Abundant Pathogenic Variation in the New Hybrid Rust *Melampsora ×columbiana* on Hybrid Poplar

George Newcombe, Brigid Stirling, and H. D. Bradshaw, Jr.

First author: Department of Forest Resources, University of Idaho, Moscow 83844; and second and third authors: College of Forest Resources, Box 354115, University of Washington, Seattle 98195.
Accepted for publication 2 July 2001.

ABSTRACT

Newcombe, G., Stirling, B., and Bradshaw, H. D., Jr. 2001. Abundant pathogenic variation in the new hybrid rust *Melampsora ×columbiana*. Phytopathology 91:981-985.

The recently described rust hybrid *Melampsora ×columbiana* was discovered as a result of its novel pathogenic variation on *Populus trichocarpa* × *P. deltoides* (T×D) hybrid poplar. To characterize this pathogenic variation, 10 commercial T×D clones, all F_1 clones, were chosen as host differentials. Fourteen mononuredinial isolates of Pacific Northwestern field collections of *M. ×columbiana*, from 1996 to 1998 inclusive, were determined to be 13 distinct pathotypes. In contrast, four Southeastern isolates of *M. medusae* could not be distinguished on the same T×D host differentials, although they can be distinguished as pathotypes using *P. deltoides* differentials. The first three pathotypes of *M. ×columbiana* (*Mxc1*, *Mxc2*, and *Mxc3*) and a Mississippi isolate of *M. medusae* were inoculated onto a three-generation T×D pedigree, formerly used to characterize the *Mmd1* gene for resistance to *M. medusae*. Resistance to the *Mxc3* pathotype mapped to the same linkage group (group Q) as the *Mmd1* gene. In contrast, linked genes for resistance to *Mxc1* and to *Mxc2* were located on linkage group O, and were unlike *Mmd1* and *Mxc3* in that they were inherited from *P. deltoides*. The latter two genes resembled *Mmd1* and *Mxc3* in that infection type was correlated with quantitative traits such as uredinial density and latent period. Pathogenic variation in *M. ×columbiana* matches resistance genes from both *P. trichocarpa* and *P. deltoides* and reveals the vulnerability to hybrid rust of commercial T×D hybrid poplar clones.

Additional keywords: gene-for-gene theory, host-parasite specificity.

Populus trichocarpa Torr. & A. Gray × *P. deltoides* Marshall (T×D) clones have been the mainstay of short-rotation, hybrid poplar culture in the Pacific Northwest in recent decades. Unfortunately, commercial T×D clones that were initially resistant to leaf rust have become susceptible in recent years. Initially, the sole problem was *Melampsora medusae* Thuem., introduced into the region in 1991. However, hybridization between *M. occidentalis* H. Jacks. and *M. medusae* generated a new leaf rust population on hybrid poplar in the Pacific Northwest in the mid-1990s (9) with new potential for virulence. *M. occidentalis* and *M. medusae* are thought to have coevolved with *P. trichocarpa* and *P. deltoides*, respectively. Thus, their hybrid, *M. ×columbiana* G. Newc., combines parental rust genomes (i.e., *M. occidentalis* and *M. medusae*) that match those of the dominant commercial hybrid poplar clones of the region.

This is reflected in new pathogenic variation. For example, University of Washington clones 49-177 and 15-29, resistant to *M. medusae*, were widely planted in the 1990s for short-rotation intensive culture. Now their susceptibility to *M. ×columbiana* makes them an unlikely choice for the establishment of new clonal plantations (B. Stanton, *personal communication*).

The magnitude of the pathogenic variation in *M. ×columbiana* has, however, been unclear. One of the parental rusts, *M. occidentalis*, alternates in the Pacific Northwest between its aecial host, *Pseudotsuga menziesii* (Douglas fir), and its telial host, *P. trichocarpa* (15). *M. medusae* alternates in the eastern United States between its aecial host, *Larix laricina* (eastern larch), and its telial host, *P. deltoides*. *M. ×columbiana* presumably alternates between Douglas fir and hybrid poplar (9). The possibility of overwintering on poplar (i.e., bypassing the aecial host) notwithstanding, sexual recombination intrinsic in host alternation does not necessarily produce pathogenic variation. Indeed, there is no indication of pathogenic variation in *M. occidentalis* that would affect hybrid poplar in any way, in spite of its obligate alternation with Douglas fir (9). Instead, the new virulence of *M. ×columbiana* on hybrid poplar clones such as 49-177 and 15-29 likely pertains to the absence of an avirulence allele that is common, or perhaps fixed, in *M. medusae*.

