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Melampyrum sylvaticum, a new alternate host for pine stem rust *Cronartium flaccidum*

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Abstract: *Cronartium flaccidum* was observed for the first time on *Melampyrum sylvaticum*. Uredinia and telia were found on *M. sylvaticum* growing in Scots pine (*Pinus sylvestris*) stands representing both moist and subdry forest site types. The finding suggests that the occurrence of *Cronartium flaccidum* is much wider in northern Fennoscandia than has been reported over the past century.

Key Words: Cronartium rust, occurrence, *Pinus sylvestris*, Scots pine

The heteroecious *Cronartium flaccidum* (Alb. & Schw.) Wint. causes Cronartium rust on Scots pine (*Pinus sylvestris* L.) in Europe (Lagerberg, 1912; Jørstad, 1928; Ferdinandsen and Jørgensen, 1938–39; Rennerfelt, 1943; Wilson and Henderson, 1966). The autoecious form of *C. flaccidum*, *Peridermium pini* (Pers.) Lev., which causes resin top on pines, is considered to be more common than the heteroecious form in northern Fennoscandia due to lack of known alternate hosts in this region (Liro, 1908; Rainio, 1926; Jørstad, 1928; Kari, 1936; Rennerfelt, 1943; Roll-Hansen, 1973; Kaitera et al., 1994).

Reports of *C. flaccidum* in Finland are few, with some reported cases on *Pedicularis* spp., *Vincetoxicum hirundinaria* Medicus and *Paeonia* spp. (Liro, 1908; Hylander et al., 1953). *Pedicularis* spp. occur sporadically throughout Finland on peatlands and along lake shores (Hämet-Ahti et al., 1984), and these are the only known species acting as potential alternate hosts for *C. flaccidum* of practical importance. Liro (1906, 1907) considered *Pedicularis* spp. as being the primary alternate hosts for *C. flaccidum* in Finland. The aim of this study was to investigate the occurrence of *C. flaccidum* on known hosts and potential alternate hosts in diseased Scots pine stands in northern Finland.

Spore and fruitbody collection and measurements.—Eighteen Scots pine stands suffering from resin top or Cronartium rust, representing moist (*Hylocomium-Myrtillus* type), subdry (*Empetrum-Myrtillus* type) or dry (*Myrtillus-Calluna-Cladonia* type) forest site types, were checked at the end of July and early August 1997 for uredinia and telia production on ground vegetation in northern Finland. Species occurring in large numbers in the stands and apparently bearing *Cronartium* telia (e.g., Ferdinandsen and Jørgensen, 1938–39) were closely examined. About 50 plants per stand of *Melampyrum sylvaticum* L. were collected and brought to the laboratory for microscopic examination.

Fifty measurements of uredinia, telia and spores were made per location using a stereomicroscope for uredinia and telia and a compound light microscope for spores. Leaves of alternate hosts bearing *Cronartium* sp. fruitbodies, were deposited to Botanical Museum, University of Oulu, where the identification of the respective plant species was confirmed.

DNA analysis.—Pieces of *M. sylvaticum* leaves bearing *Cronartium* sp. telia were used to confirm the identification of the rust species using DNA sequences. This was done, because also another *Cronartium* species, *C. ribicola* J. C. Fisch., occurs rarely on some introduced pine species (not on Scots pine) in the southernmost part of Finland (Liro, 1908) and could, therefore, theoretically be found on alternate hosts in Finland. DNA was isolated from aecia and telia as described by Vainio et al. (1998). The protocol included cell disruption, phenol-chloroform (1:1) extractions, a chloroform:isoamyl alcohol (24:1) extraction, precipitation with polyethylene glycol and drying. The DNA was resuspended into 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA. The ITS-region (incl. the 5.8S rRNA gene) was amplified using primers ITS1-F (specific for fungi) and ITS4-B (specific for basidiomycetes) as described by Gardes and Bruns (1993), except that Dynazyme thermostable polymerase (Finnzymes, Finland) was used and the ITS1-F primer contained a 40 bp CG-clamp at its 5′end. The amplification products were digested with restriction enzyme *Alu*I as suggested by the manufac-

