A Leaf Rust Epidemic of Hybrid Poplar Along the Lower Columbia River Caused by *Melampsora medusae*

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ABSTRACT

Newcombe, G., and Chastagner, G. A. 1993. A leaf rust epidemic of hybrid poplar along the lower Columbia River caused by *Melampsora medusae*. Plant Dis. 77:528-531.

Melampsora medusae caused an epidemic of leaf rust of hybrid poplar (*Populus trichocarpa* × *P. deltoides*) along the Columbia River in western Oregon and Washington in 1991. Although native to North America, *M. medusae* had not attacked hybrid poplars previously in the Pacific Northwest. The initial disease focus was a commercial plantation of 11 clones planted in monoclonal blocks of 5–50 ha near Scappoose, Oregon. By early fall, six hybrid clones infected by *M. medusae* were severely rusted and partially defoliated, four other clones were moderately rusted without leaf loss, and one clone was only lightly rusted. Ramets of the same clones were less severely rusted in other commercial plantations and a nursery within a 100-km radius of Scappoose. Two monouredinial isolates from Oregon and one from Kentucky of *M. m. deltoidae* were tested to determine host range on poplar. The isolate from Kentucky was distinguished from the isolates from Oregon on one clone of *P. deltoides*, three hybrid clones, and two clones of *P. tremuloides*.

Short-rotation, intensive culture of hybrid poplars is increasingly practiced by forest-products companies in the Pacific Northwest. Large-scale plantations on idled agricultural land are a relatively new venture, presenting new challenges. The rapid growth of hybrid poplars can assure a steady supply of high yields of desirable feedstock for pulp. Hybrid poplar pulp might also eventually be used as a biofuels feedstock for ethanol conversion (1).

The black cottonwood (*Populus trichocarpa* Torr. & Gray) is a fast-growing tree native to the Pacific Northwest and amenable to clonal propagation (3). First-generation hybrids of *P. trichocarpa* × *P. deltoides* J. Bartram ex Marsh. tend to be even faster-growing (4,9). Selected hybrid clones in large operational plantations established in the early 1980s along the lower Columbia River were unaffected by poplar leaf rust until the epidemic of 1991. *Melampsora occidentalis* H. Jacks., commonly seen on *P. trichocarpa*, is generally not observed on hybrids, and *M. medusae* Thuem. had never been observed on hybrids in the Pacific Northwest (2).

The objective of this study was to determine the distribution of poplar leaf rust in Oregon and Washington, to rate disease severity, and to identify the causal rust species. Further characterization, at the forma specialis level, was done once *M. medusae* was identified as the primary causal rust species.

Accepted for publication 30 December 1992.

MATERIALS AND METHODS

Identification of the leaf rust species. Morphology and surface ornamentation of urediniospores of field samples were examined with scanning electron microscopy (SEM) and light microscopy (LM). Length and width of urediniospores were measured with LM. For SEM, urediniospores were air-dried, mounted on aluminum stubs, and sputter-coated with approximately 30 nm of gold/palladium. The specimens were then examined with a JEOL 840A SEM. For LM, the urediniospores were mounted either in clear nail polish without a coverslip (for observation of surface ornamentation [6]) or in water (for measurement of length and width). A Kentucky isolate of *M. m. deltoidae* from *P. deltoides* clone ST 535 (obtained from L. Shain of the University of Kentucky) was examined for comparative purposes.

Mapping the epidemic. On 23 and 24 October 1991, a survey for incidence of leaf rust in hybrid poplar plantations along the lower Columbia River was carried out at the following sites: Skamokawa and Puget Island, Washington, and Westport, Midland, Clatskanie, Goble, Scappoose, and Woodburn, Oregon. Each of these sites, with the exception of the nursery in Woodburn, consists of monoclonal blocks. At each site, five leaves bearing uredinia were collected from each infected clone and brought back to the laboratory for identification and measurement of urediniospores. Ten urediniospores from each of five uredinia on a leaf were measured. Rust severity of 10 trees spaced evenly within monoclonal blocks of 11 clones at Scappoose was rated according to the method of Schreiner (7). Severity of rust infection on leaves was estimated as light, medium, or heavy and given a numerical value of 1, 5, or 25, respectively. Then the per-