The simplest hypothesis would be a single new pathotype with virulence toward the *Mmd1* gene from *P. trichocarpa* that confers resistance to *M. medusae* (8). The alternative hypothesis is that many new pathotypes of *M. ×columbiana* are interacting with additional genes for resistance originating from both *P. trichocarpa* and *P. deltoides*.

Our primary objective was to distinguish between the above two hypotheses. We inoculated a set of T×D host differentials with 14 putative pathotypes of *M. ×columbiana*. Three of the latter were used to inoculate the mapping pedigree so that genes for resistance other than *Mmd1* could be discovered.

MATERIALS AND METHODS

Host differentials to distinguish putative pathotypes. T×D clones were chosen for differentials because of their importance in commercial poplar culture. They are listed in Table 1. Ramets of these clones were grown in a greenhouse to produce detached leaves for inoculations.

Collection of other rust isolates. Isolates of *M. ×columbiana* were collected in the field during 1996 to 1998 inclusive (Table 2). Isolates were previously determined (9) as *M. ×columbiana*, except for pathotype *Mxc13* collected in 1998. *Mxc13* was also determined to be *M. ×columbiana* by G. Newcombe with the

Corresponding author: G. Newcombe; E-mail address: georgen@uidaho.edu

Publication no. P-2001-0816-04R

TABLE 1. Origins of *Populus trichocarpa* × *P. deltoides* (T×D) F_1 clones used as host differentials to characterize the pathogenic variation of *Melampsora ×columbiana*[a]

Clone	Provenance of female T parent	T parental clone	Provenance of male D parent	D parental clone
DTAC7				
55-263	Granite Falls, WA	93-968	Texas	TEXS7C4
15-29	Chilliwack, British Columbia	CHI 80-1	Stoneville, Mississippi	ST 1
24-128	Monroe, WA	MON 80-2	Stoneville, Mississippi	ST 1
50-197	Granite Falls, WA	93-968	Illinois	ILL 005
49-177	Orting, WA	ORT 81-3	Texas	TEX S7C1
47-174	Orting, WA	ORT 80-1	Missouri	MO 243
44-143	Orting, WA	ORT 80-1	Illinois	ILL 005
184-401	Randle, WA	RAN 91-568	Oklahoma	OK 17-10
23-91	Monroe, WA	MON 80-1	Stoneville, Mississippi	ST 70

[a] All but DTAC7 were produced by R. F. Stettler at the University of Washington in Seattle.

methods described previously (9). Briefly, *Mxc13* data (urediniospore length, spine density, and echinulation pattern) were subjected to a discriminant analysis that included all isolates and specimens of "Discriminant analysis II" of the previous paper (9). In addition to the *Mmd1* and Mississippi isolates of *M. medusae*, four other isolates of this species were employed: 97-3, 97-4, 97-5, and 97-6 (9).

Determinations of pathotypes. Pathotypes were determined by detached-leaf culture. Leaves detached from greenhouse-grown plants (i.e., differentials of Table 1) were placed abaxial side up on filter paper soaked in 10 ppm of gibberellic acid (5,8), lightly misted with distilled water, and inoculated with a cotton swab that had been rubbed in urediniospores. Inoculated detached leaves were incubated at 20°C under constant illumination at 35 μE m^{-2} s^{-1}.

Infection types. The leaves were scored for infection type from 10 to 14 days postinoculation. In each host clone–rust isolate inoculation, the infection type was judged to be resistant or susceptible as previously described (9). The resistant infection type comprises four categories: 0 = no macroscopic flecking or uredinia; 1 = necrotic flecking and a few tiny uredinia; 2 = small uredinia surrounded by necrotic flecking; and 3 = small- to medium-sized

TABLE 2. Collection data for the 13 pathotypes of *Melampsora ×columbiana* characterized in this study