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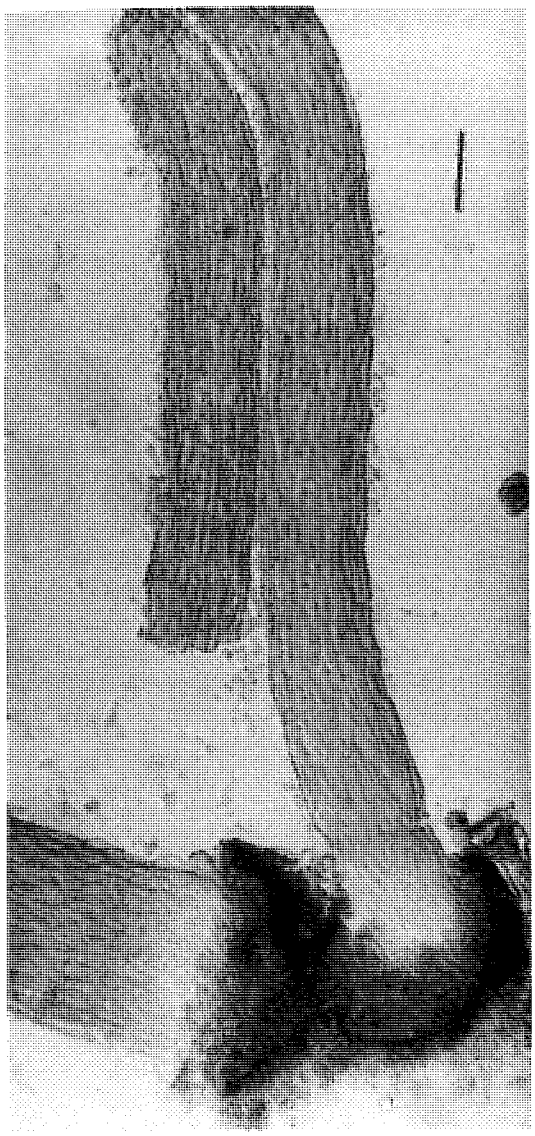

FIG. 1. Telial columns of *Cronartium flaccidum* with basidia. Scale bar = 40 μm.

turer of the enzyme (Boehringer Mannheim, Germany). *Cronartium flaccidum* aecia from Italy and Finland, as well as *C. ribicola* aecia and a piece of *Ribes nigrum* L. leaf bearing *C. ribicola* telia from Finland, were used as positive controls, and pieces of *M. sylvaticum* leaves without any fungal fruitbodies as negative controls.

Results.—Neither uredinia nor telia of *Cronartium* sp. were observed on any other plant except *M. sylvaticum*. Both uredinia and telia of *Cronartium* sp. were found in abundance (almost all individuals infected) on *M. sylvaticum* in two stands representing a moist forest site type. The respective uredia and telia occurred rarely (only a few individuals bearing uredinia or telia) on *M. sylvaticum* in patches of a moist forest site within subdry forest site type in two locations.

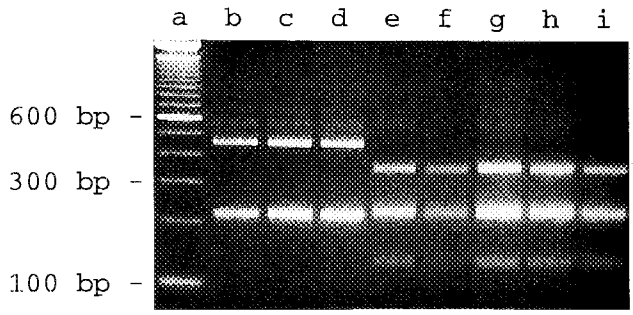

FIG. 2. Restriction fragment length polymorphism analysis (*AluI* digestion) of PCR-amplified ITS. The samples lanes from left to right are as follows. a. 100 bp DNA ladder. b. *Cronartium ribicola* telia on *Ribes*. c, d. *Cronartium ribicola* aecia on *Pinus strobus*. e, f. telia from *Melampyrum sylvaticum*. g–i. *Cronartium flaccidum* aecia on *Pinus sylvestris*. The sizes of three markers are shown on the left.