Table 1. Measurements of *Melampsora medusae* urediniospores from different hybrid poplar (*Populus trichocarpa* × *P. deltoides*) clones and locations affected by the fall 1991 epidemic of leaf rust and an isolate of *M. m. deltoides* from *P. deltoides* in Kentucky[a]

Clone[b]	Taxon[c]	Location	Spore length (μm)		Spore width (μm)	
			Range	$\bar{x}$	Range	$\bar{x}$
23-91	T × D	Scappoose, Oregon	30–37	34	15–17	16
195-529	T × D	Scappoose, Oregon	30–37	33	15–18	17
199-574	T × D	Scappoose, Oregon	29–33	31	14–18	15
47-174	T × D	Scappoose, Oregon	25–37	33	15–17	16
47-174	T × D	Skamokawa, Washington	25–37	33	13–17	15
47-174	T × D	Midland, Oregon	27–35	31	14–17	16
47-174	T × D	Goble, Oregon	27–35	30	14–16	15
11-11	T × D	Woodburn, Oregon	25–32	29	15–19	18
ST 535	D	Kentucky	25–34	29	15–18	16

[a] Measurements were made with an ocular micrometer of fresh mature urediniospores mounted in water. A total of 250 spores per clone were measured (five leaves × five uredinia per leaf × 10 spores per uredinium).

[b] Clone numbers, with one exception (ST 535 from Stoneville, Mississippi), refer to the University of Washington/Washington State University Poplar Program 1991 Clone Register, which has further information on the geographic origins of parents.

[c] T × D = *P. trichocarpa* × *P. deltoides*, D = *P. deltoides*.

centage of leaves thus rusted was estimated at <25, 26–50, 51–75, or >75 and given a numerical value of 1, 2, 3, or 4, respectively. Multiplication of the two numerical values gave a Schreiner rating ranging from 0 (highly resistant) to 100 (highly susceptible). On 1 November 1991, additional samples were received from IFA Nurseries Inc. in Toledo, Washington, for identification purposes.

Plant propagation and care. Known clones of species and hybrids of poplar were rooted as cuttings in soil with periodic misting and grown in a greenhouse with supplemental lighting at 21 C and a 14-hr photoperiod.

Detached leaf inoculations. Young leaves (the fourth from the shoot apex) were surface-disinfested in a solution of 1% NaOCl for 1 min, rinsed in distilled water for 5 min, cut to fit into 9-cm petri dishes, and rinsed in distilled water. Leaves were then placed abaxial side up in 9-cm petri dishes on filter paper saturated with a 100 μg/ml solution of gibberellic acid. Urediniospores from previously inoculated leaves were collected and suspended in distilled water in a test tube. The suspension was vortexed for 30 sec and diluted to approximately 5×10^4 spores per milliliter. The germinability of spores in the suspension was checked on dishes of 1.5% water agar amended with 100 μg/ml each of streptomycin and chloramphenicol, and the dishes were incubated for 24 hr in the dark at 15 C and scored as above. Spore suspension was then brushed onto the leaves with an alcohol-sterilized artist's brush. Leaves used as controls were brushed with distilled water only. Petri dishes containing inoculated and control leaves were placed in the dark at 15 C for 24 hr, then placed in continuous light (approximately 100 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 20 C.

After 16 days, clones were rated as resistant if no sporulating uredinia were present on any of the inoculated leaves, susceptible if sporulating uredinia were consistently present, and intermediate if a few uredinia sometimes formed but sporulated only rarely.

