance factors on a consensus genetic map and alignment on the *P. trichocarpa* genome sequence for chromosome 19 (v2.0). Genetic distances in cM are indicated on the left of the linkage groups. The gene models are designated by their number without the prefix POPTR_0019s. Resistance gene analog domains are abbreviated as follows: B for BEAF and DREF DNA-binding finger (BED), L for leucine-rich repeat (LRR), n for nuclear localization sequence (NLS), N for nucleotide-binding site (NBS) and T for Toll interleukin 1 receptor (TIR). STK stands for serine threonine kinase. Position of the Mer locus (Cervera et al. 2001) is deduced from marker RGAm4-1 designed from a *P. deltooides* BAC sequence developed for positional cloning of Mer (Lescot et al. 2004). For marker details, see Bresson et al. (2011).

Although R_{US} maps to a clearly distinct area, all three loci map on the same chromosome (XIX, fig. 3b) which happens to be particularly rich in NBS-LRR resistance gene analogs (Kohler et al. 2008).

A combined transcriptomic and proteomic approach has been carried out in two contrasted groups of poplar half-sib genotypes possessing the resistant R_{US} allele (i.e., heterozygous $R_{US}r_{US}$ genotypes) or deprived from it (i.e., $r_{US}r_{US}$ genotypes) to determine molecular markers associated to the R_{US} -mediated QR. Interestingly, whereas almost no genes were induced at 2 and 4 days after inoculation

in the $r_{US}r_{US}$ bulk compared to mock-inoculated leaves, the $R_{US}r_{US}$ bulk was characterized by a strong induction of many marker genes typically associated to qualitative resistance (Rinaldi et al. 2007), suggesting a delay in defense reaction activation for QR. Several of these “marker” genes were confirmed by proteomic and by RTqPCR expression profiling (e.g., thaumatin-like protein, glutathione *S*-transferase).

