First Report of the Eurasian Poplar Leaf Rust Fungus, *Melampsora larici-populina*, in North America

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ABSTRACT

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Melampsora larici-populina, native to Eurasia, was found in October 1991 in hybrid poplar (*Populus trichocarpa* × *P. deltoides*) plantations along the Columbia River near Woodland, Washington, and Scappoose, Oregon. Clavate to broadly ellipsoid urediniospores measured 30–49 × 13–16 μm and were echinulate except for an apical smooth patch. Telia were exclusively epiphyllous. Detached leaf inoculations were used to investigate the telial (poplar) host range of three different monouredinial isolates in the laboratory. Clones known to be susceptible in Europe and Australia to *M. larici-populina* (i.e., *P. nigra* var. *italica* and *P.* × *euramericana* cv. I-488) were susceptible in these tests, as were 20 clones of *P. trichocarpa*, the native black cottonwood of the Pacific Northwest. In general, the pattern of susceptibility among 50 clones representing many poplar taxa, including interspecific hybrid classes, was in accord with what is known of the host range of *M. larici-populina* in Europe and Australia. Pathogenic variation among the three isolates was not observed.

Of the many *Melampsora* species that cause leaf rust of poplars, *M. medusae* Thuem. and *M. larici-populina* Kleb. are the two most destructive, because each has a wide host range that includes many species and interspecific hybrids. *M. medusae* is native to North America but has been introduced throughout most of the world where poplars are grown (20). *M. larici-populina*, native to Eurasia and introduced into Australia (20), was reported by Gäumann (5) to occur in North America; this has never been confirmed (2–4,9,11,14,15).

Although *M. occidentalis* H. Jacks. is endemic to the Pacific Northwest, it is usually found only on the native black cottonwood (*Populus trichocarpa* Torr. & Gray). Relatively few hybrids of *P. trichocarpa* × *P. deltoides* J. Bartram ex Marsh. (planted in the Pacific Northwest for intensive, short-rotation culture) are attacked by *M. occidentalis*. *M. medusae*, although native to North America, was not found in hybrid poplar plantations west of the Cascade Mountains until 1991 (10.)

An epidemic of leaf rust occurred in previously rust-free hybrid poplar plantations along the Columbia River west of the Cascades in the fall of 1991. The extent of the epidemic was mapped and the leaf rust fungi responsible were identified at different locations and on different clones. Only *M. medusae* was found (10) until late in October, when a *Melampsora* species was found that differed from the five species reported on *Populus* in North America (6). The objective of this study was to identify this *Melampsora* species.

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MATERIALS AND METHODS

Morphology and surface ornamentation of urediniospores. Leaves of hybrid poplar (i.e., *P. trichocarpa* × *P. deltoides*) clone 49-177 bearing uredinia were collected on 28 October 1991, near Woodland, Washington, and Scappoose, Oregon. (Clone number refers to the University of Washington/Washington State University Poplar Program 1991 Clone Register, which has further information on the geographic origins of parents.) These two locations are on opposite sides of the Columbia River. For comparative purposes, urediniospores of *M. occidentalis* on *P. trichocarpa* near Puyallup, Washington, were also collected. The urediniospores were observed with both scanning electron microscopy (SEM) and light microscopy (LM) (12), as previously described (10).

Measurements of spores, paraphyses, and sori. Urediniospores and paraphyses were mounted in water on a glass slide and measured with an ocular micrometer at a magnification of 400×. Twenty urediniospores from each of 10 different leaves from each site (for a total of 400 urediniospores) were measured. The apical thickness of 50 paraphyses from the 20 leaves was measured. Telia were collected on 7 January 1992 at Woodland and sectioned freehand with a razor blade. A total of 16 different telia on eight leaves (two telia per leaf) were sectioned and mounted in water on glass slides so that 10 teliospores per telium could be measured. The diameters of single, isolated uredinia and telia (at least 100 of each) on leaves were measured with an ocular micrometer on a dissecting microscope.

Independent confirmation of identification. Field-collected uredinial and telial samples and inoculated leaf pieces bearing uredinia were submitted to M. Palm of the U.S. Department of Agriculture (Beltsville, MD) and Y. Hiratsuka of Forestry Canada (Edmonton, Alta.) for identification.

