MYCOLOGICAL RECORDS 2: *NEUROSPORA INTERMEDIA* TAI

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ABSTRACT

Neurospora intermedia Tai and its *Chrysonilia* von Arx anamorph are reported from debarked *Pinus radiata* D. Don logs. Species of *Neurospora* forming a surface mould on timber appear to have a wide geographic distribution and do not represent a threat to the soundness of timber.

Keywords: *Neurospora intermedia*; fungi; Ascomycetes; *Pinus radiata*.

INTRODUCTION

A shipment of debarked *Pinus radiata* logs from Mt Maunganui and Wellington, New Zealand, was quarantined by the US Department of Agriculture on arrival at the port of Sacramento. When the ship's hold was opened the logs were found to be coated with an orange "candy floss"-like fungus. The New Zealand Ministry of Forestry arranged for a sample to be sent to NZ FRI for identification. The logs had been processed at two debarking facilities in Wellington and one in Mt Maunganui. The logs from the Wellington facilities had been dipped in anti-sapstain chemicals, either copper-8 quinolinolate or 2(thiocyanomethylthio) benzothiazole, and the Mt Maunganui logs were sprayed with a mixture of didecyldimethyl ammonium chloride and 3-iodo-2-propynyl butyl carbamate. The logs were loaded on to the MV Pac Ocean which departed New Zealand 1 January 1994 and arrived at the Port of Sacramento, California, on 20 January 1994. The shipment was released from quarantine in mid-February, well before the final identification of the fungus was made in April 1994.

The sample, which consisted of a block of wood with surface mould, had been wrapped in thick brown paper, placed in a plastic bag and airfreighted to New Zealand. In transit perithecia developed amongst the orange mycelium.

IDENTIFICATION METHODS

Standard mycological methods of investigation were used. Perithecia and hyphae to be studied were mounted in 10% ammonia. Ascospore rib patterns were studied after irrigation of the slide with Giemsa stain (Frederick *et al.* 1969).

Small fragments of orange mycelium and perithecia from the wood block were transferred to 3% malt extract agar (MEA) and incubated in the dark at room temperature. Perithecia developed and released ascospores which adhered to the petri dish lid. These were collected with a sterile loop and suspended in 20 ml of sterile distilled water. Four drops of suspension were transferred to each of two MEA plates and incubated at 25°C. To test the effect of heat treatment the remaining suspension was then placed in a 50°C water bath for 30 min and a further four drops were transferred to each of two MEA plates and incubated at 25°C. Individual germinating ascospores were picked off the plate with a sterile needle and transferred to fresh MEA plates and incubated in the dark at room temperature. Single ascospore isolates were mated by cutting 5 × 5-mm squares from the margin of the actively growing mycelia of the two isolates and placing them, approximately 10 mm apart, on MEA plates. These were sealed with cling film and incubated in the dark at room temperature.

RESULTS

In the initial isolation attempt, cultures derived from either mycelial fragments or whole perithecia covered the plates in 2 days with scant pale-orange mycelia. Perithecia were produced within 7 days and ejaculated ascospores were found adhering to the petri dish lid within 14 days. These ascospores were used to prepare the single ascospore isolates. The cultures derived from single ascospores failed to produce any perithecia; however, paired isolates produced perithecia as did the multi-ascospore isolation plate. Macroconidiospores as seen on the original wood block were not produced in the petri dishes, but the hyphae pushed past the cling film seal and produced a fan of hyphae in the cavity between stacked plates. In this drier environment the hyphae produced macroconidiospores. It was also noted that the mixing of mycelia outside the plates initiated perithecia production under the cling film and later at the periphery of the single ascospore plates. Microconidiospores were not seen.

Non-heat-treated ascospores did not germinate and plates were quickly overrun by yeast and other filamentous fungi. The heat-treated ascospores all germinated.

DESCRIPTION

Mycelium rapidly growing on 3% malt extract agar, reaching edge of a 90-mm plate in 2 days, orange to sordid buff; perithecia developing and maturing in 14 days in the dark at room temperature. **Perithecia** aggregated to scattered, partially submerged to superficial, smooth to tomentose, brown, becoming dark brown to black at maturity, globose, 369–697 µm diam.; ostiole papillate, to 200 µm long (Fig. 1D). **Asci** cylindrical, long-stalked, 203.5–261.1 × 15.4–19.2 µm, 8-spored; apex with distinct, inamyloid ring (Fig. 1C). **Physes** very distinct in young perithecia, becoming less so with age, thin-walled, septate (Fig. 1B). **Ascospores** uniseriate, 1-celled, oval, 21.9–25.7 × 12.1–14.4 µm, initially hyaline, becoming yellow-brown, then dark brown and opaque at maturity, germ pore at both ends; wall ornamented with c. 20 longitudinal, anastomosing, dark brown ribs, 1–3 µm wide (Fig. 1A). **Macroconidiospores** thallic, 3–7 × 3–30 µm, sub-globose to cylindrical, conspicuous thickening of septa before disarticulation, after disarticulation septal walls bulging, with small striated scar (Fig. 1E). **Microconidiospores** not seen.