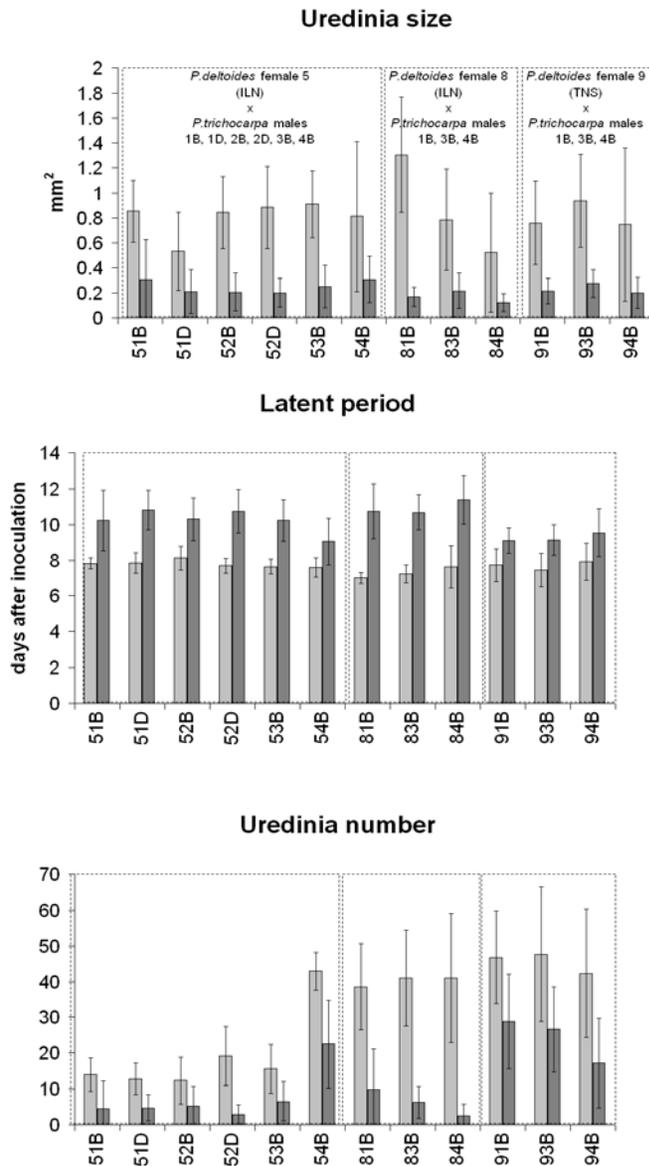


Figure 4—Family means (+/- associated standard errors) for three epidemiological components in 12 *P. deltooides* x *P. trichocarpa* F₁ families involving three distinct *P. deltooides* mothers of different origins (ILN, TNS) after leaf-disk inoculation with an *Mlp* strain able to sporulate in presence of any complete resistance factor inherited from *P. deltooides* segregating in this material. For each family, a distinction is made between genotypes carrying such qualitative resistance factor (in black) and genotypes lacking it (in grey). Presence of segregating qualitative resistance factors was revealed using incompatible stains of the pathogen. See Dowkiw and Bastien (2007) for further details.

Regarding the genetics of the pathogen’s side of the interaction, not much is known yet, even on the genetic determinism of virulence (i.e., the pathogen’s matching piece to qualitative resistance). One reason is that progenies are difficult to obtain due to the heteroecious life cycle of the pathogen

(i.e., the need to perform its sexual stage on a different host plant, here larch) and to its strict biotrophic status. However, recent sequencing of the *Mlp* genome opens the way for new genomics tools and makes the popular rust interaction a model pathosystem for forest pathology (Duplessis et al. 2011a). Most pathogenicity effectors described so far in fungal biotrophic pathogen encode small cysteine-rich secreted proteins (SSP) (Stergiopoulos and de Wit 2009). The *Mlp* genome contains a total of 1,184 SSP-encoding genes, which are mostly specific to this rust fungus (i.e., no homologs are found in other fungi, including the wheat stem rust; Duplessis et al. 2011b). More than 50 percent of these SSP genes are expressed during the successful colonization of poplar leaves representing candidate pathogenicity factors (Duplessis et al. 2011b; Hacquard et al., in press)

Thinking at Larger Scales

One of the reasons for the rapid spread of newly adapted strains of *Mlp* lies within the spatial and temporal organization of host diversity over the country. Poplar is a perennial host that is essentially cultivated as monoclonal stands, and less than 10 cultivars are being significantly used by growers. In this context, we investigated to what extent the population genetic structure of *Mlp* can be impacted by the deployment of resistant poplar cultivars over the country.

Since 1982, the interamerican hybrid poplar cv. 'Beaupré' carrying the rust resistance gene *Mer* was broadly planted in France and remained immune for 12 years, but once overcome by virulence 7 of the pathogen, severe rust outbreaks occurred (Xhaard et al. 2011). Using both phenotypic (i.e., virulence profiles) and genotypic (i.e., microsatellites) markers, we showed that *Mlp* isolates carrying virulence 7 were widely distributed all across France and displayed a specific genetic signature consistent with a history of selection and drastic demographic changes resulting from the resistance breakdown (Xhaard et al. 2011, fig. 5). This study illustrates how poplar cultivation has influenced the spatial and genetic structure of the pathogen, and has led to the spread of virulence alleles in most pathogen populations. As a consequence, resistance management should certainly be thought about at a continental scale in order to maximize its sustainability (McDonald and Linde 2002).

Alternative Breeding Strategies

Urged by growers to deliver new cultivars, breeders explore multiple (possibly combinable), very pragmatic, strategies to delay pathogen adaptation.

Pure *P. deltoides* genotypes, although defeated, always show much lower field susceptibility to the pathogen in our nursery in Orléans than their interspecific hybrid progenies. This may result from favorable gene associations that happen to be broken in these hybrids. Constitutive resistance traits related to leaf anatomical characteristics may be particularly affected by hybridization with *P. trichocarpa* and *P. nigra*. As a short-term solution, pure *P. deltoides* cultivars selected for productivity in northern climatic conditions under traditional cultivation practices will be released in 2013. They all carry defeated qualitative resistances but repeatedly showed high QR levels under natural rust infections. Backcrossing interamerican F₁ hybrids to the *P. deltoides* species is also being considered.