Pathotype	Earlier isolate designation (9)	Host (on which the isolate was first collected)	Site	Year
Mxc1	96-1	T×D F_2 hybrid 331-1104	Clatskanie, OR	1996
Mxc2	96-2	T×D F_1 hybrid 52-225	Woodland, WA	1996
Mxc3	96-3	T×D F_2 hybrid 331-1128	Puyallup, WA	1996
Mxc4	97-2	*P. deltoides* – wild tree	St. Paul, MN	1997
Mxc5	97-8	T×D F_1 hybrid 49-177	Snohomish, WA	1997
Mxc6	97-11	T×D F_1 hybrid 49-177	Clatskanie, OR	1997
Mxc7	97-12c and 97-12d	T×D F_1 hybrid 55-263	Corvallis, OR	1997
Mxc8	97-16	T×D F_1 hybrid family 545	Puyallup, WA	1997
Mxc9	97-21	T×D F_1 hybrid 50-194	Boardman, OR	1997
Mxc10	97-22	T×D F_1 hybrid 50-197	Boardman, OR	1997
Mxc11	97-23	D×N cv. Carpaccio	Puyallup, WA	1997
Mxc12	97-24	D×M cv. Eridano	Puyallup, WA	1997
Mxc13	Not included in the earlier study (9)	T×D F_1 hybrid 15-29	Halsey, OR	1998

TABLE 3. Resistance (R) or susceptibility (S) to pathotypes of *Melampsora ×columbiana* compared with its parental species (i.e., *M. medusae* and *M. occidentalis*)

		Hybrid poplar host differentials[c]									
Pathotype[a]	Collection[b]	DTAC7	55-263	15-29	24-128	50-197	49-177	47-174	44-143	184-401	23-91
Mmd1	SE(GA)	S	R	R	S	R	R	S	S	R	R
Mmd1	SE(SC)	S	R	R	S	R	R	S	S	R	R
Mmd1	SE(VA)	S	R	R	S	R	R	S	S	R	R
Mmd1	SE(VA)	S	R	R	S	R	R	S	S	R	R
Moc	(WA)	R	R	R	R	R	R	R	R	R	R
Moc	(WA)	R	R	R	R	R	R	R	R	R	R
Mxc1	(OR-1996)	S	S	S	S	R(3)	S	R(1)	S	R(3)	S
Mxc2	(WA-1996)	S	S	R(3)	S	R(3)	S	S	S	S	R(1)
Mxc3	(WA-1996)	S	R(2)	R(1)	S	R(3)	R(3)	S	S	R(2)	S
Mxc4	St. Paul, MN	S	R	R	R	R	R	S	R	R	R
Mxc5	Snohomish, WA	R(3)	S	R(3)	S	R(3)	S	R(2)	R(2)	R(3)	R(2)
Mxc6	Clatskanie, OR	R(3)	R(3)	R(3)	R(3)	R(2)	S	R(3)	R(3)	S	R(0)
Mxc7	Corvallis, OR	R(1)	S	S	R(0)	R(3)	S	R(0)	R(0)	R(0)	R(0)
Mxc7	Corvallis, OR	R(1)	S	S	R(0)	R(3)	S	R(0)	R(0)	R(0)	R(0)
Mxc8	Puyallup, WA	S	R(0)	R(1)	S	S	S	R(3)	S	R(3)	S
Mxc9	Boardman, OR	S	R(1)	R(1)	R(3)	R(3)	R(3)	S	S	R(3)	R(3)
Mxc10	Boardman, OR	R(3)	R(3)	R(2)	S	S	R(3)	R(3)	R(3)	S	R(3)
Mxc11	Puyallup, WA	S	R(3)	R(1)	R(3)	R(3)	R(3)	S	S	R(1)	S
Mxc12	Puyallup, WA	R(3)	R(2)	R(1)	S	R(3)	R(3)	S	R(3)	R(2)	S
Mxc13	Halsey, OR	R(0)	S	S	S	R(2)	S	R(0)	R(3)	R(0)	R(0)

[a] *M. medusae* = *Mmd*; *M. occidentalis* = *Moc*; and *M. ×columbiana* = *Mxc*. Resistance to *Mxc* pathotypes from the Pacific Northwest is further characterized by an average infection type in brackets.

[b] Southeastern (SE) collections in 1997 of *Mmd* were from *Populus deltoides*. Washington (WA) collections in 1997 of *Moc* were from *P. trichocarpa*. The *Mxc4* pathotype was collected in Minnesota on *P. deltoides*; and all other *Mxc* pathotypes were collected on T×D hybrid poplar.

[c] T×D clones from the UW/WSU Hybrid Poplar Program, with the exception of DTAC7.

uredinia with chlorotic and necrotic flecking. The susceptible infection type, 4, was characterized by medium- to large-sized uredinia without peripheral chlorosis or necrosis.

