

## SHORT RESEARCH NOTES

**First record of *Nematospora coryli* in Australia and its association with dry rot of *Citrus***

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**Abstract.** *Nematospora (Eremothecium) coryli* was isolated from *Citrus* and identified for the first time in Australia. This insect-transmitted yeast was associated with dry rot in cultivated and native *Citrus* fruits. Although *N. coryli* is known as a serious seed pathogen of many tropical and sub-tropical plants, evidence is presented that it has been present and undetected in Queensland for at least ninety years.

In July 2004, a yeast culture that had developed on tissue culture medium from mandarin seeds used for embryonic tissue culture was forwarded to the Queensland Department of Primary Industries, Plant Pathology Herbarium (BRIP) for identification. The yeast grew on the seeds despite the seeds having been extracted from fruit under sterile conditions. The same yeast was subsequently isolated on potato-dextrose agar containing 0.02% streptomycin (PDA + S) from fruits of *Citrus reticulata* (mandarin cv. de Nules), *Citrus limon* (lemon) and *Citrus australis* (native lime) that had dry flesh and brownish, shrivelled seeds (Fig. 1) from several locations in eastern Australia between Bundaberg, Queensland, and Gosford, New South Wales.

Microscopic examination of discoloured seeds showed a waxy-yeasty deposit on the surface. Preparation of microscope slides in acid fuchsin in lacto-glycerol showed the presence of characteristic cylindrical-obtuse asci, 57–68 × 6–9 µm (Fig. 2C and D), each containing 4–8 needle-shaped ascospores, 40–50 × 2 µm. Ascospores (Fig. 2E and F) were two celled with a long, slender, flexuous, whip-like appendage, 10–25 µm long, arising from one end. The two cells stained differentially in acid fuchsin in lacto-glycerol with only the appendaged cell staining (Fig. 2E

and F). Spores were arranged in two groups within the ascus with the non-appendaged ends oriented towards the poles of the ascus (Fig. 2D). Mycelium was septate, with swollen fusiform to clavate cells (Fig. 2A), and the more or less globose yeast cells (Fig. 2A, B and D) were 18–22 µm diam.

Growth in culture on PDA + S for 7 days resulted in colonies, which were white to cream, butyrous, smooth, slightly raised, with a narrow, mycelial fringe. The fungus was also readily found in the pulp, remote from the seed, of affected citrus fruits. These characters enabled identification of the fungus as *Nematospora coryli* Peglion, which is described and illustrated in Barnett *et al.* (2000) and de Hoog *et al.* (2000). Dried specimens and living cultures have been deposited as BRIP 44998 (from mandarin), BRIP 45001 (from lemon) and BRIP 45029 (from native lime).

*N. coryli* was transferred to the genus *Eremothecium* by Kurtzman (1995) who argued that the genera *Ashbya*, *Eremothecium*, *Holleya* and *Nematospora* did not differ sufficiently at the molecular level to be maintained as separate genera. We use the name *Nematospora*, primarily because that is the name by which the fungus is most widely known in the phytopathological literature. A second