Plant propagation and care, detached leaf inoculations and incubations, and rating of disease phenotypes. 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. 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 described previously (10). Spore suspension was then brushed onto the leaves with an alcohol-sterilized artist's brush. Inoculated leaves of cv. I-488 were included in each experiment as a susceptible check. Leaves used as controls were brushed with distilled water only. In each experiment (conducted on three separate occasions), five detached leaves of each of 10 clones were inoculated. 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 qualitatively as resistant if no sporulating uredinia were present on any of the inoculated leaves and susceptible if sporulating uredinia were consistently present.

Selection of monouredinial isolates for inoculation purposes. Rusted leaves of hybrid poplar clone 49-177 were collected in Woodland and Scappoose. The

urediniospores were used to inoculate surface-sterilized leaf disks of greenhouse-grown 49-177. When uredinia formed on disks, isolated uredinia (two from Scappoose and one from Woodland) were selected and increased on 49-177. Thus, three monouredinial isolates, 138-91-S and 144-91-S from Scappoose and 143-91-W from Woodland, were available for use in further experiments to determine the host range.

Determination of host range in *Populus*. Even though the *Populus* host range of *M. larici-populina* has never been comprehensively studied, *P. nigra* L. var. *italica* Münchh. (an Italian selection from a European species) and *P.* × *euramericana* (Dode) Guinier cv. I-488 are specifically known to be susceptible (6). Clones of *P. trichocarpa* (native to the Pacific Northwest) tend to be susceptible when planted in regions where *M. larici-populina* is endemic (7,17,18). *P. alba* L. (native to Eurasia), belonging to subsection *Albidae* Dode of the section *Leuce* Duby, is known to be resistant (6). *P. deltoides* (native to eastern and central North America), *P. maximowiczii* A. Henry (native to Japan and northeastern Asia), and hybrids of *P. trichocarpa* × *P. deltoides* and of *P. trichocarpa* × *P. maximowiczii* are variable for resistance to *M. larici-populina* (1,6,7,17).

To determine whether host range conformed to expectations for *M. larici-populina*, and to test for variability among the three monouredinial isolates, the following were grown, inoculated, and rated: two known susceptible clones, *P. n. italica* and *P.* × *euramericana* cv. I-488; one clone of the resistant *P. alba*; 20 clones of *P. trichocarpa*; three clones of *P. deltoides*; 16 hybrid clones of *P. trichocarpa* × *deltoides*; three clones of *P. maximowiczii*; and five clones of *P. trichocarpa* × *P. maximowiczii*.

RESULTS AND DISCUSSION

Morphology and measurements of urediniospores, paraphyses, and sori. Urediniospores from both Scappoose, Oregon, and Woodland, Washington, tended to be clavate to broadly ellipsoid. Some were oval to ovate and a few were obovate. Apices were rounded and bases were often truncate. Spore contents were yellow and walls were hyaline. When examined with the SEM, the wall surface of the urediniospore was echinulate except for a smooth patch 5–9 μm wide on, or slightly to one side of, the apex (Fig. 1A). The spines were largest toward the base and progressively smaller toward the smooth patch. The echinulation and apical smooth patch were also visible in the LM. In contrast, the larger urediniospores of *M. occidentalis* were evenly echinulate (Fig. 1B).

Urediniospores measured 30–49 × 13–16 μm, whereas the apical wall thickness of uredinial, mostly capitate paraphyses varied from 5 to 11 μm. Uredinia on host clone 49-177 from Woodland and Scappoose were hypophyllous and varied from 300 to 400 μm in diameter.

Isolated telia were epiphyllous, and 70% (73/104) were from 200 to 300 μm in diameter. Composite, or confluent, sori (i.e., a number of telia fused together) were fairly common. Teliospores measured 32–47 × 5–10 μm.

On the basis of morphological and morphometric grounds, the poplar leaf rust fungus found near Woodland and Scappoose matched the literature description of *M. larici-populina* (21,22). Both M. Palm and Y. Hiratsuka (*personal communications*) confirmed the identification of *M. larici-populina* on the same basis. In addition, the uredinial and telial specimens from Woodland matched specimens in the Arthur Herbarium of *M. larici-populina* from South America, Europe, Australia, South Africa, and Japan (Y. Hiratsuka, *personal communication*). A specimen of *M. larici-populina* on clone 49-177 (*P. trichocarpa* × *P. deltoides*) is held by the Mycological Herbarium of Washington State University as WSP No. 69582.