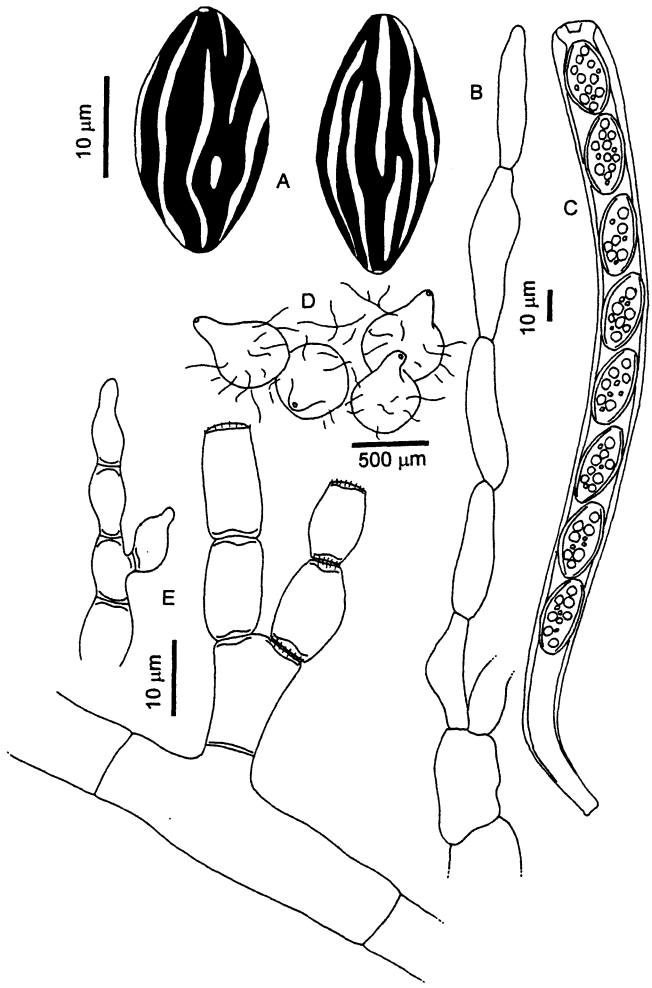

FIG. 1–A: ascospores; B: physis; C: ascus; D: perithecia; E: macroconidiospores.

Specimen examined: block of wood from a debarked log of *Pinus radiata* D.Don exported from New Zealand, on the MV Pac Ocean, and quarantined in Sacramento, USA, 8.ii.1994, *L.Farnworth*, culture **NZFS** 274.

DISCUSSION

Based on studies of morphology and anatomy and experimental observations, the fungus was identified as the heterothallic species *Neurospora intermedia* Tai with a *Chrysonilia* von Arx (= in part *Monilia* Persoon) anamorph or asexual state (Tai 1935; Frederick *et al.* 1969; von Arx 1981; Hawksworth *et al.* 1983). The large size of the perithecia and asci exclude this isolate from *N.sitophila* Shear & Dodge, and the small ascospores exclude it from *N.crassa* Shear & Dodge (Frederick *et al.* 1969; Subramanian 1971).

A search of the literature found only scant mention of *Neurospora, Chrysonilia,* and *Monilia* on timber surfaces. Cartwright & Findlay (1958, p.300) listed *Monilia* as a sapstain fungus sporulating on wood surfaces. Webster (1980, p.327) noted "In nature these species of *Neurospora* colonise burnt ground and charred vegetation, and are also found in warm humid environments such as wood-drying kilns and bakeries where they can cause serious trouble because of their rapid growth and sporulation". Two recent papers by Shaw (1990, 1993) reported blooms of *N.sitophila* on steam-heated (to 127°C), debarked logs of *Pinus elliottii* Engelmann var. *elliottii* in Queensland, Australia. Anecdotal information suggests that blooms of orange mould are not uncommon on logs in New Zealand.

Shaw (1990) reviewed the literature for the natural occurrence of heterothallic *Neurospora* teleomorphs and could not find a single example. This is surprising when the isolates from this study produced perithecia so freely both on the original wood block and in culture.

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