Melampyrum spp. as alternate hosts for *Cronartium flaccidum* in Finland

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Abstract

Distribution and frequency of *Cronartium flaccidum* on *Melampyrum* spp. was studied on Scots pine throughout Finland. Leaves of the alternate hosts were collected, and the frequency of *Cronartium* telia was recorded. Morphological dimensions of fruitbodies and spores were measured, and some telial samples were identified genetically. Telia were observed for the first time on *M. pratense* and *M. nemorosum* in natural forests, and on *M. arvense* in Finland. Telia occurred in 22 % of the *M. sylvaticum*-stands, 3 % of the *M. pratense*-stands, 12 % of the *M. nemorosum*-stands, and in the *M. arvense*-stands investigated. Geographically, telia were lacking on *M. sylvaticum* and *M. pratense* in southern Finland, but they were relatively common on these species in northern Finland, whereas 92 % of the *M. sylvaticum*-stands and 30 % of the *M. pratense*-stands bore plants with telia in the area. The proportions of stands with telia, plants with telia per stand and telia-bearing leaves per plant were greater on *M. sylvaticum* than on the other *Melampyrum* spp.

Introduction

Pine stem rusts, *Cronartium flaccidum* (Alb. & Schwein) G. Winter and *Peridermium pini* (Pers.) Lév cause severe damage on Scots pine (*Pinus sylvestris* L.) in Europe (Gäumann 1959). Genetic analysis suggests that gene flow occurs between these two rusts, and that they, therefore, belong to the same species (Hantula *et al.* 2004). In Finland, *P. pini* is more common than *C. flaccidum* based on population studies conducted with aeciospores (Hantula *et al.* 1998, Kaitera *et al.* 1999). Geographically, *C. flaccidum* has been found locally in northern Finland in the late 1990s (Kaitera & Hantula 1998), but there are several findings of the rust in natural forests in the southern coast of Finland and in the Åland archipelago both on Scots pine and on alternate hosts since the 1800s (Liro 1908, Kaitera & Nuorteva 2003a, b).

Common alternate host genera for *C. flaccidum* in Finland are *Vincetoxicum, Pedicularis, Melampyrum* and *Paeonia* (Liro 1908, Hylander *et al.* 1953, Kaitera *et al.* 1999). In genus *Melampyrum*, the rust has been found on *M. sylvaticum* L. in northern Finland (Kaitera & Hantula 1998, Kaitera 2000), and in artificial inoculations, *M. sylvaticum* L. (Kaitera 1999, Kaitera & Nuorteva 2003a, b), *M. nemorosum* L. (Kaitera & Nuorteva 2003a, b), *M. pratense* L. (Kaitera 1999, Kaitera & Nuorteva 2003b) and *M. arvense* L. (Kaitera & Nuorteva 2003b) have been shown to be susceptible to the rust. According to some old reports (Rennerfelt 1943; Hylander *et al.* 1953), *C. flaccidum* occurs also on *M. arvense* and *M. cristatum* L. in natural forests in Sweden.

In Scandinavia, there are five *Melampyrum* species growing in natural forests (Hultén 1950; Hämet-Ahti *et al.* 1984), which also grow elsewhere in Europe (Hegi 1974). Only two species, *M. pratense* and *M. sylvaticum*, are common and widely-spread in Scandinavia, and thus, may play significant roles as alternate hosts in natural forests. The aim of this study was to clarify the distribution and frequency of *C. flaccidum* on *Melampyrum* spp. in Finland.

Materials and methods

Old leaves of *Melampyrum* spp. were collected systematically throughout Finland in Scots pine stands infected by pine stem rusts in 1998–2002. For a more thorough description of e.g. the data collection, see Kaitera *et al.* (2005). Data of damaged stands collected in private forest owners' land was used as basis for the sample collection. The data included 338 *M. pratense*-, 111 *M. sylvaticum*-, 17 *M. nemorosum*-, one *M. cristatum*- and one *M. arvense*-stand. Geographically, 33 % of stands with *M. pratense* and 25 % of those with *M. sylvaticum* occurred in northern Finland. The corresponding proportions were 46 % and 57 % in southern Finland.