None of the five *Melampsora* species previously reported in North America (4)

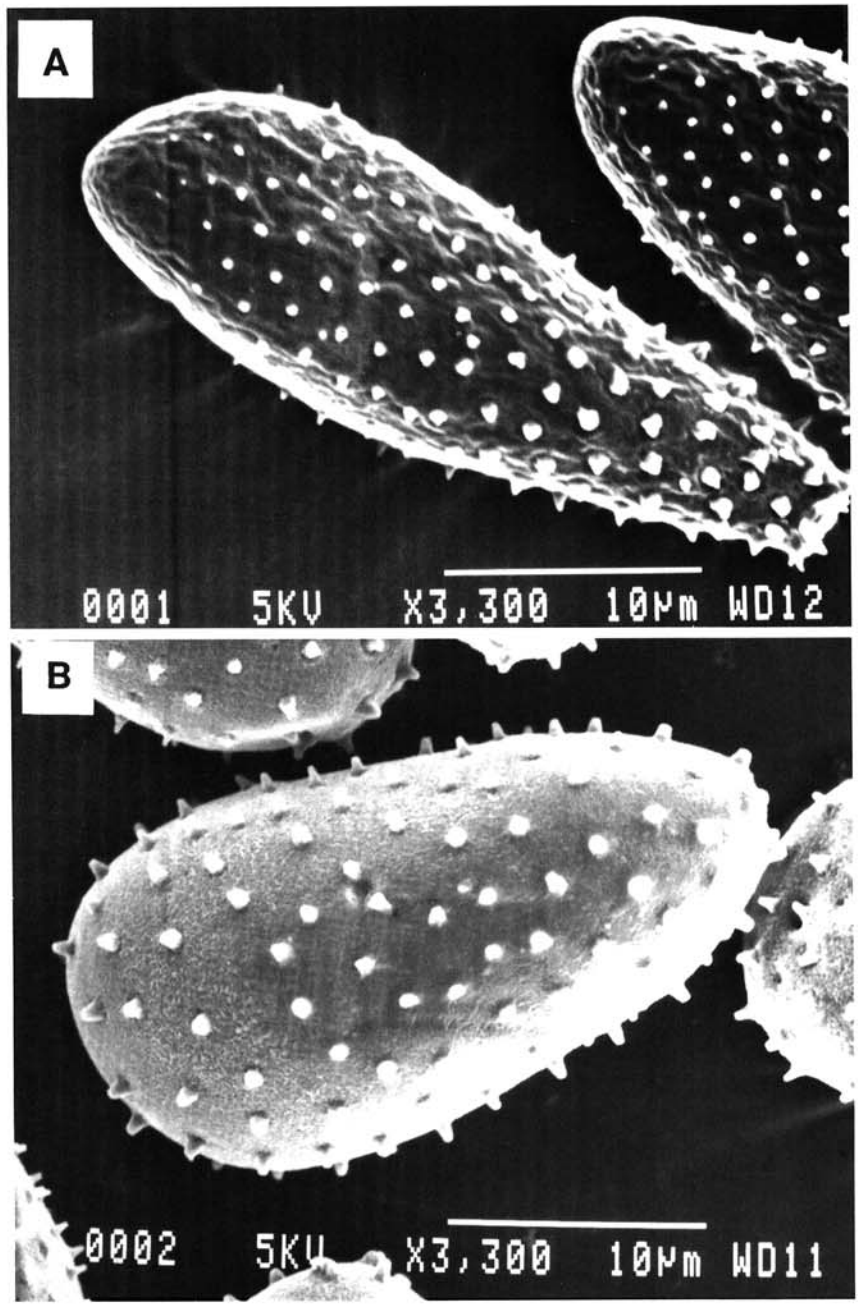

Fig. 1. (A) SEM of a urediniospore of *Melampsora larici-populina* from hybrid poplar clone 49-177 near Woodland, Washington, showing the clavate shape, with the spines largest toward the base and progressively smaller toward the apical smooth patch. **(B)** SEM of a urediniospore of *M. occidentalis* from *Populus trichocarpa* near Puyallup, Washington, showing the uniform echinulation and greater spore width.

possesses echinulate urediniospores with apical smooth spots and epiphyllous telia. The only *Melampsora* species other than *M. larici-populina* that attack poplars and have echinulate urediniospores with smooth apices are *M. allii-populina* Kleb. and *M. multa* Shang, Pei & Yuan. *M. allii-populina* is sympatric with *M. larici-populina* in Europe and Asia (19), whereas *M. multa* is present in the province of Liaoning in northeastern China (13). The former possesses uredinial paraphyses without apical wall thickening, urediniospores without bilateral wall thickening, and hypophyllous telia (19), whereas the latter has uredinial paraphyses with a very thick wall at the apex (8–16 μm vs. 5–10 μm for *M. larici-populina*) and large telia (300–800 μm in diameter) that are single-layered when epiphyllous and single-, bi-, or tri-layered when hypophyllous (13).