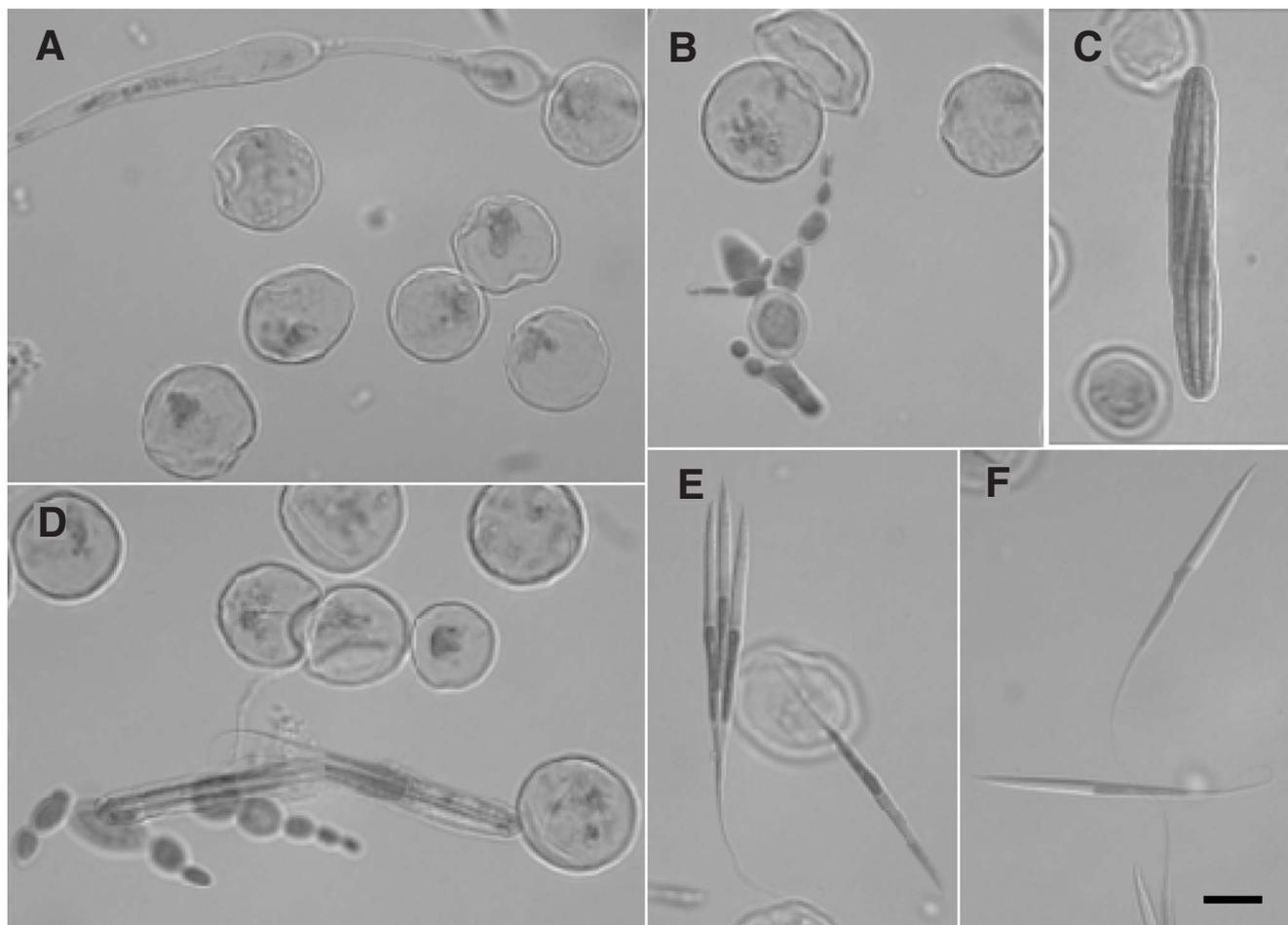


**Fig. 1.** *Nematospora coryli*, symptoms on mandarin cv. De Nules, BRIP 44998.

species, *Nematospora sinecauda* Holley, has been recorded in Australia by Oram *et al.* (2003) as the cause of a seed rot of Indian mustard (*Brassica juncea*).

Early literature reported that *Nematospora coryli* caused a serious disease on a range of species and varieties of *Citrus* (Fawcett 1936). For example, it was reported to cause desiccation, dry rot and premature fruit drop of oranges, grapefruit and tangerines in California in the 1920s (Fawcett 1929). More recent literature fails to report *Nematospora coryli* as a pathogen of *Citrus* (Klotz and Fawcett 1948; Cook 1975; Reuther *et al.* 1978; Timmer *et al.* 2000, 2003).

*N. coryli* is known as a serious pathogen of seeds of many species of tropical and sub-tropical plants, including *Gossypium hirsutum* (where it is the cause of internal boll rot or Stigmatomycosis), *Anacardium occidentale*, *Coffea* spp., *Corylus avellana*, *Crotalaria* spp., *Cajanus cajan*, *Dolichos lablab*, *Phaseolus* spp., *Tephrosia vogelii* and *Vigna*



**Fig. 2.** *Nematospora coryli*, BRIP 44998. (A) Hyphae and globose yeast cells. (B) Globose and budding yeast cells. (C) Ascus. (D) Disintegrating ascus and ascospores, and globose yeast cells. (E and F) Ascospores stained in acid fuchsin. Scale bar = 10 µm.

*sinensis* (Mukerji 1968), *Lycopersicon esculentum* (Batra 1973), *Glycine max* (Heinrichs *et al.* 1976) and *Macadamia* (Zúñiga *et al.* 1988). The fungus is usually transmitted by sap-sucking pentatomid (Hemiptera) insects and the yeast always enters through insect punctures (Mukerji 1968). The yeast survives in adults through the insect's life (Kulik and Sinclair 1993). *N. coryli* has been recorded in Africa, Asia, Europe and North and South America (Mukerji 1968) but has not previously been reported in Australia.

There is strong circumstantial evidence that *N. coryli* has been present in Queensland for at least 90 years. Jarvis (1914) described a gumming disease of lemon fruits associated with feeding by spined citrus bug (*Biprorulus bibax*). Although he attributed the likely cause to a bacterial infection, the symptoms he described and illustrated are identical to those we have observed. Spined citrus bug was particularly abundant and damaging during the present fruit production season in the Bundaberg area (M. W. Smith, unpublished). Dry-fleshed mandarins and lemons have been a persistent problem in Australian citrus production for at least a decade, but the cause has remained unclear with diagnoses as boron deficiency (Anderson 1988) or damage caused by spined citrus bug (Mo 2003).