Uredinial density and latent period. Uredinial density of each inoculated leaf was estimated in the following categorical manner: 0 = no uredinia; 1 = 1 to 5 uredinia; 2 = 5 to 10 uredinia; 3 = 10 to 20 uredinia; 4 = 20 to 40 uredinia; 5 = 40 to 80 uredinia; and 6 = >80 uredinia. Latent period was recorded in days by observing all inoculated plants daily.

New resistance genes. Determinations of new resistance genes employed the mapping pedigree (1,2) in whole-plant inoculations conducted at the Center for Urban Horticulture greenhouse at the University of Washington. The pedigree comprises the F_2 family 331, its F_1 parents, and its *P. trichocarpa* and *P. deltoides* grandparents, 93-968 and ILL-129, respectively. These inoculations, with a Mississippi isolate of *M. medusae*, and with three pathotypes of *M. ×columbiana* (*Mxc*, *Mxc2*, and *Mxc3*) were as previously described (9); briefly, they involve transfer of inoculum on a cotton swab to abaxial surfaces of leaves that have received prior misting. The leaves were scored for infection type from 10 to 14 days postinoculation, for latent period from 5 to 14 days postinoculation, and uredinial density at 14 days postinoculation.

Statistics. The three pathotypes, *Mxc1*, *Mxc2*, and *Mxc3*, and their corresponding resistance genes were compared by computing a simple matching dichotomy coefficient ($a + d/a + b + c + d$, where a and d represent the paired clones for which the values of both variables agreed, and b and c represent nonmatching values) for the reactions of all clones in the three-generation pedigree. Resistance (infection types 0, 1, 2, and 3) and susceptibility (infection type 4) provided the dichotomy.

RESULTS

Pathogenic variation on hybrid poplar differentials. *M. medusae* isolates were virulent on some T×D differentials (Table 3) as expected. All four isolates were virulent on the same four differentials and avirulent on the same six. These isolates were previously distinguished as pathotypes on *P. deltoides* differentials (9), but resistance in hybrid poplar, presumably from *P. trichocarpa*, blocks those distinctions. As expected, isolates of *M. occidentalis* were avirulent on all T×D differentials. In contrast, *M. ×columbiana* displayed abundant pathogenic variation on the differentials.

Pathotypes of *M. ×columbiana* (*Mxc*). Of the 14 isolates listed in Table 2, only isolates 97-12c and 97-12d from the same leaf from clone 55-263, near Corvallis, OR, proved to be the same pathotype (i.e., *Mxc7*). The other 12 isolates were distinct pathotypes as shown in Table 3. Isolates 97-8 and 97-11 (*Mxc5* and *Mxc6*, respectively) were collected from the same commercial clone, 49-177, from different sites in 1997, yet they were distinguished on 3 of the 10 differentials: 55-263, 24-128, and 184-401.

Of the 10 host differentials, poplar clone 50-197 was susceptible to only two pathotypes, *Mxc8* and *Mxc10*. The other nine differentials were each susceptible to three or more pathotypes. Conversely, no pathotype was virulent on all 10 differentials (Table 3).

New resistance genes. Inoculation with the *Mxc3* pathotype produced results that were indistinguishable from the earlier *Mmd1* segregation (7,8). In other words, all F_2 clones resistant to the *Mmd1* pathotype were also resistant to the *Mxc3* pathotype. Similarly, all F_2 clones susceptible to the former were susceptible to the latter. The *Mxc3* and *Mmd1* pathotypes were distinguished, however, when inoculated onto the F_1 host differentials (Table 3). The *Mxc3* pathotype was virulent on clone 23-91, whereas the *Mmd1* pathotype, as represented by four isolates of *M. medusae*, was avirulent. It appears prudent, therefore, to consider *Mxc3* a resistance gene that may be distinguished from *Mmd1* (12). In addition, resistance to the *Mxc1* and *Mxc2* pathotypes segregated in the three-generation poplar pedigree in a pattern distinct from that of *Mxc3* (Table 4).

