National Poplar Rust Disease Survey 2009-10

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Abstract

Leaf samples bearing a heavy rust load were collected from a range of infected poplar clones (mostly Euramericana hybrids or *P. nigra*) and in most of the regions nationally. Collections were made late in the season during February-March 2009 and March-April 2010. Identification of the rust species was made from 37 separate samples taken in two years and geographically apart. All rust samples were identified as belonging to M*elampsora larici-populina.* More importantly, there was no evidence of hybridisation or new rust species based on the urediniospore appearance. Previous evidence of *Melampsora medusae-populina,* a unique interspecific hybrid (Spiers & Hopcroft 1985, appears not to be any more extensive and probably reflects the limited range of *Melampsora medusae*.
Future research will endeavour to develop a DNA marker-based technique for identifying M*elampsora* rust races/species, since we are uncertain how definitive urediniospore ID is in distinguishing rust mutation and changing virulence.

Introduction

The rust fungi (*Uredinales)* are one of the largest groups of fungi, accounting for one-third of the teleomorphic species of Basidiomycetes (Hawksworth et al. 1995).

Rust fungi found on poplars (and also on willows) belong to the genus *Melampsora*. Rust caused by *Melampsora* is one of the most important leaf diseases of poplars. Internationally some 13 species and two hybrids of *Melampsora* have been described on *Populus,* of which three have been identified in New Zealand, namely *Melampsora larici-populina*, *Melampsora medusae* and *Melampsora medusae-populina,* a unique interspecific hybrid. Willows (*Salix* spp.) host four rust (*Melampsora*) species, *Melampsora coleosporioides, Melampsora epitea* var *epitea* and two unidentified species attacking willows *Salix viminalis* and *Salix daphnoides/Salix incana* x open pollinated hybrids respectively.

Rusts pose the greatest disease challenge to willows and poplars internationally. It is the same in New Zealand. Rust fungi have complex life cycles involving up to five different spore stages. These fungi often require two unrelated host plants to complete their full life cycles. The second hosts in New Zealand include conifers (willow rust *Melampsora epitea*) and larch trees (*Melampsora larici-populina*). However, they do not require a secondary host and can re-infect without going through all five stages. The urediniospore stage is what is seen in the nursery. Urediniospores are capable of producing many cycles of the same form during the season, causing disease epidemics on poplars. Where leaf rusts are common and severe, poplar and willow leaves may become distorted, wither and drop prematurely, reducing growth up to 30%. The more susceptible a tree is to leaf rust(s), the more rapidly the disease develops and the sooner defoliation starts. Growth loss due to rust is often masked by the normally rapid growth of poplars and willows and can best be measured by comparing the growth of infected trees with comparable healthy trees protected by fungicide sprays. Continued defoliation of successive flushes of growth by leaf rusts decreases vigour and indirectly increases the likelihood of winter injury as well as susceptibility to other diseases, e.g. silver leaf fungus. Rust infection should be anticipated in the nursery by starting a spray control programme 2-3 weeks before the infection normally occurs. Figure 1 shows the location of the rust infection inside the leaf and the visible rust is due to the spores erupting through the leaf surface. Consider the effect of this on the internal leaf physiology:

The surface of urediniospores (Figure 2) is covered with spines (echinulae: ‘Echidna’ is the Australian spiny anteater). Rust species are identified from their location on the leaf (either the underside or both sides), and the size and spinyness of their urediniospores. Urediniospores of *Melampsora larici-populina* have a smooth surface at the apex, while those of *Melampsora medusae* have a smooth surface on the equatorial zone. The hybrid rust race identified by Adrian Spiers had smooth areas at the apex and equator.