The detection of *N. coryli* on native lime poses the intriguing prospect that this fungus is native to Australia and has moved from that host onto cultivated citrus. The native lime on which it was detected was collected in a Brisbane garden, adjacent to dry rainforest along an ephemeral creek where native lime is endemic. This site also represents the southern most point of the distribution of native lime. Curiously, a second species of *Nematospora*, probably undescribed, was also isolated in this sample.

The detection of *N. coryli* in citrus fruit represents a major breakthrough in understanding and managing a problem that has affected fruit quality and hampered breeding and commercial rootstock seed production in Australia for many years (J. Owen-Turner, Citrus Consultant, personal communication). Because of its wide host range and its association with damage caused by Hemipteran insects, *N. coryli* may have been undetected on a range of crops in Australia. The presence of *N. coryli* may explain damage associated with Hemiptera feeding in Australia on grain legumes (Swaine and Ironside 1983), cotton (Pyke and Brown 1996) and macadamia (Ironside 1996). As nothing is known about the aetiology or epidemiology of citrus dry rot in Australia, work needs to be done to determine firstly, whether *N. coryli* occurs in Australia on some of the other known hosts as well as on native and weedy plants and secondly, the insects in Australia that transmit *N. coryli*, so that disease management strategies can be developed.

## References

- Anderson CA (1988) Mineral deficiencies and toxicities. In 'Compendium of citrus diseases.' (Eds JO Whiteside, SM Garnsey, LW Timmer) (APS Press: USA)
- Barnett JA, Payne RW, Yarrow D (2000) 'Yeasts: characteristics and identification.' 3rd edn. (Cambridge University Press)
- Batra LR (1973) Nematosporaceae (Hemiascomycetidae): taxonomy, pathogenicity, distribution and vector relations. Technical Bulletin No. 1469, ARS, USDA, Washington, DC.
- Cook AA (1975) 'Diseases of tropical and subtropical fruits and nuts.' (Hafner Press: New York)
- de Hoog GS, Kurtzman CP, Phaff HJ, Miller MW (2000) In 'The yeasts, a taxonomic study.' 4th edn. (Eds CP Kurtzman, JW Fell) (Elsevier: Amsterdam)
- Fawcett HS (1929) *Nematospora* on pomegranates, citrus and cotton in California. *Phytopathology* **19**, 479–482.
- Fawcett HS (1936) 'Citrus diseases and their control.' (McGraw-Hill: New York)
- Heinrichs EA, Lehmann PS, Corso IC (1976) *Nematospora coryli*, yeast spot of soybeans in Brazil. *Plant Disease Reporter* **60**, 508–509.
- Ironside DA (1996) 'Insect pests of macadamia in Queensland.' (QDPI: Brisbane)
- Jarvis E (1914) A gumming disease affecting lemon fruits. *Queensland Agricultural Journal* **1**, 345–348.
- Klotz LJ, Fawcett HS (1948) 'Color handbook of citrus diseases.' (University of California Press: Berkeley, CA, USA)
- Kulik MM, Sinclair JB (1993) Yeast spot (*Nematospora* spot). In 'Compendium of soybean diseases.' (APS Press: USA)
- Kurtzman CP (1995) Relationships among the genera *Ashbya*, *Eremothecium*, *Holleya* and *Nematospora* determined from rDNA sequence divergence. *Journal of Indian Microbiology* **14**, 523–530.
- Mo J (2003) 'Spined citrus bug.' Available online at: [www.agric.nsw.gov.au/reader/cit-spined.htm](http://www.agric.nsw.gov.au/reader/cit-spined.htm)
- Mukerji KG (1968) *Nematospora coryli*. CMI Descriptions of Plant Pathogenic Fungi and Bacteria **184**.
- Oram RN, Edlington JP, Halsall DM, Veness PE (2003) *Pseudomonas* blight and *Nematospora* seed rot of Indian mustard. In 'Proceedings of the 13th biennial Australian research assembly on Brassicas.' Tamworth, NSW, 8–12 September 2003.
- Pyke BA, Brown EH (1996) 'The cotton pest and beneficial guide.' (Cotton Research & Development Corporation: Narrabri, Australia)
- Reuther W, Calavan EC, Carman GE (1978) 'The citrus industry. Vol. IV. Crop protection.' (University of California: Berkeley, CA, USA)
- Swaine G, Ironside DA (1983) 'Insect pests of field crops.' (QDPI: Brisbane)
- Timmer LW, Garnsey SM, Broadbent P (2003) Diseases of citrus. In 'Diseases of tropical fruit crops.' (Ed. RC Ploetz) (CABI Publishing: UK)
- Timmer LW, Garnsey SM, Graham JH (2000) 'Compendium of citrus diseases.' 2nd edn. (APS Press: USA)
- Zúñiga D, Vargas E, Umaña G (1988) Diagnostico y aspectos preliminares de la epidemiologia de las pudriciones del fruto de la macadamia (*Macadamia integrifolia*) en Turrialba. *Agronomía Costarricense* **12**, 45–51.

Received 1 July 2004, accepted 26 July 2004