A sample of plants (50 in number) of *M. pratense*, *M. sylvaticum* and *M. nemorosum* were collected per stand close to the infected trees. A sample of similar size of *M. cristatum* and *M. arvense* were checked in the field. The plant leaves were checked for *Cronartium* telia in the field and in the laboratory. The number of telia per leaf and the length and width of fully developed telia, teliospores and urediniospores were measured under microscopes. A few telial samples per host and stand were identified genetically. In about 100 samples, of which 80 % were *M. sylvaticum* leaf samples, DNA was isolated from telia (Vainio *et al.* 1998), the ITS region was amplified using primers ITS1-F and ITS4-B (Gardens & Bruns 1993), and the amplification products were digested. The amplification products from *M. pratense* and *M. nemorosum* were sequenced, and blast searches were made to find most similar sequences in Genbank. For a more thorough descriptions of the used protocols, see Kaitera *et al.* (2005).

Results

Telia occurred in 22 % of the investigated *M. sylvaticum*-stands, and in 3 % of the *M. pratense*-stands, and they located mainly in northern Finland. Ninety-two percent of the *M. sylvaticum*-stands and 30 % of the *M. pratense*-stands included plants carrying telia in northern Finland, while telia were lacking on these alternate hosts in southern Finland. Telia were also found in 12 % of the *M. nemorosum*-

stands, and in the investigated *M. arvense*-stand, but not in the *M. cristatum*-stand. The mean proportion of plants bearing telia per stand was significantly higher for *M. sylvaticum* than for *M. pratense* and *M. nemorosum*. The mean proportion did not differ significantly between site types for either *M. sylvaticum* or *M. pratense*, but was significantly higher in young development classes compared to older ones for *M. sylvaticum*. Variation in the number of leaves bearing telia per plant was highest for *M. sylvaticum*, while 38 % of the infected plants bore telia on 3–13 leaves per plant. Telia occurred less frequently on the rest of the *Melampyrum* spp. The average number of telia per leaf varied between 12.3–16.2 among the *Melampyrum* spp., but it did not differ significantly between *M. sylvaticum* and *M. pratense*. The average width of telia and length of teliospores were significantly greater on *M. pratense*, and the average width of teliospores was greater on *M. arvense* compared to those on the other *Melampyrum* spp. The PCR amplifications of leaves with telia resulted in single amplification products of about 900 bp. After digestion with restriction enzymes followed by gel electrophoresis, the banding pattern for *Cronartium flaccidum* was observed. Based on this pattern, 50–60 % of the samples of *M. sylvaticum*, *M. pratense* and *M. nemorosum* were identified as *C. flaccidum*. The ITS sequences of the samples determined and compared to GenBank gave the highest similarities to *P. pini* and *C. flaccidum*. For a more thorough description of the results, see Kaitera *et al.* (2005).

Discussion and conclusions

The present study confirmed that *Melampyrum* spp. are important alternate hosts for *C. flaccidum* in natural forests in Finland. This is due to the frequencies of *M. sylvaticum* and *M. pratense* bearing telia especially in northern Finland. These findings are also the first ones on *M. pratense*, *M. nemorosum* and *M. arvense* in natural forests, and correspond well with the susceptibility of these species to *C. flaccidum* under inoculation experiments (Kaitera 1999; Kaitera & Nuorteva 2003a, b). The rust is also more common than the aeciospore studies (Hantula *et al.* 1998; Kaitera *et al.* 1999) have suggested. The distribution is, however, strongly concentrated in northern Finland, whereas no telia were found on *M. sylvaticum* or *M. pratense* in southern Finland. Telia were also more common in stands belonging to young development classes compared to older ones on *M. sylvaticum*, which may lead to increasing numbers of epidemics in young pine stands in the future. The high variation in morphological characteristics of telia and different spores corresponds well with the reported dimensions of natural samples in the litterature (Liro 1908; Gäumann 1959; Kaitera & Hantula 1998). The lower dimesions are probably due to the high number of dry, late-summer samples among all studied samples. Molecular analysis of the telial samples of *M. sylvaticum*, *M. pratense* and *M. nemorosum* confirmed that the telia were of *C. flaccidum*. Some samples could not be identified probably due to low numbers of telia in the samples or small numbers of DNA in the teliospores after karyogamy and meiosis.

