

# Temperature Effects on Germination of Uredospores of *Melampsorium betulinum* and on Rust Development

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## ABSTRACT

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Uredospores of *Melampsorium betulinum* germinated in vitro at temperatures of 1–20 C, with an optimum at 10 C. Uredospores were killed when exposed to 30 C for 6 hr or 35 C for 30 min. Uredospores did not germinate during incubation for 24 hr at 25 C or 3 hr at 30 C but did germinate during a subsequent incubation for 24 hr at 10 C. Uredospores germinated within about 3 hr at optimum conditions. Uredia developed on detached leaf disks at 10–20 C and 100% relative humidity. Uredia developed on attached and detached leaves within 13–14 days at 12 C. The presence of uredospores beneath dormant bud scales and stem infection by *M. betulinum* are reported. Sixteen of 40 dormant European birch trees from a rust-infected nursery became infected with *M. betulinum* when vegetative growth was forced in February of the following year.

Birch rust causes discolored foliage and defoliation of birch (*Betula*) trees in landscapes and nurseries. The nomenclature and authority of the birch rust fungus were disputed until 1970, when Boerema (2) designated the proper binomial to be *Melampsorium betulinum* Kleb. Synonyms and descriptions of *M. betulinum* are well documented (3,4,8,10). Hiratsuka (4) reviewed the host range and the world distribution of *M. betulinum*. In North America, birch rust occurs mostly above the 39th parallel.

The rust fungus is known to overwinter on or in buds, and Liro suggested in 1907 (4) that spores or mycelium occur in or on the buds of young birch trees. Only the uredial stage has been reported in North America. The alternate hosts in Europe are Larch (*Larix europaea* DC., *L. kaempferi* Sarg.), and *Alnus* species (3,4,6). Although important for the control of this rust, the effects of temperature on spore germination and incubation periods have not been reported.

This study was undertaken to determine 1) the time and temperature ranges for uredospore germination, 2) the thermal-death point of uredospores, and 3) latent period of the fungus.

## MATERIALS AND METHODS

In all spore germination and inoculation tests, uredia were brushed to remove

senescent spores and brushed again 48–72 hr later to collect young spores of a uniform age. Sterile distilled water was added to prepare a suspension at  $10^3$  uredospores per milliliter. Spore concentration was determined using a hemacytometer.

In preliminary tests, water-washed agar (WWA) was a favorable medium for germination of uredospores. WWA was prepared by washing 20 g of Difco-Bacto agar with 5 L of distilled water. For this purpose, the agar was placed on four layers of cheesecloth in a filter funnel under vacuum. After preparation of 2% WWA, the solidified medium in petri dishes was seeded with 1 ml of freshly prepared uredospore suspension per dish. Four seeded dishes per treatment were placed in a plastic box lined with wet paper towels to maintain 100% relative humidity. The boxes were placed in constant-temperature chambers at 1, 5, 10, 15, 20, 25, and 30 C. The temperature of the medium in the dishes in the chambers stabilized within 15–30 min. Spore counts were made at 3, 6, and 24 hr.

Thermal death of uredospores was determined by seeding WWA in petri dishes (60 × 15 mm) with 0.5 ml of spore suspension and exposing three dishes per treatment to 25 or 30 C for 3, 6, or 24 hr followed by exposure to 10 C for 24 hr. Spores (150/petri dish) were then counted as germinated or not. In another experiment, uredospores were exposed to 10 or 35 C for 0.5, 1, 2, 3, and 4 hr followed by exposure to 10 C for 24 hr. Germination of 100 spores per petri dish was assessed.

Inoculation experiments were conducted on leaf disks of *B. pendula* Roth (European birch) in 24-well Costar (Cambridge, MA) cluster dishes (no. 3524) held in constant-temperature chambers at 5, 10, 15, 20, 25, and 30 C.

Three replicates of 12 paired leaf disks were used per temperature. Rustfree paired leaf disks (8 mm diameter) were cut with a cork borer. Each pair consisted of disks from the opposite sides of a leaf midrib. Each disk was placed abaxial surface up in a well containing a 12.6-mm-diameter Schleicher and Schnell analytical paper (no. 740-E) moistened with 0.3 ml of sterile distilled water. One leaf disk of each pair was brush-inoculated with 48-hr-old uredospores; the other disk was the untreated control. Three cluster dishes, each containing 12 paired disks, were placed in a plastic box lined with moistened paper towels in each of the constant temperature treatment chambers. After 22 days, the number of sporulating uredia on each leaf disk was determined.

Incubation period was determined using 1-yr-old, disease-free, nursery-grown *B. pendula* seedlings planted one per 15-cm pot in a greenhouse to break dormancy. When leaves were fully unfolded, the trees were sprayed with a freshly prepared uredospore suspension applied to both leaf surfaces until beads of water were evident. Seedlings were incubated in mist in a growth chamber at 12 C for 24 hr. The mist atomizer was then turned off and the relative humidity ranged from 70 to 100% for the remainder of the experiment. Seedlings were examined daily for symptoms.