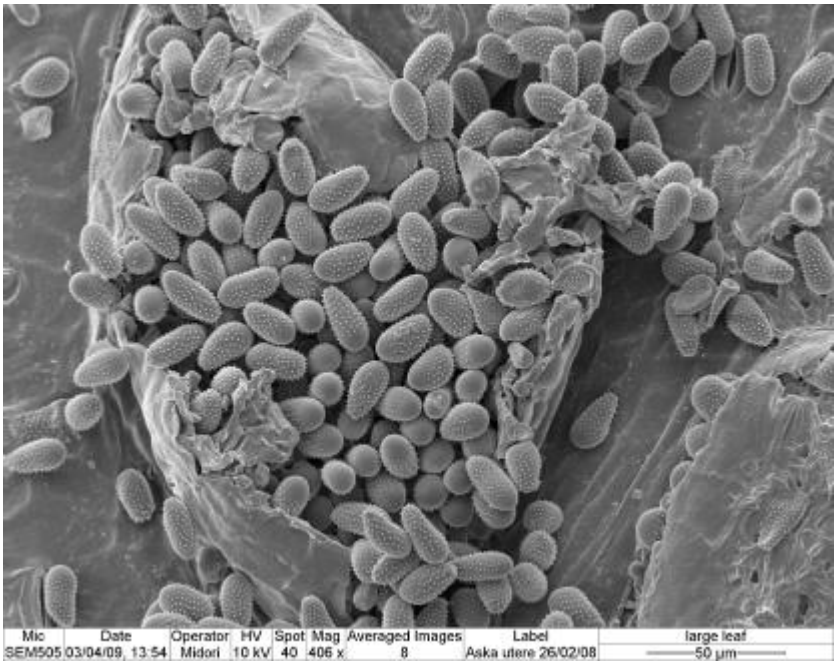

Figure 1. *Melampsora* sp. urediniospores emerging out from the inside of a poplar leaf collected in 2009.

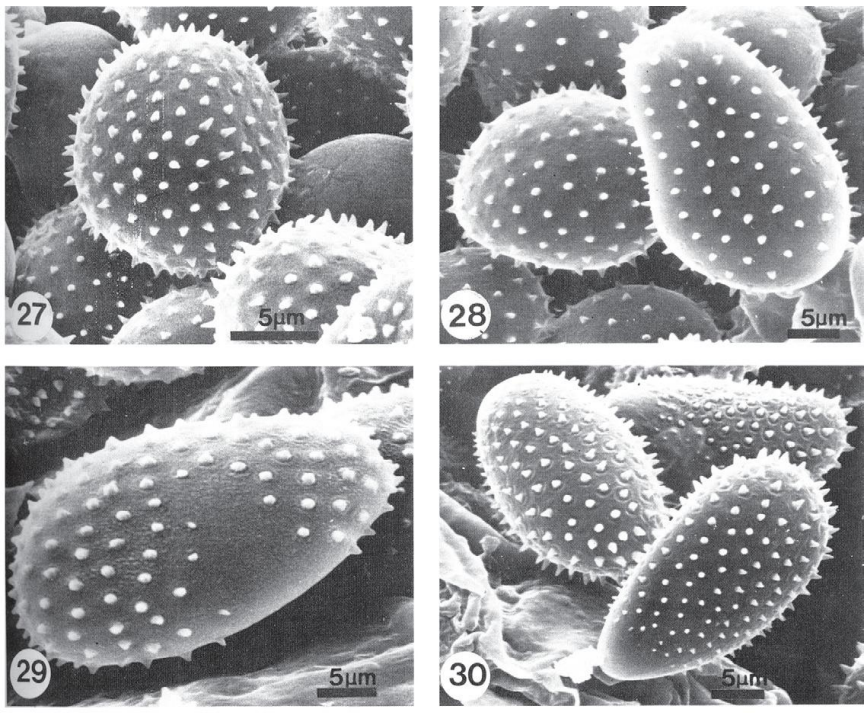

Figure 2. Uniformly echinulate urediniospores of *Melampsora epitea* (27) and *M. coleosporioides* (28), echinulate urediniospores of *M. medusae* with smooth equatorial patch (29) and *M. larici-populina* with smooth apical patches (30), (Spiers & Hopcroft 1985).

Materials and Methods

A national assessment of the status of rust species was initiated to determine:
1. If any new species have arrived in New Zealand
2. Whether further hybridisation has occurred between known rust species
3. The relative frequency of the different species.