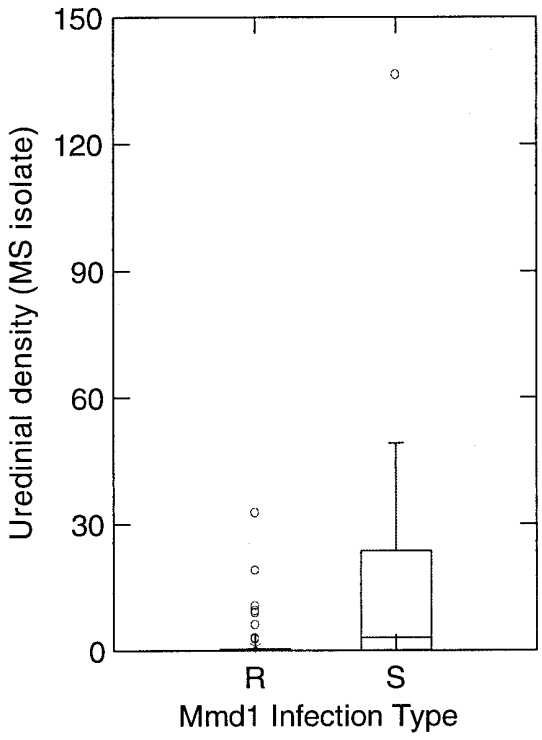

Fig. 1. Distribution of mean uredinial density (uredinia per square centimeter) of F_2 clones inoculated with a Mississippi isolate of *Melampsora medusae*. Clones are shown grouped by prior *Mmd1* infection type (R = resistant; S = susceptible) and are represented by box plots. Note that only R outlying clones share the uredinial densities of the S box or midrange. However, a total of six resistant clones (the three '0's, and three other clones with a low-infection type) were found among the clones with the *Mmd1* susceptible infection type.

TABLE 4. Infection types for three pathotypes of *Melampsora ×columbiana*, (*Mxc1*, *Mxc2*, and *Mxc3*) when inoculated onto a three-generation hybrid poplar pedigree in which the *Mmd1* gene for resistance was previously identified (7,8)

	Three-generation T×D pedigree				
Pathotype	*Populus trichocarpa* clone 93-968	*Populus deltoides* clone ILL-129	F_1	F_2	Linkage group for resistance gene
Mxc1	S	R	21R:1S (F_1 parents of F_2: 53-242 = R; 53-246 = R)	$10R_0$:31R:18S (Expected, 1:2:1 for incompletely dominant R; chi-squared = 2.27; $P = 0.32$)	O
Mxc2	S	R	10R:12S (53-242 = R; 53-246 = S)	26R:35S (Expected, 1:1 for R from *Mxc2mxc2* heterozygote, 53-242; chi-squared = 1.33; $P = 0.25$)	O
Mxc3	R	S	11R:11S (53-242 = R; 53-246 = R)	39R:21S (Expected, 3R:1S; chi-squared = 3.2; $P = 0.07$)	Q

[a] R_0 = resistant infection type of 0 (no symptoms); R = resistant infection types of 1, 2, or 3; and S = susceptible infection type of 4.

The Mississippi isolate of *M. medusae*. All F_2 clones previously shown to be resistant to the *Mmd1* isolate of *M. medusae* (8) were also resistant to the Mississippi isolate (Fig. 1). Among susceptible F_2 clones lacking the *Mmd1* dominant allele were six clones that were resistant to the Mississippi isolate.

Phenotypes of the new resistance genes, *Mxc1* and *Mxc2*. Previous work demonstrated that the *Mmd1* gene for resistance to *M. medusae* is associated with reduced uredinial density and diameter, longer latent period, and reduced infection efficiency (7). The *Mxc1* and *Mxc2* genes appear to be similarly associated with quantitative traits (Table 5). Correlations among *Mxc1* infection type, uredinial density, and latent period were all significant. The same was true for *Mxc2*, except for the correlation between infection type and latent period.

Linkage mapping of the new resistance genes, *Mxc1* and *Mxc2*. A contingency test indicated that the resistance genes to the *Mxc1* and *Mxc2* pathotypes are significantly linked (i.e., chi-square = 11.65; 1 df; $P < 0.001$). Not surprisingly, contingency testing of the *Mxc3* locus versus *Mxc1* and *Mxc2* did not reveal significant linkage ($P = 0.29$ and 0.87, respectively).

Simple matching dichotomy coefficients (Table 6) also reflect these relationships among the three loci. Roughly two thirds of the clones inoculated with *Mxc1* reacted the same way (either resistance or susceptibility) when inoculated with *Mxc2*. In contrast, only half of the clones inoculated with *Mxc1* or *Mxc2* reacted the same way when inoculated with *Mxc3*.