Melampyrum spp. did not bear uredinia or telia of *Cronartium* sp. in fourteen locations.

The average diam of uredinia was 100.3 and 98.8 μm in two samples (range 79.0–138.3 μm for both samples) (herbarium specimens Oulu F032175 and Oulu F032176) from the respective locations. The average length of telia was 536.8 μm (158–1185 μm) and 555.4 μm (138–1067 μm), and average width was 76.6 μm (39.5–118.5 μm) and 87.3 μm (59.3–158.0 μm) for the two samples (FIG. 1). The respective average figures were for urediniospore length 23.8 μm (17.0–31.8 μm) and 22.6 μm (15.9–28.6 μm), for urediniospore width 17.9 μm (12.7–21.2 μm) and 12.7 μm (12.7–22.3 μm), for teliospore length 45.1 μm (31.8–61.5 μm) and 48.7 μm (31.8–63.6 μm), for teliospore width 10.2 μm (6.4–12.7 μm) and 12.7 μm (6.4–19.1 μm), and for basidiospore diam 6.8 μm (4.2–10.6 μm) and 8.4 μm (4.2–12.7 μm).

The PCR-amplification from all aecia as well as samples from *M. sylvaticum* and *R. nigrum* leaves with telia resulted in single amplification products of about 900 and 950 bp (not shown). In contrast, no amplification product was observed from *M. sylvaticum* leaves without uredinia or telia. When the amplified fragments were digested with restriction enzyme *AluI*, two banding patterns were observed (FIG. 2). The amplification product of ITS from aecia and telia of *C. ribicola* showed two bands with apparent sizes of 220 bp and 450 bp, whereas the other samples (*C. flaccidum* aecia from Scots pine and telia from *M. sylvaticum*) showed three bands with apparent sizes of 130 bp, 230 bp and 350 bp. The 220 bp and 230 bp bands appeared to be twice as intense as the other bands, and assuming these two represent double restriction fragments, the summed fragment sizes of the two patterns were 890 and 940 bp, indicating that the digestions were complete. The differ-

ence between the patterns obtained from *C. flaccidum* aecia and *C. ribicola* aecia or telia showed that the two species can be separated by a restriction fragment length polymorphism analysis of PCR-amplified ITS. Therefore, the grouping of telia from *M. sylvaticum* with *C. flaccidum* aecia confirmed their identification as *C. flaccidum*.

Discussion.—This is the first report of *C. flaccidum* on *Melampyrum* in Finland and the first report on *M. sylvaticum* in Europe (Hylander et al., 1953; Gäumann, 1959). Uredinia, telia, urediniospore, teliospore and basidiospore sizes observed in this study were within the range of corresponding figures reported earlier for *C. flaccidum* (Liro, 1908; Gäumann, 1959; Wilson and Henderson, 1966).

The finding suggests that *C. flaccidum* may be common on the moist forest site type in Finland. Resin top disease caused by the autoecious *P. pini* is believed to occur most commonly in stands representing subdry or dry forest site types, typically consisting of Scots pine (Kaitera and Jalkanen, 1995), but heteroecious *C. flaccidum* was also (but seldom) found on these forest site types. This suggests that heteroecious *C. flaccidum* may occur throughout Finland. This is based on the wide distribution of *Melampyrum* spp., extending even into the northernmost part of Finland (Hämet-Ahti et al., 1984). The distribution of *Melampyrum* spp. in northern Fennoscandia (Hultén, 1950) also suggests that heteroecious *C. flaccidum* is common in the other countries in the region, too, as has been reported earlier (Rennerfelt, 1943; Roll-Hansen, 1973). There is also reason to believe that *C. flaccidum* may be found on *Melampyrum pratense* L., *M. cristatum* L., *M. arvense* L. and *M. nemorosum* L. in Finland. Only the first one of the latter species can, however, play an important role as an alternate host, as it occurs rather commonly throughout the country, the other species occurring rarely only in the southernmost part of Finland (Hämet-Ahti et al., 1984). Final proof that *C. flaccidum* can infect *M. sylvaticum* can, however, only be obtained if the aeciospores of *C. flaccidum* are inoculated and can become established on *M. sylvaticum*.

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