Poplar rust leaf samples were collected for different poplar clones and from RC nurseries in different regions in 2009. Rust leaf samples were also collected from Northland, Auckland, Bay of Plenty, Coromandel, Central North Island, Eastland, Hawke's Bay, Taranaki/Wanganui, Manawatu, Wellington, Wairarapa, Gisborne, Waikato, Bay of Plenty, Nelson/Tasman, Marlborough, Canterbury, Otago, Southland and Southern Lakes during the months of March and April 2010. The rust leaf samples were air dried. Dry samples mounted on double-sided tape on aluminium specimen stubs, sputter coated with gold, and studied using an FEI Quanta 200 Scanning Electron Microscope. Digital images of urediniospores saved were from different geographical regions.

We identified the species from already published *Melampsora* sp. urediniospore images (Figure 2).

Results

Urediniospores from all 17 samples evaluated in 2009 and 20 samples from 2010 had smooth apical patches as seen in Figure 3a, 3b, 3c and 3d. The length of the spores was around 25 µm, and width 12 µm, smaller than published dimensions of 30-50 x 14-22 µm (Pei & McCracken 2005).

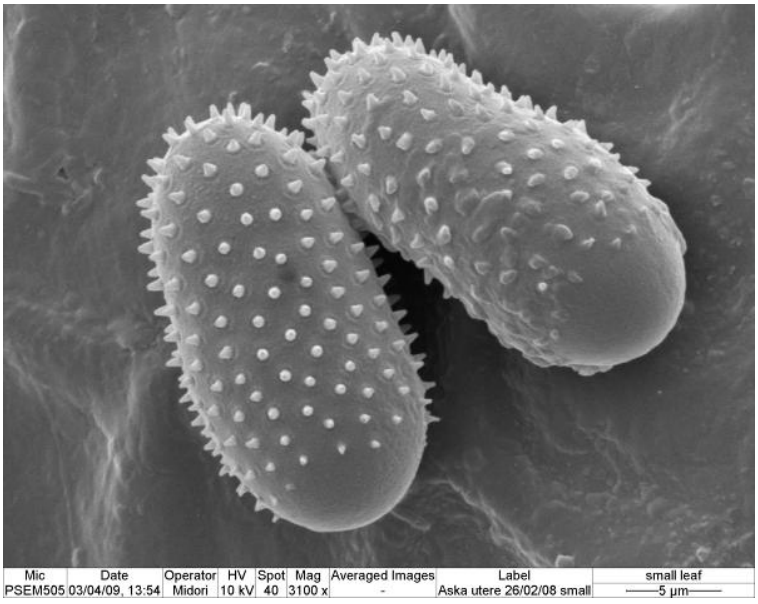

Figure. 3a. *Melampsora larici-populina* urediniospores on underside of 'Crowsnest' poplar leaf collected from Aokautere Nursery, Manawatu collected in 2009.

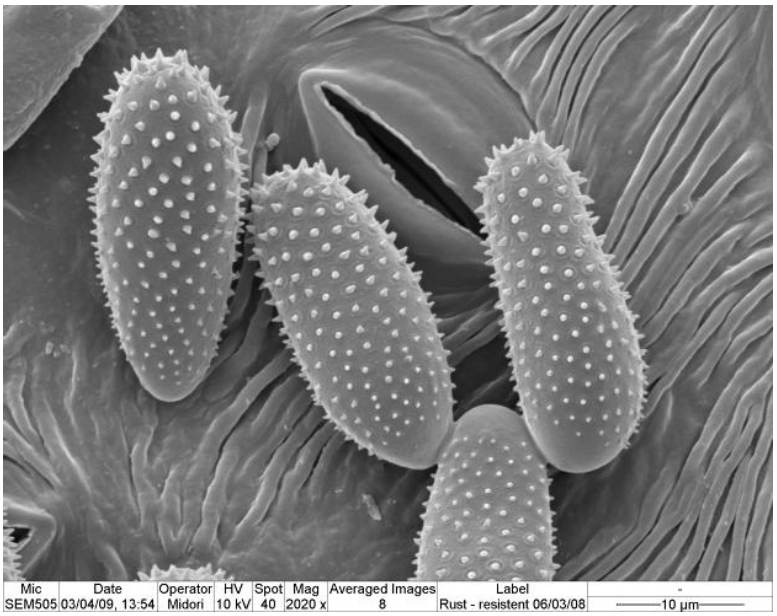

Figure 3b. Urediniospores of *Melampsora larici-populina* collected from leaf of experimental *Poplar trichocarpa x P. nigra* at Plant & Food Research, Manawatu, collected in 2009. The size of the spores compares with the size of the leaf breathing hole, the stoma.