Testing at the forma specialis level. Plants included in this test were three clones of *P. deltoides* from Minnesota known to be susceptible to *M. m. deltoidae* (M. Ostry, *personal communication*) and five clones of *P. tremuloides* Michx. from Oregon known to be susceptible to an unidentified leaf rust (R. Cameron, *personal communication*), probably *M. m. tremuloidae*. Other accessions of *P. deltoides, P. nigra* L. var. *italica* Münchh., and five hybrid *P. trichocarpa* × *P. deltoides* clones were used as well. Three isolates of *M. medusae* were used: the Kentucky isolate from *P. deltoides* described above, one isolate from rusted hybrid clone 11-11 in Woodburn, Oregon, and one isolate from hybrid clone 49-177 in Goble, Oregon; clone numbers refer to the University of Washington/Washington State University Poplar Program 1991 Clone Register, which has further information on the geographic origins of parents. Isolates were further selected and increased as monouredinial isolates before the above set of plants were inoculated, using the technique described above. The set was inoculated on three separate occasions; each time, five detached leaves per clone were inoculated and one leaf served as an uninoculated control.

RESULTS AND DISCUSSION

Identification. *M. medusae* is distinguished from other *Melampsora* species on *Populus* by its urediniospores, which are echinulate except for an equatorial smooth patch (11). This equatorial smooth patch was visible in both the SEM (Fig. 1A) and the LM on all urediniospore samples listed in Table 1 as well as on the urediniospores of *M. m. deltoidae* from Kentucky (Fig. 1B). The urediniospores were generally obovate to oval with bilateral wall thickening as reported for *M. medusae* (10,11,13). Capitate paraphyses with uniformly thickened walls, typical of uredinia of *M. medusae*, were also noted. In addition, urediniospore dimensions (Table 1) of all samples, including those from Kentucky, also fit published descriptions of *M. medusae* (8,10,11,13). A specimen of *M.*