To detect rust in dormant leaf buds, field-grown, 1-yr-old seedlings of *B. pendula* with at least 50% of their leaves infected with rust in 1981 were used. The dormant leaf buds were excised, cleared, and stained using the technique of Shipton and Brown (7). Buds were dissected on a microscope slide so that each bud scale and leaf primordium could be examined microscopically for rust spores and/or mycelium. Uredospores were compared with the description and measurements given by Wilson and Henderson (8). The source of *M. betulinum* in the field was checked in an experiment using 40 dormant birch trees from a rust-infected nursery. The trees were transplanted and placed in a fiberglass greenhouse in February to force vegetative growth and to isolate them from additional sources of rust.

## RESULTS

The temperature range for uredospore germination was 1–20 C with an optimum of 10 C (Fig. 1). Uredospores generally

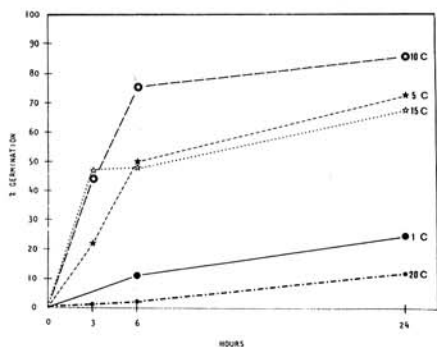
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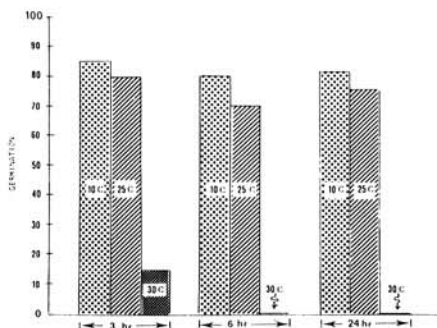
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germinated within 3 hr at 5–20 C. Inhibition of germination of spores in groups or clusters, as reported for other rust fungi (1,5,8,9), was not observed with *M. betulinum*. Germ tube lengths varied considerably within each temperature treatment. For example, after 24 hr at 10 C, the germ tube lengths varied from 93 to 150  $\mu\text{m}$ .

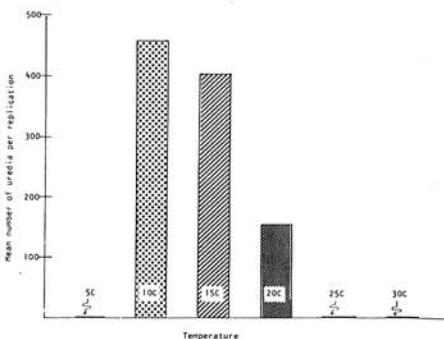
Uredospores incubated for 6 and 24 hr at 30 C followed by 24 hr at 10 C did not germinate (Fig. 2); neither did those incubated for 30 min or more at 35 C. Uredospores did not germinate within 24



**Fig. 1.** Germination of uredospores of *Melampsorium betulinum* on 2% water-washed agar after 3, 6, and 24 hr of exposure to 1, 5, 10, 15, and 20 C.



**Fig. 2.** Germination of uredospores of *Melampsorium betulinum* on water-washed agar when spores were exposed to 10, 25, and 30 C for 3, 6, and 24 hr followed by exposure to constant temperature of 10 C for 24 hr.



**Fig. 3.** Mean numbers of sporulating uredia of *Melampsorium betulinum* appearing on three replicates of 12 leaf disks exposed to constant temperatures of 5, 10, 15, 20, 25, and 30 C for 22 days after inoculation.

hr at 25 C or within 3 hr at 30 C but did so if subsequently incubated 24 hr at 10 C. Germination during 24 hr at 10 C after 24 hr at 25 C was not significantly different ( $P=0.01$ ) from germination at continuous 10 C.

Uredia developed on leaf disks at temperatures between 10 and 20 C (Fig. 3). Although a few more pustules developed at 10 C than at 15 C, no statistical difference was evident. A significant suppression of uredial formation occurred at 20 C. No uredia developed at 5, 25, or 30 C or on uninoculated leaf disks.

One-year-old seedlings of *B. pendula* inoculated and held at 12 C developed uredia on leaves and succulent stems within 13–16 days (Fig. 4A). Infection on birch stems by *M. betulinum* has not been