Table 1. Host range of three monouredinal isolates of *Melampsora larici-populina* from western Washington and Oregon

Host clone (species range)	Disease rating[a]
Populus nigra var. *italica* (Europe)	S
P. trichocarpa (Pacific Northwest)	
8-1038	S
93-968	S
ORT 81-3	S
147-947	S
147-991	S
147-1012	S
147-955	S
95-875	S
236-1590	S
236-1821	S
236-1825	S
236-1826	S
235-1810	S
235-1850	S
245-1852	S
245-1849	S
250-2179	S
250-2176	S
250-2174	S
258-1872	S
P. × *euramericana* cv. I-488	S
P. alba (Eurasia)	R
P. deltoides (eastern and central North America)	
IL-005 (73-005-02)	R
IL-129 (59-129-17)	R
TX S7C1	R
P. trichocarpa × *P. deltoides*	
11-5	R
11-11	R
47-174	R
55-264	R
52-237	R
180-706	S
49-177	S
24-305	S
15-30	S
22-87	S
44-146	S
44-132	S
50-197	S
17-50	S
DTAC-7	S
23-91	S
P. maximowiczii (Japan)	
290-124	S
290-4	S
290-6	R
P. trichocarpa × *P. maximowiczii*	
284-22	S
263-11	S
282-183	R
269-63	S
278-299	S

[a] S = susceptible interaction (uredinia formed and sporulated on five replicate leaves or leaf pieces in each of three separate experiments); R = resistant interaction (uredinia never formed).

Host range. Our results (Table 1) were as expected for all three isolates of *M. larici-populina*. The two known susceptible clones, *P. n. italica* (an Italian selection of a European species) and *P.* × *euramericana* cv. I-488, were susceptible, whereas the clone of *P. alba* (native to Eurasia) expected to be resistant was, in fact, resistant. The finding that all 20 clones of *P. trichocarpa* (native to the Pacific Northwest) were susceptible confirms previous field observations of *P. trichocarpa* planted in Europe, where *M. larici-populina* is endemic (7,17). Expected variability in reaction to *M. larici-populina* was observed among *P. trichocarpa* × *P. deltoides* hybrid clones, *P. maximowiczii* (native to Japan and northeastern Asia) clones, and *P. trichocarpa* × *P. maximowiczii* clones (1,6,7,17). The fact that the three clones of *P. deltoides* (native to eastern and central North America) were resistant is also in keeping with expectations of a high proportion of resistant individuals in the eastern cottonwood (7,17). These results further confirm the identification of *M. larici-populina*.

The three isolates did not differ in host range (i.e., there was no pathogenic variation). It is possible they could have been distinguished if a greater number of host clones had been tested, although the set of 50 clones should have been adequate. It is also possible that our three field isolates represent inadequate sampling of the variation occurring at Woodland and Scappoose. Furthermore, because *M. larici-populina* was discovered late in the season just before leaf fall, there was no time to map its distribution. *M. larici-populina* could have been present at other sites and on clones other than the one (i.e., 49-177) from which our three isolates were collected. Sampling from other sites, and particularly from other clones, might have yielded pathogenic variants. In New Zealand, pathogenic variation was observed in 1979, 6 yr after the introduction of *M. larici-populina* (8).

If *M. larici-populina* becomes established in the Pacific Northwest, it is likely that its distribution would ultimately match that of the uniformly susceptible and relatively abundant native black cottonwood, *P. trichocarpa*. Thus, scattered plantings of hybrid and ornamental poplars, such as the susceptible Lombardy poplar, would not escape infection from *M. larici-populina*.