Medium-term solutions will rely on a more careful exploration of the genetic variability available in the European species *P. nigra* that co-evolved with the pathogen and on the simultaneous release of several unrelated cultivars to generate host diversity at regional scale. A collection of 2,300 *P. nigra* genotypes, most of them originating from different French natural populations, but also from Italy, Germany, and the Netherlands, have recently been screened for rust resistance under natural and controlled infection. Additional breeding traits like avoidance and tolerance (i.e., the ability to maintain growth despite being susceptible to the disease) are also being evaluated using fungicide treated vs. untreated field experiments.

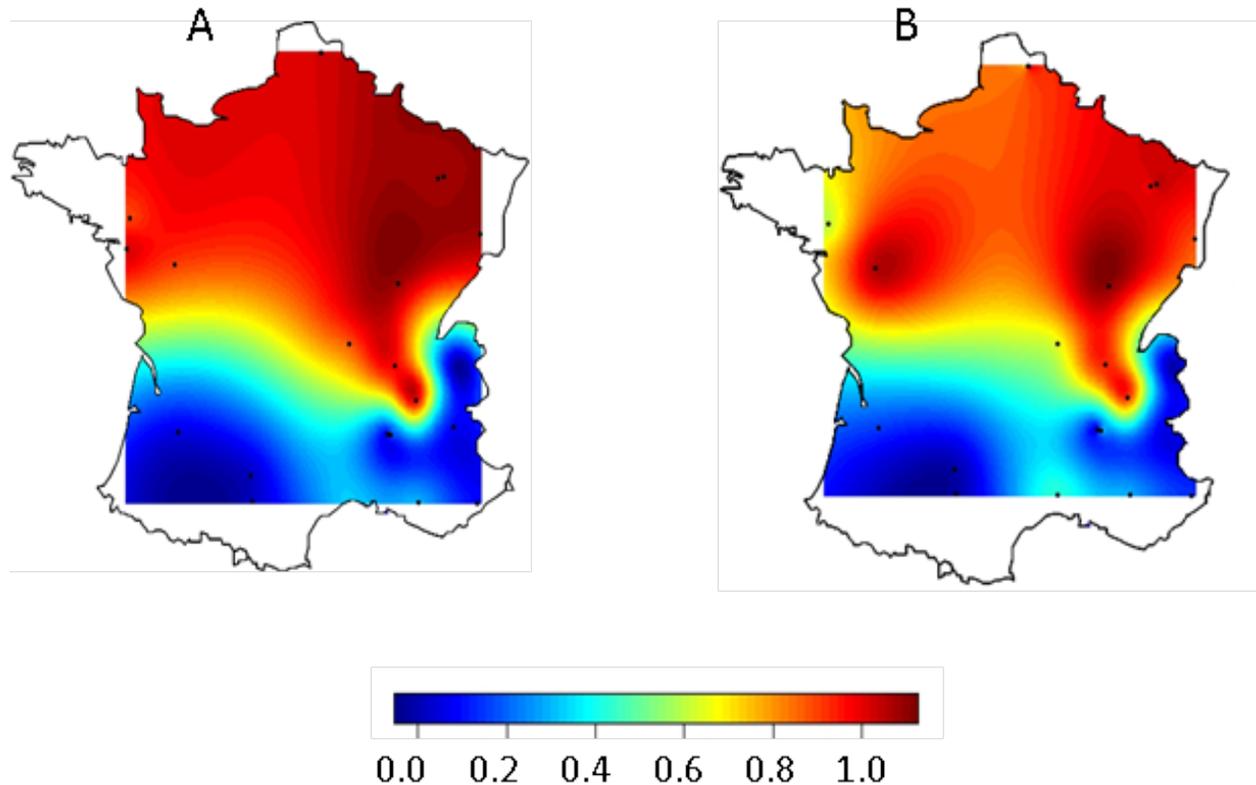


Figure 5—Spatial interpolations for the frequency of *Mlp* strains. A: belonging to the “cultivated” genetic group (i.e., “group 1” in Xhaard *et al.* 2011), and B: possessing virulence 7.

Acknowledgments

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