CSIRO PUBLISHING

www.publish.csiro.au/journals/apdn *Australasian Plant Disease Notes*, 2007, **2**, 7–9

First record of *Pythium tracheiphilum* associated with lettuce wilt and leaf blight in Australia

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Abstract. *Pythium tracheiphilum,* the cause of lettuce stunt, wilt and leaf blight, was recorded for the first time in Australia in July 2005, on gourmet lettuce at two farms at Gingin, near Perth, Western Australia. A subsequent survey of commercial lettuce crops failed to find symptoms of lettuce wilt and leaf blight at additional properties, and *P. tracheiphilum* was not isolated. *P. tracheiphilum* was, however, isolated from chicory from two home gardens, one at Fremantle, and one at Beaconsfield, near Perth, in September 2005.

Pythium tracheiphilum was described from Italy in 1965 causing a vascular wilt and stem rot of lettuce (*Lactuca sativa*) (Matta 1965). It was subsequently described as a pathogen of lettuce in other parts of Europe (Blok and van der Plaats-Niterink 1978; Zinkernagel and Krober 1978; Hall 1989; González *et al*. 2004) and North America (Tortolero and Sequeira 1978; Reeleder and Charbonneau 1987) causing stunting, wilting, root rot, stem rot and leaf blight. Losses caused by *P. tracheiphilum* are economically important, and losses up to 30% have been recorded (Zandstra *et al*. 1988). They are particularly severe during wet seasons.

P. tracheiphilum also causes leaf and head rot of maturing Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) in Denmark, with losses of 40 to 50% (Møller and Hockenhull 1997). It has also been recorded as a root pathogen of spinach (*Spinacea oleracea*) in Sweden (Larsson 1994), sugar beet (*Beta vulgaris*) in Iran (Noor *et al*. 2004) and as a cause of damping-off of pine (*Pinus halepensis*) seedlings in Algeria (Paul *et al*. 1992). Blok and van der Plaats-Niterink (1978) showed that *P. tracheiphilum* was highly pathogenic to cucumber (*Cucumis sativus*) and cauliflower (*Brassica oleracea* var. *botrytis*) in *in vitro* tests.

P. tracheiphilum is a soilborne pathogen that infects foliage by soil splash, rather than by invasion through the roots and stem (Tortolero and Sequeira 1978; Møller and Hockenhull 1997). Once introduced into a site it can be difficult to control by rotation, because the longevity of oospores is likely to be longer than the normal rotation time. Møller *et al*. (2003) have shown that leaf and head rot of Chinese cabbage can be controlled in the field by *Clonostachys rosea*, but not by other biocontrol agents or fosetyl-Al.

In Australia, a *Pythium*, initially identified as *P. tracheiphilum,* was isolated from roots of rice (*Oryza sativa*) seedlings in New South Wales (Cother and Gilbert 1993); however, re-examination of the culture (DAR 67486) using rDNA internal transcribed spacer (ITS) sequences has shown that this isolate is not *P. tracheiphilum* (J. H. Cunnington, unpubl. data).

In May 2005, following a week of heavy rain, a gourmet lettuce grower at Gingin, ~100 km north of Perth, Western Australia, noticed wilting in ~15% of his cos lettuce crop. Samples were taken in early June and sent to Crop Health Services, Knoxfield, Victoria. A *Pythium* was consistently isolated from angular leaf lesions. The rDNA ITS region was amplified by PCR and sequenced. The sequence has been deposited on GenBank (accession EF164896). Similarity searches against GenBank showed that the sequence differed from the type strain of *P. tracheiphilum* by six bases. Identification was confirmed by examination of sporangial and gametangial morphology. As there were no verified records of *P. tracheiphilum* in Australia this was considered as a new incursion and warranted further action.