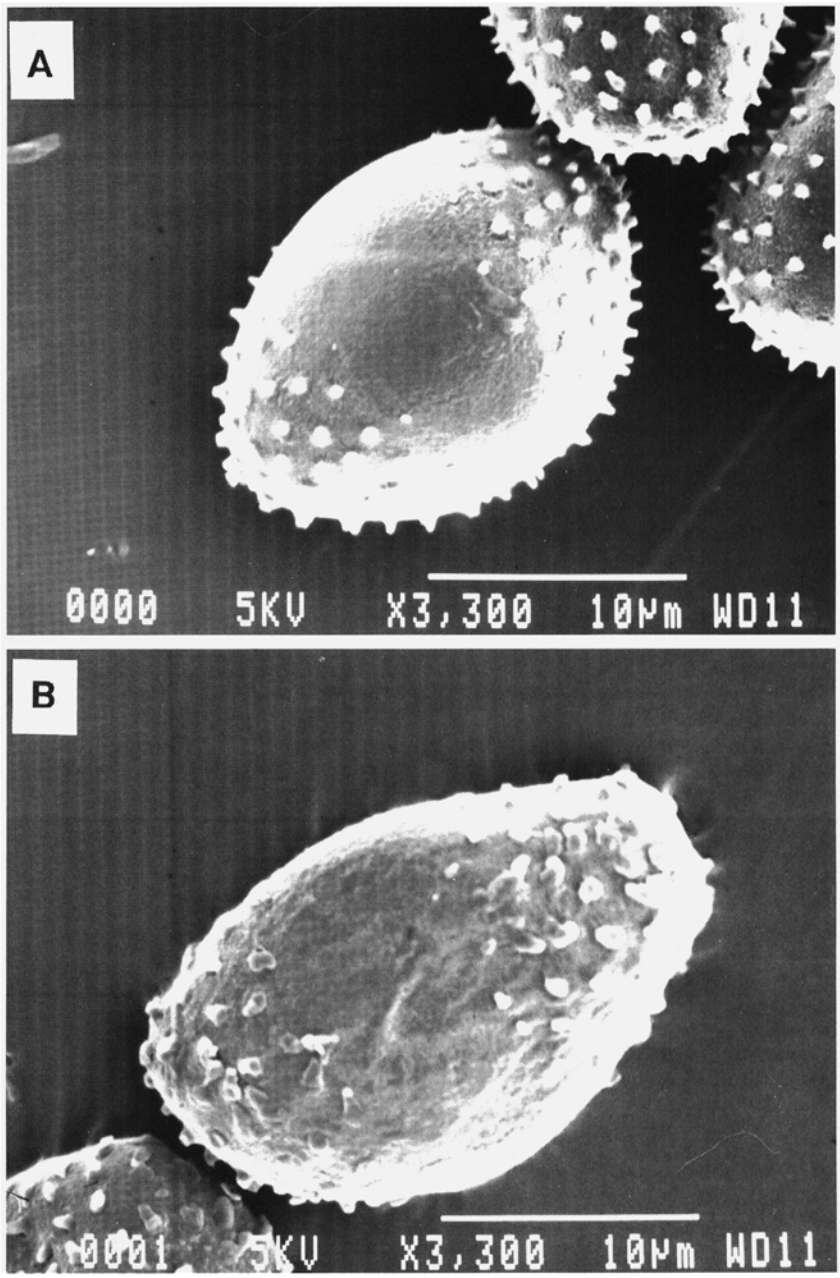

Fig. 1. (A) SEM of a urediniospore of *Melampsora medusae* from hybrid poplar clone 47-174 in Scappoose, Oregon. An equatorial smooth band is evident. **(B)** SEM of a urediniospore of a Kentucky isolate of *M. medusae* f. sp. *deltoidae* from *Populus deltoides* clone ST 535, obtained from L. Shain (University of Kentucky) in lyophilized form. The spines may have been damaged by lyophilization, but the equatorial smooth band is still evident.

medusae on clone 47-174 (*P. trichocarpa* × *P. deltoides*) is held by the Mycological Herbarium of Washington State University as WSP No. 69581.

Mapping the epidemic. *M. medusae* was not observed on 23 October 1991 in the hybrid poplar plantations at Puget Island, Washington, and Westport and Clatskanie, Oregon. Surveys on 30 October and 19 November revealed light levels of rust in Clatskanie on hybrid clone 47-174 (Bill Schuette, *personal communication*). One 2-yr-old tree of clone 47-174 in Skamokowa, Washington, was lightly rusted. At Midland, Oregon, a single uredinium was seen on clone 47-174. At Goble, Oregon, clone 47-174 was lightly rusted (none of the 30 trees surveyed rated a Schreiner score >1). Urediniospore samples from Woodburn, Oregon, and Toledo, Washington, were also identified as *M. medusae*. Only in Scappoose, Oregon, was heavy rust and defoliation caused by rust observed.

Table 2. Field rating of *Melampsora medusae* rust severity in Scappoose, Oregon, hybrid popular (*Populus trichocarpa* × *P. deltoides*) plantation in October 1991

Clone[a]	Provenance of male *P. deltoides* parents[b]	Rating[c]	Defoliation
23-91	Stoneville, Mississippi	1	No
49-177	Brazo County, Texas	25	No
52-225	Perry County, Illinois	25	No
46-158	Brazo County, Texas	25	No
184-402	Morton County, Kansas	50	No
47-174	Howard County, Missouri	100	Yes
200-604	Morton County, Kansas	100	Yes
195-529	Hughes County, Oklahoma	100	Yes
199-574	Payne County, Oklahoma	100	Yes
194-525	Morton County, Kansas	100	Yes
196-553	Texas	100	Yes

[a] Clone numbers refer to the University of Washington/Washington State University Poplar Program 1991 Clone Register, which has further information on the geographic origins of parents.
[b] The male parent from Morton County, Kansas, was the only one shared by more than one clone.
[c] According to the method of Schreiner (6). Severity of rust infection on leaves was estimated as light, medium, or heavy and given a numerical value of 1, 5, or 25, respectively. Then the percentage of leaves thus rusted on 10 ramets of a given clone was estimated at <25, 26–50, 51–75, or >75 and given a numerical value of 1, 2, 3, or 4, respectively. Multiplication of the two numerical values gave a Schreiner rating ranging from 0 (highly resistant) to 100 (highly susceptible).

