First report of *Pythiogeton ramosum* (Pythiales) in Australia

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Abstract In 2012, a project was initiated to assess if the soft rot disease of ginger in Australian fields was associated with pathogens other than *Pythium myriotylum*. Together with nine *Pythium* spp., ten isolates of a *Pythium*-like organism were also recovered from ginger with soft rot symptoms. These *Pythium*-like isolates were identified as *Pythiogeton* (*Py.*) *ramosum* based on its morphology and ITS sequences. In-vitro pathogenicity tests allowed confirmation of pathogenicity of *Py. ramosum* on excised carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*) and potato (*Solanum tuberosum*) tubers, although it was not pathogenic on excised ginger (*Zingiber officinale*) and radish (*Raphanus sativus*) rhizome/roots. In addition it was found to be pathogenic on bean (*Phaseolus vulgaris*), capsicum (*Capsicum annuum*) and cauliflower (*Brassica oleracea* var. *botrytis*) seedlings. This is the first record of *Py. ramosum* and its pathogenicity in Australia.

Keywords Ginger rhizome · *Zingiber officinale* · *Pythium* soft rot

The Australian ginger industry is small (less than 1 % contribution to world production), but it is a cash crop for around 30 full time growers, who produce approximately AUD 15.6 million at farm gate value per year (Camacho and Brescia 2009). The annual production of the whole Australian ginger industry is estimated at about 8,000 tonnes with almost half of this delivered to the domestic fresh market and the rest for the processing industry (Camacho and Brescia 2009). In 2007, farmers unexpectedly experienced severe losses (up to 100 %

in some ginger fields) due to *Pythium* Soft Rot (PSR). In 2009, *Pythium myriotylum* was reported as a causal agent responsible for the outbreak in Australia (Stirling et al. 2009). However, from our recent research, it is proposed that there is probably more than one *Pythium* sp. associated with PSR of ginger in Australian fields. In 2012, ginger with PSR symptoms caused by *Pythium* spp. was sampled from a representative selection of farms at Yandina, on the Sunshine Coast, Queensland, Australia. Along with nine *Pythium* spp. isolated from PSR ginger and soil around the ginger rhizome, ten isolates of a *Pythium*-like organism were also obtained from PSR ginger sampled from two farms (farm 1 at 26° 32'S, 152°56'E and farm 2 at 26° 33'S, 152°56'E) at Yandina. Isolations had been undertaken by excising sections (5 mm²) of rhizome with PSR symptoms, quickly surface decontaminating (around 10 s) with 25 % bleach containing 1 % HOCl, then washing twice with sterilized distilled water, blotted dry with autoclaved paper towel. The sections were then transferred on to Petri plates with corn meal agar with an amendment of 50 µg/ml Penicillin, 50 µg/ml Polymyxin, and 25 µg/ml Pimaricin (CMA + 3P). The Petri plates were incubated in the dark overnight at 27 °C, then sections taken from the growing edge of the colonies were transferred onto 1.5 % water agar, incubated again under the same conditions for another night, after which hyphal tips of each of the isolates were excised out under an inverted compound microscope (Leica) and placed onto full strength potato dextrose agar (PDA Difco).

All ten *Pythium*-like isolates were considered to be heterothallic as attempts to produce sexual structures on dual cultures were not successful. Using the keys of Jee et al. (2000) and Huang et al. (2013), these isolates were then identified as *Pythiogeton ramosum* based on the typical features of sickle-shape appressoria, terminal, subspherical, ellipsoidal, utriform, or bursiform sporangia with apical beaks (Fig. 1) which measured from 20–214×18–48 µm. Colonies on PDA and CMA media did not show any special pattern, mycelia

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Fig. 1 *Py. ramosum* growing on PDA showing an indistinctive pattern (left), terminal bursiform sporangia with different sizes and shapes of globose and elongate (right) (Bar= 20 µm)

were quite sparse on media and rarely aerial (Fig. 1). The hyphae were aseptate, hyaline, smooth, 2–5 (–7) µm wide. The hyphae also had many shorter branches which were usually bearing appressoria and growing at a right angle with the main hyphae. On solid culture media, old sporangia formed vacuoles, which had the potential to be misidentified as oospores. Two isolates of this species description were deposited with Queensland Plant Pathology Herbarium with BRIP numbers (BRIP59948 and BRIP59949). For confirmation of species identification, ITS regions were amplified and analysed. Pure DNA from the mycelial mats growing in potato broth was obtained using CTAB protocol (Doyle and Doyle 1990). A PCR reaction was carried out in a Eppendorf Mastercycler® using the two universal primers ITS1 and ITS4 (White et al. 1990) to allow amplification (approximately 860 bp) of ITS the region including the 5.8S rRNA encoding region. The Genbank database was searched (BLAST) for similar sequences resulting in 100 % homology with a *Py. ramosum* isolate JQ610190.1 from Taiwan (Huang et al. 2013). The sequences were also deposited with Genbank under KF151204 and KF151205.

Pythiogeton ramosum was first isolated from plant debris by Minden (1916) and was later commonly recovered from materials under anaerobic conditions (Czeczuga et al. 2005; El-Hissy et al. 1994; Huang et al. 2013; Nascimento et al. 2012). *Py. ramosum* is not well studied and was believed to be a saprophyte and until recently its pathogenicity on living plants had not been confirmed (Ann et al. 2006; Huang et al. 2013). In this study we initially attempted to carry out some in-vitro pathogenicity tests for our isolates to check if they were pathogenic on tested materials.

Pathogenicity tests were conducted in-vitro on ginger (*Zingiber officinale*), carrot (*Daucus carota*), radish (*Raphanus sativus*), sweet potato (*Ipomoea batatas*) and potato (*Solanum tuberosum*) rhizome/tubers. The experiment was based on descriptions of Le et al. (2010) to test the pathogenicity and extent of rotting from isolates (BRIP59948 and BRIP59949) inoculated on excised

rhizome/tuber sections (1×1×5.5 cm) at 27 °C. All tested isolates of *Py. ramosum* were shown to be non-pathogenic on excised ginger and radish pieces, but they were pathogenic on carrot, sweet potato and potato tubers. The tested tubers were decayed, soft and brown in colour and *Py. ramosum* was reisolated after placing decayed tissue onto CMA+3P. Additionally, pre-emerging damping off tests were carried out in Petri plates according to descriptions of Zhang and Yang (2000) and disease indices (scale from 0 to 1) were calculated to evaluate aggressive levels of *Py. ramosum* on some vegetable crops including bean (*Phaseolus vulgaris*), capsicum (*Capsicum annuum*), and cauliflower (*Brassica oleracea* var. *botrytis*). Disease indices recorded from 0.13 to 0.50 indicated that *Py. ramosum* was able to attack and cause pre-emerging damping off on these crop species (Fig. 2). The results from this study allowed us to conclude that *Py. ramosum* is pathogenic on several hosts and this is the first report of the pathogenicity of *Py. ramosum* on either excised rhizome/tuber sections or seeds/seedlings of the tested materials. Although *Py. ramosum* was isolated directly from soft rot ginger, it was non-pathogenic on ginger at 27 °C. Therefore, more pathogenicity tests including on living plants at different temperatures will be warranted.

Fig. 2 Cauliflower seedlings following inoculation with *Py. ramosum* (left) showing necrosis, brown rot in comparison to white and healthy seedlings in the uninoculated controls (right)

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