DISCUSSION

Thirteen distinct pathotypes of *M. ×columbiana* were readily discriminated in this limited survey. Pathogenic variation toward hybrid poplar in the Pacific Northwest appears to be abundant, in spite of the recent origin of this rust hybrid (9). It has been theorized that hybridization in the fungi leads to new host specialization (3). In contrast, isolates representing the parents of *M. ×columbiana* (i.e., *M. medusae* and *M. occidentalis*) displayed no pathogenic variation toward the hybrid poplar F_1 differentials (Table 3).

The Mississippi isolate of *M. medusae* was, however, avirulent on six F_2 clones in the pedigree that are susceptible to the *Mmd1* isolate of *M. medusae*. Pathogenic variation within *M. medusae* on F_2 clones lacking the dominant *Mmd1* allele from *P. trichocarpa* is not surprising. Studies of *M. medusae* on its native host, *P. deltoides*, have repeatedly revealed pathogenic variation (9–11). T×D F_1 differentials may also lack the dominant *Mmd1* allele, and such clones might prove resistant to at least some isolates of *M. medusae* possessing matching avirulence.

Nevertheless, it is notable that the Mississippi isolate of *M. medusae* was not virulent on any clones in the pedigree possessing the *Mmd1* allele. In contrast, the *Mxc1* and *Mxc2* pathotypes of *M. ×columbiana* were virulent on some such clones. They were also virulent on T×D commercial clones in the differential set that was resistant to all isolates of *M. medusae* (Table 3). Unfortunately, the presence or absence of the *Mmd1* gene in the T×D commercial clones of the differential set is unknown.

The pathogenic variation of *M. ×columbiana* suggested that genes for resistance other than *Mmd1* were present in the differentials. Again, this limited study readily revealed three new genes for resistance to pathotypes *Mxc1*, *Mxc2*, and *Mxc3*. The *Mxc1* and *Mxc2* genes behave phenotypically like *Mmd1* in that infection type was correlated with quantitative traits such as uredinial density and latent period. This was also true in studies of resistance to the Eurasian poplar rust, *M. larici-populina* (6). However, *Mxc1* and *Mxc2* were unlike *Mmd1* in that they were inherited from *P. deltoides*. The *Mxc3* gene could not be distinguished from *Mmd1* with the host pedigree, but their corresponding pathotypes were distinguished with the host differentials (Table 3).

Quantitative differences in aggressiveness of pathotypes toward specific hybrid poplar clones were not the focus of this study. However, such differences should be further studied. For example, clone 15-29 is reported here as susceptible to pathotype *Mxc1*, collected in 1996, and to *Mxc13*, collected in 1998. The *Mxc13* pathotype appeared to be more aggressive in this study. In any event, the rust susceptibility of 15-29 is now such that this clone is no longer planted in lower Columbia plantations (B. Stanton, *personal communication*).

The abundant pathogenic variation that prompted this study has resulted in the discovery of three new genes for resistance. Similarly, pathogenic variation of *M. larici-populina* in Europe has led to identification of two new major genes for resistance (i.e., *Mer* [4] and M03/04-480 [14], respectively). Use of an intraspecific *P. deltoides* family in Iowa led to discovery of the *Lrd1* gene for resistance to *M. medusae* (13). The total number of published major genes for rust resistance in *Populus* is now seven, making this the best studied system in this respect among forest trees. Interestingly, *P. deltoides* is the source of five of the seven genes for rust resistance that have so far been identified in *Populus* spp. The only exceptions to date are *Mmd1* and *Mxc3* from *P. trichocarpa*, genes that may be closely linked (12).

TABLE 5. Spearman rank correlations among infection type (0 to 4 scale), uredinial density (0 to 6 scale), and latent period (days) for both the *Mxc1* and *Mxc2* genes for resistance to *Melampsora ×columbiana*

	Mxc1 gene		*Mxc2* gene	
	Uredinial density	Latent period	Uredinial density	Latent period
Infection type	0.90 ($P < 0.001$; $n = 57$)	–0.54 ($P < 0.001$; $n = 37$)	0.60 ($P < 0.001$; $n = 61$)	–0.24 ($P = 0.07$; $n = 59$)
Uredinial density	…	–0.44 ($P = 0.007$; $n = 37$)	…	–0.62 ($P < 0.001$; $n = 59$)

TABLE 6. Simple matching dichotomy coefficients for three pathotypes of *Melampsora ×columbiana* (*Mxc1*, *Mxc2*, and *Mxc3*) when inoculated onto a three-generation hybrid poplar pedigree[a]

	Mxc1	*Mxc2*	*Mxc3*
Mxc1	1.00	0.672	0.534
Mxc2	0.634	1.00	0.483
Mxc3	0.476	0.451	1.00

[a] Dichotomy based on resistance (infection types 0, 1, 2, and 3) versus susceptibility (infection type 4). Coefficients above the diagonal are for the F_2 only ($N = 58$), whereas those below the diagonal are for the entire pedigree ($N = 82$).