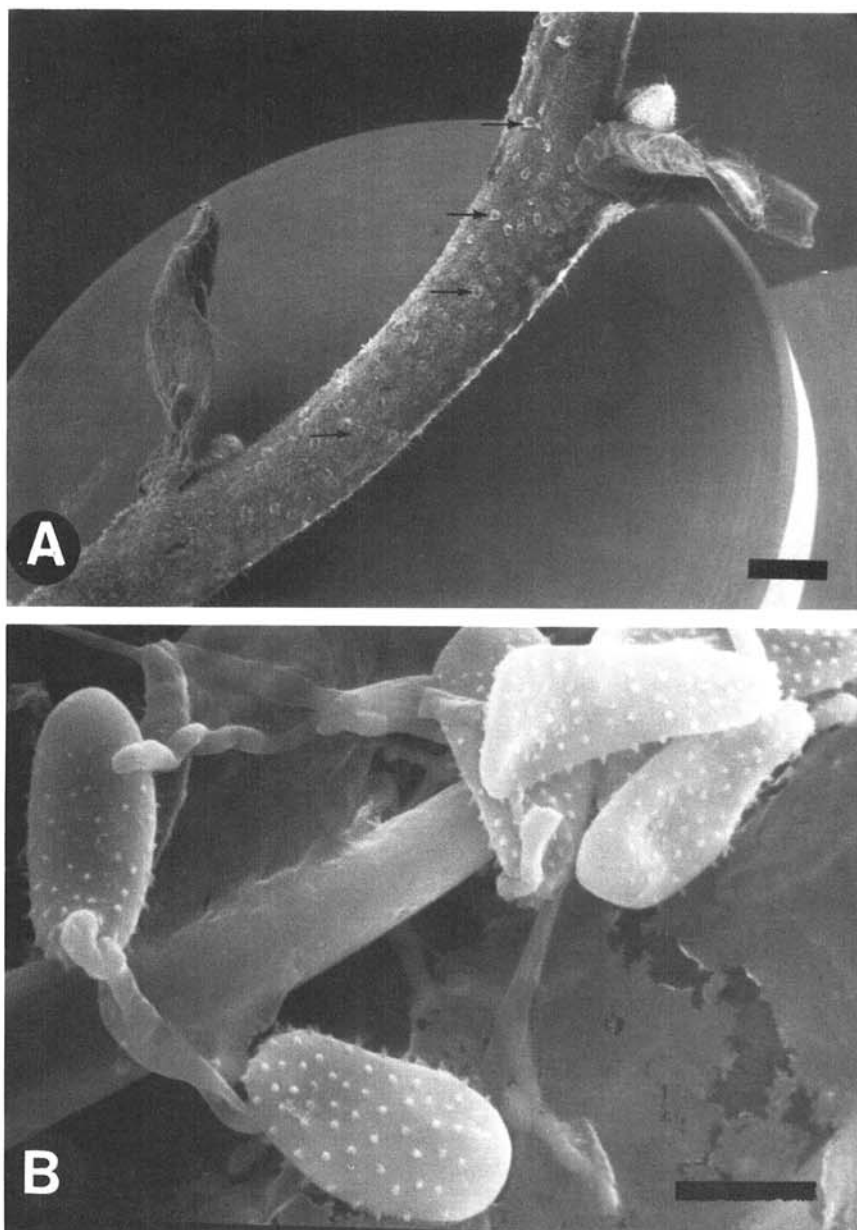
reported previously. Uredia on stems were difficult to detect because they blended with the branch and stem, causing only a slight discoloration.

Microscopic examination of dissected, cleared, and stained 1-yr-old dormant leaf buds of *B. pendula* revealed uredospores beneath the bud scales. Some tissue was stained by aniline blue, but hyphae could not be distinguished.

Of 40 dormant European birch seedlings that had been exposed to *M. betulinum* the previous season, 16 developed rust on one or more leaves after being forced in the greenhouse in February.

## DISCUSSION

Uredospores germinated at temperatures from 1 to 20 C and did so within 3



**Fig. 4.** Uredia and uredospores of *Melampsorium betulinum*. (A) Lateral branch of a European birch seedling infected with *M. betulinum*. Arrows indicate uredia. Bar = 1 mm. (B) Immature uredospores of *M. betulinum* from a uredinium on a stem as observed with the scanning electron microscope. Uredospores measure about 22  $\times$  11  $\mu\text{m}$ . Bar = 10  $\mu\text{m}$ .

hr at 5–20 C. The maximum temperature of 20 C for spore germination in vitro was similar to the maximum temperature for infection and disease development in leaf disks. Thus, cool temperatures favor development of this rust.

Although spores in groups were observed to germinate readily, the numbers of spores within groups were not determined. However, the germ tubes from these groups were numerous. Germ tube lengths varied considerably at each temperature and were not considered a useful indicator of temperature effects.

Because thermal death of uredospores occurred when the spores were exposed to 35 C for 30 min or more, or to 30 C for 6 hr or more, this could be a useful factor for disease control, provided young trees and their seeds would withstand these temperatures. Thermal death of birch seedlings and birch seed will be determined in future tests.

Stem infections were believed to be rare. However, uredia developed on

stems in this research. Thus, we may find them to be more prevalent than previously expected. It is probable that stem infections occur on cultivars with large lenticels, such as European birch, which has openings large enough to admit the germ tube.

Although uredospores and mycelia had been suggested to occur within leaf buds (4), neither had been observed previously. Uredospores were observed beneath the leaf scales. Areas stained by aniline blue indicated that mycelium was present; however, hyphae could not be discerned. Because 40% of a sample of previously infected European birch seedlings from a nursery developed rust while in isolation, the fungus may be transported to new areas via infected buds on seedlings.

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