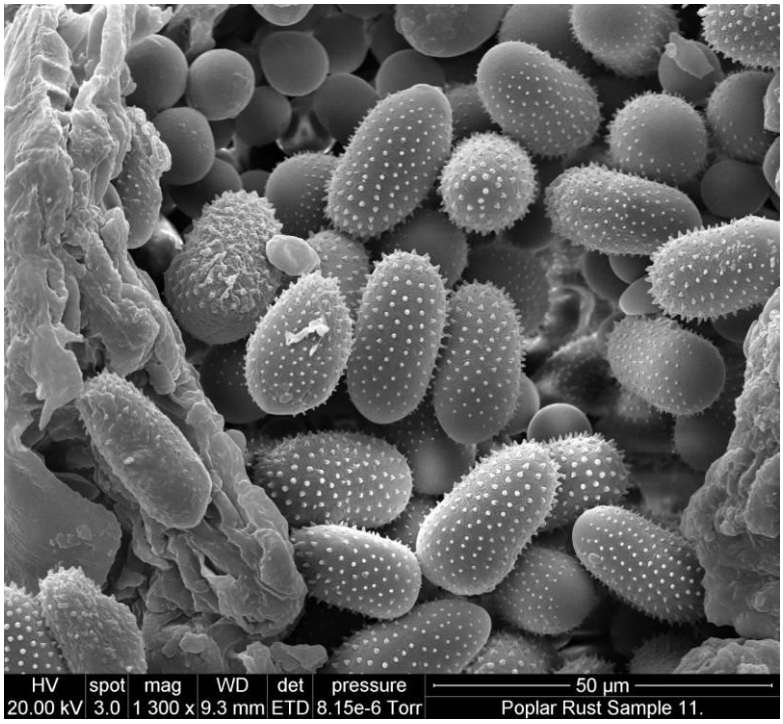

Figure 3c. Urediniospores of *Melampsora larici-populina* collected from leaf of *Populus deltoides x P. nigra* (probably 'Veronese') at Balclutha, Otago, collected in April 2010.

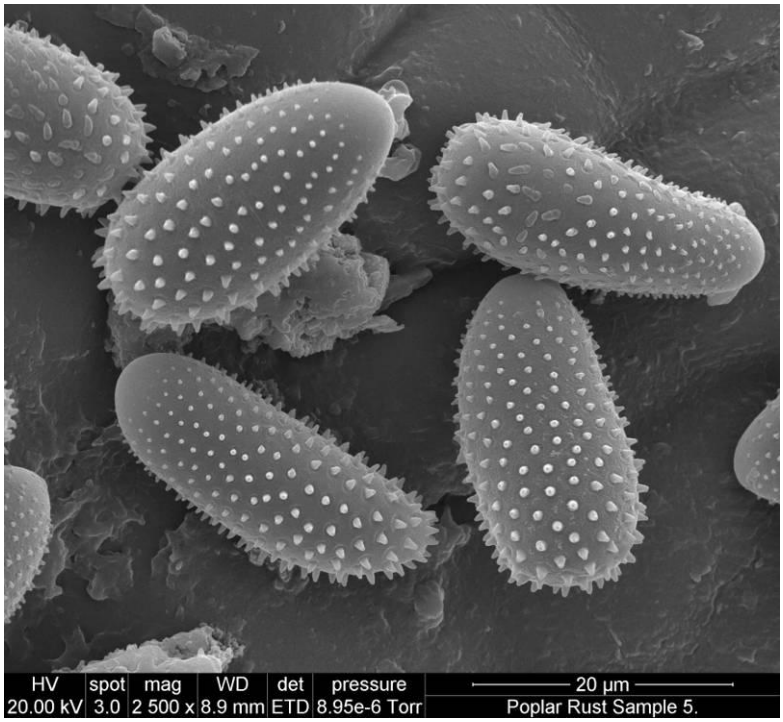

Figure 3d. Urediniospores of *Melampsora larici-populina* collected from leaf of *Populus deltoides x P. nigra* at Inglewood, Taranaki, collected in March 2010.