References

Gardes M & Bruns T D 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molec Ecol 2: 113–118.

Gäumann E 1959. Die Rostpilze Mitteleuropas. Beitr Kryptogamenflora Schweiz 12: 85–93.

Hämet-Ahti L, Suominen J, Ulvinen T, Uotila P & Vuokko S 1984. Retkeilykasvio. (In Finnish). Suomen Luonnonsuojelun Tuki Oy, Forssa.

Hantula J, Niemi M, Kaitera J, Jalkanen R & Kurkela T 1998. Genetic variation of pine stem rust in Finland as determined by Random Amplified Microsatellites (RAMS). Eur J For Path 28: 361–372.

Hantula J, Kasanen R, Kaitera J & Moricca S 2002. Analyses of genetic variation suggest the pine rusts *Cronartium flaccidum* and *Peridermium pini* belong to the same species. Mycol Res 106: 203–209.

Hylander N, Jørstad I & Nannfeldt JA 1953. Enumeratio Uredinearum Scandinavicarum. Opera Bot 1: 13–14.

Hegi G 1974. *Melampyrum*. In Illustrierte Flora von Mittel-Europa. Hartl D & Wagenitz G (eds). Carl Hanser Verlag, München, pp. 403–451.

Hultén E 1950. Atlas över växternas utbredning i Norden. (In Swedish). Generalstabens litografiska anstalts förlag. Stockholm.

Kaitera J 1999. *Cronartium flaccidum* fruitbody production on *Melampyrum* spp. and some important alternate hosts to pine. Eur J For Path 29: 391–398.

Kaitera J 2000. Analysis of *Cronartium flaccidum* lesion development on pole-stage Scots pines. Silva Fenn 34: 21–27.

Kaitera J & Hantula J 1998. *Melampyrum sylvaticum*, a new alternate host for pine stem rust *Cronartium flaccidum*. Mycologia 90: 1028–1030.

Kaitera J & Nuorteva H 2003a. *Cronartium flaccidum* produces uredinia and telia on *Melampyrum nemorosum* and on Finnish *Vincetoxicum hirundinaria*. For Path 33: 205–213.

Kaitera J & Nuorteva H 2003b. Relative susceptibility of four *Melampyrum* species to *Cronartium flaccidum*. Scand J For Res 18: 499–504.

Kaitera J, Nuorteva H & Hantula J 2005. Distribution and frequency of *Cronartium flaccidum* on *Melampyrum* spp. in Finland. Can J For Res 35: 229–234.

Kaitera J, Seitamäki L, Hantula J, Jalkanen R & Kurkela T 1999. Inoculation of known and potential alternate hosts with *Peridermium pini* and *Cronartium flaccidum* aeciospores. Mycol Res 103: 235–241.

Liro J I 1908. Uredinae Fennicae. Bidr Känned Finlands Natur och Folk 65: 447–449.

Rennerfelt E 1943. Om vår nuvarande kunskap om törskatesvampen (*Peridermium pini*) och sättet för dess spridning och tillväxt. (In Swedish with German summary: Über unsere gengenwärtige Kenntnis von Kienzopf (*Peridermium*) und die Art seiner Verbreitung und seines Wachstums). Sv Skogsvårdsför Tidskr 41: 305–324.

Vainio E J, Korhonen K & Hantula J 1998. Genetic variation in Phlebiopsis gigantea as detected with random amplified microsatellite (RAMS) markers. Mycol Res 102: 187–192.