The probability of establishment of *M. larici-populina* in North America will depend on its ability to overwinter there. It might overwinter in the uredinial state, as has been reported elsewhere (22), on persisting green leaves, on fallen leaves, as urediniospores lodged under bud scales, or as mycelium in buds. *Larix decidua* Mill. (European larch), *L. leptolepis* (Siebold & Zucc.) Gordon (Japanese larch), and *Pinus radiata* D. Don (Monterey pine) have been reported as aecial hosts (1,16,19) in Europe, Japan, and Australia, respectively. Damage to the aecial host is probably negligible to light. However, the full aecial host range of *M. larici-populina* has never been systematically investigated.

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LITERATURE CITED

1. Chiba, O. 1962. On the variation in susceptibility of poplar clones to the leaf rust caused by *Melampsora larici-populina* Klebahn. Forestry Agency, Ministry of Agriculture, Tokyo, Japan.
2. Conners, I. L. 1967. An annotated index of plant diseases in Canada, and fungi recorded on plants in Alaska, Canada and Greenland. Can. Dep. Agric. Publ. 1251.
3. Cummins, G. B. 1962. Supplement to Arthur's Manual of the Rusts in the United States and Canada. Hafner Publishing Co., New York.
4. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, MN.
5. Gäumann, E. 1959. Die Rostpilze Mitteleuropas. Beitr. Kryptogamenfl. Schweiz 12:1-1407.
6. Heather, W. A., and Chandrashekar, M. 1982. Evolutionary, epidemiological and ecological implications of forms of resistance in *Populus* spp. to *Melampsora* leaf rust. Aust. For. Res. 12:231-244.
7. Krzan, Z. 1978. Variability of poplars in resistance to infection by the rust *Melampsora larici-populina* Kleb., under field conditions. Pages 95-112 in: The Mechanism of Poplar Leaf Resistance to Fungal Infection. R. Siwecki, ed. Polish Academy of Sciences, Warsaw.
8. Latch, B. J., and Wilkinson, A. G. 1980. New poplar clones help distinguish races of *M. larici-populina* Kleb. in New Zealand. Australas. Plant Pathol. 9:112-113.
9. Lowe, D. P. 1969. Check list and host index of bacteria, fungi and mistletoes of British Columbia. For. Res. Lab. Inf. Rep. BC-X-32.
10. Newcombe, G., and Chastagner, G. A. 1993. A leaf rust epidemic of hybrid poplar along the lower Columbia River caused by *Melampsora medusae*. 77:528-531.
11. Parmelee, J. A. 1960. The fungi of Ontario. I. Uredinales. Can. Dep. Agric. Publ. 1080.

12. Preece, T. F., and Hick, A. J. 1990. An Introductory Scanning Electron Microscope Atlas of Rust Fungi. Farrand Press, London.
13. Shang, Y., Pei, M., and Yuan, Z. 1986. A new rust fungus on poplars. Pages 180-184 in: Acta Mycol. Sin. Suppl. 1.
14. Shaw, C. G. 1973. Host fungus index for the Pacific Northwest—I. Hosts. Wash. State Agric. Exp. Stn. Bull. 765.
15. Shaw, C. G. 1973. Host fungus index for the Pacific Northwest—II. Fungi. Wash. State Agric. Exp. Stn. Bull. 766.
16. Spiers, A. G. 1975. Species of *Melampsora* infecting conifers in New Zealand. Plant Dis. Rep. 59:486-488.
17. Steenackers, M. 1988. Breeding poplars for rust resistance—recent advances. Faculty of Forestry, University of Gent, Belgium. 53(2A):417-422.
18. Steenackers, V. 1969. The state of knowledge in breeding rust-resistant poplars. Pages 419-430 in: Biology of Rust Resistance in Forest Trees. Proc. NATO/IUFRO Advanced Study Inst. U.S. Dep. Agric. For. Serv. Misc. Publ. 1221.
19. Taris, B. 1968. Contribution a l'étude des rouilles des *Populus* observées en France. Ann. Epiphyt. 19:5-54.
20. Van Kraayenoord, C. W. S., Laundon, G. F., and Spiers, A. G. 1974. Poplar rusts invade New Zealand. Plant Dis. Rep. 58:423-427.
21. Walker, J. 1975. *Melampsora larici-populina*. No. 479 in: Descriptions of Pathogenic Fungi and Bacteria. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, England.
22. Walker, J., Hartigan, D., and Bertus, A. L. 1974. Poplar rusts in Australia with comments on potential conifer rusts. Eur. J. For. Pathol. 4:100-118.