ACKNOWLEDGMENTS

This research was supported by the Consortium for Plant Biotechnology Research (OR22072-17), the U.S. Department of Energy, Biomass Energy Technology Division, and by six forest-products companies (i.e., Pacifica Poplars Inc., Scott Paper Ltd., Fort James Corp., Union Camp Corp., Westvaco Corp., and Potlatch Corp.). We thank J. Whisler, J. Staley, R. Barham, and B. Watson for their technical help.

LITERATURE CITED

1. Bradshaw, H. D., Jr. 1998. Case history in genetics of long-lived plants: Molecular approaches to domestication of a fast-growing forest tree: *Populus*. Pages 219-228 in: Molecular Dissection of Complex Traits. A. H. Paterson, ed. CRC Press, Boca Raton, FL.
2. Bradshaw, H. D., Jr., Villar, M., Watson, B. D., Otto, K. G., Stewart, S., and Stettler, R. F. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. Theor. Appl. Genet. 89:167-178.
3. Brasier, C. M. 2000. The rise of the hybrid fungi. Nature 405:134-135.
4. Cervera, M. T., Gusmão, J., Steenackers, M., Peleman, J., Storme, V.,

Vanden Broeck, A., Van Montagu, M., and Boerjan, W. 1996. Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. Theor. Appl. Genet. 93:733-737.
5. Larson, P. R., and Isebrands, J. G. 1971. The plastochron index as applied to developmental studies of cottonwood. Can. J. For. Res. 1:1-11.
6. Lefèvre, F., Goué-Mourier, M. C., Faivre-Rampant, P., and Villar, M. 1998. A single gene cluster controls incompatibility and partial resistance to various *Melampsora larici-populina* races in hybrid poplars. Phytopathology 88:156-163.
7. Newcombe, G. 1998. Association of *Mmd1*, major gene for resistance to *Melampsora medusae* f. sp. *deltoidae*, with quantitative traits in poplar rust. Phytopathology 88:114-121.
8. Newcombe, G., Bradshaw, H. D., Jr., Chastagner, G. A., and Stettler, R. F. 1996. A major gene for resistance to *Melampsora medusae* f. sp. *deltoidae* in a hybrid poplar pedigree. Phytopathology 86:87-94.
9. Newcombe, G., Stirling, B., Mcdonald, S. K., and Bradshaw, H. D., Jr. 2000. *Melampsora* ×*columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*. Mycol. Res. 104:261-274.
10. Prakash, C. S., and Heather, W. A. 1986. Inheritance of resistance to races of *Melampsora medusae* in *Populus deltoides*. Silvae Genetica 35:74-77.
11. Prakash, C. S., and Thielges, B. A. 1987. Pathogenic variation in *Melampsora medusae* leaf rust of poplars. Euphytica 36:563-570.
12. Stirling, B., Newcombe, G., Vrebalov, J., Bosdet, I., and Bradshaw, H. D., Jr. Suppressed recombination around the *Mxc3* locus, a major gene for resistance to poplar leaf rust. Theor. Appl. Genet. (In press.)
13. Tabor, G. M., Kubisiak, T. L., Klopfenstein, N. B., Hall, R. B., and McNabb, H. S., Jr. 2000. Bulked segregant analysis identifies molecular markers linked to *Melampsora medusae* resistance in *Populus deltoides*. Phytopathology 90:1039-1042.
14. Villar, M., Lefèvre, F., Bradshaw, H. D., Jr., and Teissier Du Cros, E. 1996. Molecular genetics of rust resistance in poplars (*Melampsora larici-populina* Kleb/*Populus* sp.) by bulked segregant analysis in a 2×2 factorial mating design. Genetics 143:531-536.
15. Ziller, W. G. 1974. The tree rusts of western Canada. Can. For. Serv. Publ. No. 1329.