Discussion

All rust samples were identified as belonging to *M. larici-populina.* More importantly, there was no evidence of hybridisation or new rust species based on the urediniospore appearance. If secondary host trees are not close, the rust sexual cycle will not be completed, there will be no hybridisation and new rust infections will be from asexually produced spores, which have a very low variability from generation to generation.

It is easy to observe rust-susceptible poplar clones in autumn, since they have lost almost all their leaves except for a tuft of leaves at the very top, not yet infected with the rust fungus. Many of the older poplar trees are susceptible, dating back to before the rust *M. larici-populina* arrived in New Zealand in 1973. Poplar and willow clones bred and released after 1980 were selected for high resistance to rust and these show up on the landscape as still in green leaf in late autumn. Rust races are considered to evolve and it is likely that poplar and willow clones will become less resistant to rust with time. For this reason, new hybrids are being bred that are selected for resistance to the current strains of rust (which may or may not be the same as previous strains). The time between initial selection and eventual commercial release is of the order of 12-15 years. Fortunately, our research suggests rust races do not change rapidly.

When replacing old poplar and willow trees, select those clones suitable for your region. Wetter climates favour rust, whereas drier climates inhibit rust spore germination (Figure 4). In addition to other characteristics such as vigour, form, fodder and timber potential, it is important to replant with clones having a high rust resistance, particularly in wetter climates.

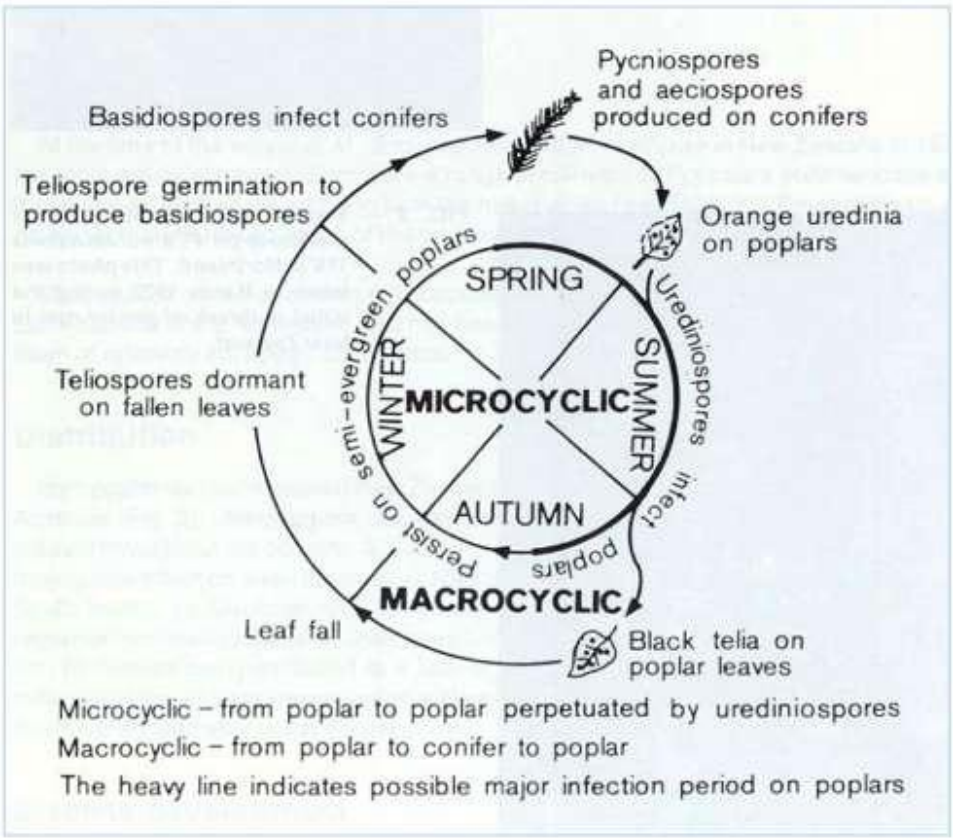

Fig. 4 - Life-cycle of the poplar leaf rusts.