The affected property was visited in July 2005. The gourmet lettuce crop was poor, with abundant *Sclerotinia* being present. In addition, ~1% of the cos crop showed leaf blight symptoms, some of which were associated with the midrib, others were V-shaped starting close to the leaf tip (Fig. 1). Some plants had discoloured vascular bundles in the stem. Symptomatic plants were returned to the laboratory for isolation. Soil from the

 10.1071/DN07004 1833-928X/07/010007

Fig. 1. Symptoms of lettuce leaf blight from which *Pythium tracheiphilum* was isolated.

affected beds was also collected, as were irrigation water samples from surface dams. No symptoms of leaf blight were seen in other lettuce types growing on this property.

Seven other gourmet and iceberg lettuce properties were surveyed in market gardening areas north and south of Perth in July 2005. Symptomatic plants were sampled and returned to the laboratory for isolation.

In the laboratory, small pieces of necrotic lesions, or discoloured vascular tissue were surface sterilised with 70% ethanol for 5 s, rinsed in sterile deionised water, blotted dry, and plated onto water agar, half strength potato dextrose agar or *Pythium* selective agar (Davison and McKay 1998). Soil and irrigation water were baited with either small pieces of lettuce leaves, freshly germinated lettuce seedlings or cotyledons of *Eucalyptus sieberi* (Marks and Kassaby 1974). The baits were surface sterilised as above, and plated onto *Pythium* selective agar. Any *Pythium* spp. isolated were hyphal tipped, and subsequently identified morphologically using standard keys (van der Plaats-Niterink 1981; Dick 1990).

P. tracheiphilum was isolated from necrotic lesions in the leaf lamina and at the leaf base from three cos plants from the original affected property. It was not isolated from either the baited soil or the baited irrigation water. *P. tracheiphilum* was also isolated from one asymptomatic cos lettuce from the adjacent property. It was not isolated from sampled lettuce from other surveyed farms.

Attempts to find the source of the infection have been unsuccessful. Both infested properties have been growing lettuce for at least 2 years. Lettuce are either directly sown into raised beds, or planted with seedlings grown on the property. Lettuce is harvested by hand as either whole heads or as loose-leaves for the fresh salad market. Crop debris is sprayed with glyphosate and the new crop planted directly into the raised beds. There is no crop rotation. Metham sodium is used for basic weed and disease control on one property. This method of cultivation indicates that *P. tracheiphilum* is unlikely to have been introduced on infected seedlings; the most likely route is from contaminated seed, even though we are unaware of any reports of seed transmission of this pathogen.

Both infested properties have introduced management, cultural and chemical strategies to contain the outbreak. These strategies include: minimising soil movement on the farm, washing down farm machinery and equipment to minimise the spread of soil on the farm, minimising outside vehicle access to the farm, deep ploughing to bury crop residues and waste, avoiding excessive irrigation, improved crop rotation, soil fumigation with metham sodium before lettuce crops, and the use of metalaxyl.

In September 2005, *P. tracheiphilum* was isolated from leaf blight symptoms from samples of chicory (*Cichorium intybus*) from a home garden in Fremantle and another home garden in Beaconsfield, both within the Perth metropolitan area. The identification was confirmed by ITS sequence data. A trace-back showed that the householders were not acquainted with each other and failed to determine how this pathogen was introduced. Both householders had not purchased commercial seedlings; their plants had been grown from commercial seed purchased 2 years earlier and they had subsequently used their own seed.

Both of these incidents raise the possibility that the source of the infection was seedborne inoculum. Even though there is no record of *P. tracheiphilum* being seedborne, oospores could contaminate the surface of the testa, or the seed could be contaminated with infested soil.

The initial isolate has been deposited in the culture collection of the Department of Agriculture and Food Western Australia Plant Pathogen Collection (WAC) as WAC 14346. Subsequent cultures have been deposited as 14342–4, 14347–8, 14421 and 14547. Duplicates of some of these are held in the Victorian Plant Pathology Herbarium (VPRI).

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Manuscript received 15 June 2006, accepted 12 December 2006