Table 3. Determination of forma specialis of two isolates of *Melampsora medusae* from Oregon and an isolate of *M. m. deltoidae* from Kentucky[a]

Taxon	Clone[b]	Isolate[c] 139-91	142-91	77-34
Populus tremuloides	No. 1	R	R	R
	No. 3	S	S	S
	No. 4	R	S	R
	No. 5	S	S	R
	No. 6	R	R	R
P. nigra	var. *italica*	S	S	S
P. deltoides	MN 11044	S	S	S
	MN 11016	S	S	S
	MN 11288	S	S	S
	MN 288-5	I	I	I
	IL 129 (59-129-17)	S	S	S
	IL 005 (73-005-02)	S	S	I
	TX S7C4	S	S	S
	TX S7C1	R	R	R
P. trichocarpa × *P. deltoides* hybrids				
Male parents				
MO 64-243-03	47-174	S	S	S
TX S7C4	55-253	S	S	R
IL 129 (59-129-17)	53-248	S	S	R
IL 129 (59-129-17)	53-239	S	S	R
ST 70	23-91	I	I	I

[a] Monouredinial isolates were used to inoculate detached surface-sterilized leaves incubated at 20 C on filter paper moistened with 100 μg/ml of gibberellic acid in petri dishes.
[b] Five *P. tremuloides* clones are known to be susceptible to rust, although *Melampsora* sp. infecting them in Oregon has not been identified (Ron Cameron, *personal communication*). MN 11044, MN 11016, and MN 11288 are known to be susceptible to *M. medusae* in Minnesota (Mike Ostry, *personal communication*).
[c] Isolate 139-91 from Woodburn, Oregon, from *P. trichocarpa* × *P. deltoides* hybrid clone 11-11; isolate 142-91 from Goble, Oregon, from *P. trichocarpa* × *P. deltoides* hybrid clone 49-177; and isolate 77-34 from Kentucky from *P. deltoides* clone ST 535. R = resistant interaction (uredinia never formed); S = susceptible interaction (uredinia formed and sporulated on five replicate leaves in each of three separate experiments); I = intermediate interaction (sometimes a few uredinia formed but only rarely sporulated).

Field rating of rust severity. Six hybrid clones in the plantation at Scappoose had severe rust (Schreiner scores of 100) and varying degrees of defoliation on 23 October 1991 (Table 2). Another five clones in Scappoose were less severely rusted and not defoliated. Clone 47-174, while among the worst affected clones in Scappoose, was only lightly rusted at other sites, indicating either pathogenic variation or lower inoculum levels.

In the laboratory, there was no evidence of pathogenic variation between the two isolates from Oregon (Table 3), except on aspen clone No. 4. Field results (Table 2) and laboratory results (Table 3) agreed for clones 23-91, the least rusted of clones at Scappoose and intermediate in response to laboratory inoculation, and 47-174, among the most susceptible clones at Scappoose and susceptible in the laboratory. Thus the laboratory findings indicate that differences in the level of rust on clone 47-174 noted at the various sites within a 100-km radius of the initial disease focus at Scappoose were most likely the result of lower inoculum levels or less favorable conditions for infection.

Testing at the forma specialis level. Two formae speciales for *M. medusae* were established in 1988 on the basis of host preference (8). *M. m. deltoidae* was isolated from, and identified as pathogenic primarily on, *P. deltoides*, whereas *M. m. tremuloidae* was isolated from, and pathogenic only on, *P. tremuloides*.