Future work

Detecting microorganisms by **polymerase chain reaction** ()mplification of DNA extracted from environmental samples has an advantage over culture techniques, which require recovery and growth of active organisms (Johnston & Aust 1994; Sivakumaran et al. 1997). Extraction of total DNA followed by PCR amplification was shown to be a senstive method for detecting specific bacteria in soil samples (Hilger & Myrold 1991). Such an approach developed for bacterial DNA can also be used to detect fungal DNA (Tebbe & Vahjen 1993).

Eukaryotic ribosomal DNA genes have high copy numbers as well as interspersed conserved and variable DNA sequences (White et al. 1990), making them ideal targets for species identification by PCR amplification coupled to restriction enzyme analysis. The internal transcribed spacer (ITS) region of fungal ribosomal DNA (Figure 5) has been used for resolving species of other genera (Gaskell et al. 1992).

Genomic DNA was extracted using standard CTAB methods (Doyle & Doyle 1990). The universal primers ITS 1 (5′-TCCGTAGGTGAACCTGCGG3′) and ITS 2 (5′-GCTGCGTTCTTCATCGATGC-3′) will be used to amplify the internal transcribed spacer region 1 (ITS 1) between the 18S and 5.8S ribosomal genes (White et al. 1990).

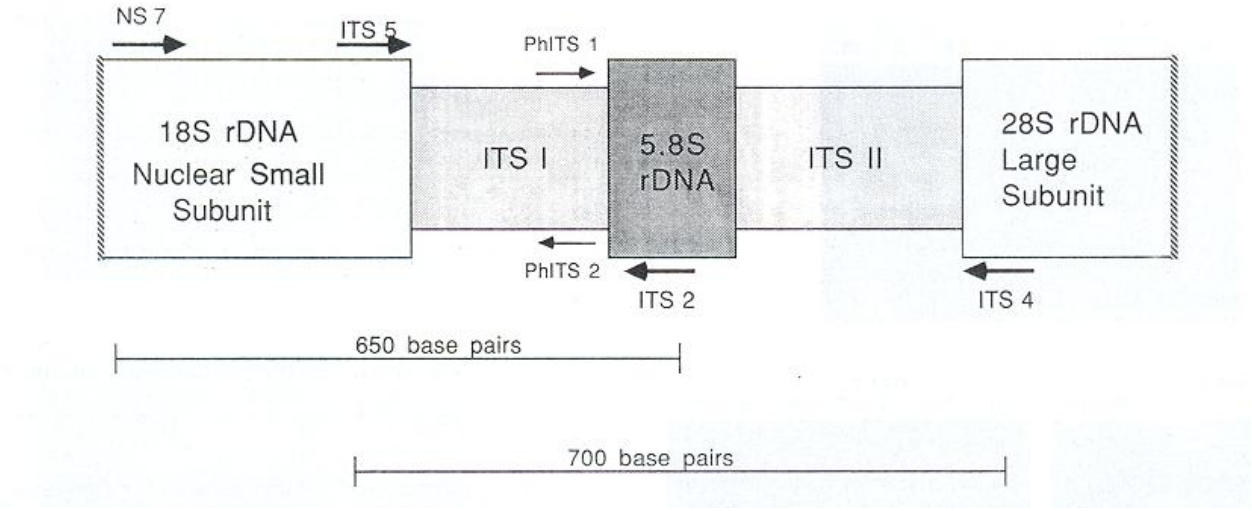

Figure 5. A schematic representation of the priming sites that will be used in this study.

References

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