Isolates of *M. medusae* from hybrid poplar have not been identified as to forma specialis prior to our work. The isolates of *M. medusae* from hybrid poplar in Oregon and the isolate of *M. m. deltoidae* from Kentucky were pathogenic on the three known susceptible clones of *P. deltoides* from Minnesota as well as on three other *P. deltoides* clones (Table 3). The three isolates were also pathogenic on aspen. Shain (8) reported very low levels of infection (e.g., 0–0.7 infection per leaf disk) on two of six clones inoculated with his Kentucky-cottonwood collection of *M. medusae* and on only one of six clones inoculated with his collection from Michigan. Although the set of aspen clones in the present study differs from that used by Shain (8), the isolate from Kentucky of *M. m. deltoidae* obtained from Shain was pathogenic on only one of the five aspen clones, in keeping with expectations. In contrast, one isolate from Oregon was pathogenic on three of five aspen clones

and the other was pathogenic on two of five. It is difficult to compare infection levels, as 17-mm-diameter disks were used in the previous study (8) and entire detached leaves or large leaf pieces were used in our study. It is also noteworthy that the isolates from hybrid poplar from Oregon were pathogenic on all four hybrid clones (Table 3), whereas the isolate of *M. m. deltoidae* from Kentucky was pathogenic on only one of the four.

Since *M. m. tremuloidae* was unable to attack *P. deltoides* in the previous study (8), the two isolates from Oregon are clearly more similar to *M. m. deltoidae*, represented in this study by the isolate from Kentucky. However, the greater telial host range (i.e., aspen and hybrid clones) of the isolates from Oregon in this limited study indicate the need for further research. It may be that isolates of *M. medusae* from hybrid poplar will have to be assigned to a third forma specialis characterized by a wider telial host range than is the case for *M. m. deltoidae*.

M. medusae, although native to North America, probably occurred until 1991 only in its forma specialis *tremuloidae* (8) on *P. tremuloides* in the Pacific Northwest west of the Cascade Mountains. *P. deltoides*, host of *M. m. deltoidae*, does not occur naturally in the area, and hybrid poplars had been unaffected by *M. medusae*. Given the fairly widespread hybrid poplar culture in the area for the past 10 yr or longer, it seems unlikely that *M. m. tremuloidae* is pathogenic to hybrids. *M. medusae* strains pathogenic to hybrids may have been introduced into this part of the Pacific Northwest from elsewhere in North America or from Europe, Asia, Australia, or New Zealand, where *M. medusae* is known as an introduced pathogen of hybrids (11). A non-North American source is likely, given the fact that *M. larici-populina* Kleb., native to Eurasia and never before found in North America, was identified at two of the surveyed sites (i.e., Woodland and Scappoose) in late fall of the 1991 epidemic (5). The probability of the simultaneous introduction into hybrid poplar plantations of two poplar leaf rust species from two different sources seems small, whereas the probability of both rusts being from the same non-North American source seems greater.

Whether the population of *M. medusae* observed in 1991 will establish itself in the Pacific Northwest is difficult to assess. Although Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) is known to be susceptible to *M. medusae* isolates from *P. tremuloides* (12) and is very common in the Pacific Northwest, its susceptibility to the *M. medusae* that occurred in 1991 on hybrid poplars in the Pacific Northwest is unknown. The same argument applies to other reported conifer hosts of *M. medusae* (12). Urediniospores or mycelia in dormant buds represent other possible modes of overwintering (11) that need to be assessed under local conditions.

ACKNOWLEDGMENTS

We thank Dale Blum of the Department of Botany, University of Washington, for help with the SEM, and Valerie McQuarrie-Baker, Linda Parks, Kathy Riley, Bill Schuette, John Staley, and Nickii Storkus for technical assistance. We thank M. E. Ostry of the USDA Forest Service, Ron Cameron of Oregon State University, and L. Shain of the University of Kentucky for contributing research materials. This study was supported with funds from the Washington Technology Center. Hybrid poplar genetic research and clonal collection and its maintenance were supported by the U.S. Department of Energy, Biomass Energy Technology Division (supplement to grant 19X-43382C). We are grateful to R. F. Stettler of the University of Washington and J. D. Rogers of Washington State University for reviewing the manuscript. PPNS 0130, Project 0939, Department of Plant Pathology, Washington State University, Agricultural Research Center, Pullman 99164.

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