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Observation of *Tylenchorhynchus ewingi* in association with cotton soils in Australia

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Abstract. We report for the first time the recovery of the plant parasitic nematode *Tylenchorhynchus ewingi* Hooper 1959 from field soils used to grow irrigated cotton (*Gossipium hirsutum*) in New South Wales, Australia. The observation was made while investigating the possible existence of an association between nematodes and Verticillium wilt of cotton. No interaction was evident between nematode population sizes, species composition or the number of nematodes in cotton roots and the severity of Verticillium wilt. The samples collected over the course of the investigation contained few plant parasitic nematodes. Although the plant parasitic nematode population was small it was comprised almost entirely of the stunt nematode, *Tylenchorhynchus ewingi*. This is the first observation of this nematode in an Australian cotton production system.

Verticillium wilt, caused by *Verticillium dahliae*, is an economically important fungal disease of cotton in Australia, particularly in the Namoi valley, New South Wales (NSW). Interactions between vascular wilts of cotton and root lesion nematodes have long been established in the United States of America (USA) and India (Prasad and Padaganur 1980; Gazaway and Mclean 2003), but there is little to no knowledge of nematodes in Australian cotton production systems. We undertook a pilot study in the Namoi Valley during the 2004–05 cotton season to establish if there is an association between severity of Verticillium wilt of cotton and occurrence of plant parasitic nematodes (Knox *et al*. 2006). That study resulted in the first observation of the spiral nematode, *Helicotylenchus dihystera* (Cobb, 1891) Sher, 1961 in cotton roots in Australia, but nematode frequency was not correlated with the incidence of Verticillium wilt. However, sampling was conducted late in the growing season and any interaction between the nematodes and *V. dahliae* during earlier stages of disease development would not have been observed. To establish if earlier season interactions were occurring between nematodes and *Verticillium*, observations were taken three times over the course of the following cotton season (2005–06) and reported herewith.

The two field locations investigated in the 2004–05 season were sampled again in the 2005–06 season; one in the lower Namoi valley (30°06.640S; 149°33.666E, GPS Datum AGD 66) and the other in the upper Namoi Valley (E; 31°11.725S; 150°25.909E). Six sample sites in each field were visited at 2 days before planting, and at 64 and 146 days after planting. GPS was used to return to the same sample sites as those assessed in the previous season. Incidence of Verticillium wilt within the cotton crop was assessed at 64 and 146 days after planting. This was done by cutting the stems of 10 plants over 1 m at each sample site and assessing the cut-stem tissue for discolouration and necrosis on a scale of 0–4 as previously described (Knox *et al*. 2006). Soil samples were collected at each sample point by taking three cores (15 cm diameter × 20 cm deep) between the assessed plant stand on the planting hill. The three cores were pooled and returned to the laboratory for assessment. Live cotton roots were extracted from 1 kg of each soil sample, cleared and stained with acid fuchsin. Nematode occurrence and frequency in the stained roots was assessed under the dissecting microscope (× 120 magnification). Soil (150 g) from each sample was sent to Biological Crop Protection (Moggill, Queensland) where nematodes were extracted in Whitehead trays for trophic group identification.

Helicotylenchus dihystera was not observed in any of the Whitehead tray assayed soil samples, even though it had been isolated from the roots of cotton at the upper Namoi site in the previous season (Knox *et al*. 2006). Plant parasitic nematodes accounted for only 3% of the total nematode population recovered from soil and was mostly comprised of a stunt nematode, later identified as *Tylenchorhynchus ewingi*

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(Dr J. Nobbs, South Australian Research and Development Institute). Of the 24 soil samples assessed, 21 were found to contain stunt nematodes, with its abundance accounting for 98% of the total plant parasitic nematodes recovered. When the samples were divided into the upper and lower regions of the Namoi further differences were observed. In the lower Namoi, 94% of soil samples contained *T. ewingi* (mean of 18 recovered per 150 g of soil), whilst for the upper Namoi the value was 75% (mean of three *T. ewingi* per 150 g).

Verticillium wilt was not observed at the lower Namoi field location in the 2005–06 crop, nor in the previous crop. At the upper Namoi location Verticillium wilt was not observed until the third sampling at 146 days post sowing and occurred at five of the six sampling sites within the field with a mean disease severity of 2.5 (disease severity scale of 0–4). This compared with a mean severity rating of 1.3 for the previous season with disease occurrence at four of six sample sites.

The frequency of nematodes within the stained cotton roots was lower than in the 2004–05 season samples. Nematodes were only observed in root material from the third and final sampling. An average of 1.4 g of fresh root material was recovered from each of the 1 kg samples with approximately one nematode observed in every 8 g of root material. Partial dissection of the stained root samples revealed that, based on anterior morphology, 45% of these endophytic nematodes were the spiral nematode *H. dihystera*, with the remainder belonging to bacteriophagous and mycophagous functional groups. Further identification was not possible due to the tissue fixation and preparation methodology utilised in this study. Nematodes recovered from both soil and root samples, at either field location, did not correlate with the incidence or severity of Verticillium wilt, suggesting that an interaction between Verticillium wilt and nematodes within cotton farming systems in the Namoi valley is unlikely.

The decrease in nematode numbers observed in cotton roots between the two seasons is consistent with the successive use of the biocide Aldicarb (Temik, Bayer Crop Science) at commercial rates of 7 kg/ha (Spurr and Sousa 1974). The wheat rotations, which preceded the 2004–05 cotton crops, did not have Aldicarb applied and (are likely to have) supported greater nematode populations. In cotton, Aldicarb application provides systemic control of the early season sucking pests, true wireworm (*Agrypnus variabilis*) and false wireworm (*Pterohelaeus darlingensis*). The impact of these sucking pests within the cotton industry has increased with the introduction of Bt insecticidal cotton varieties, probably resulting from decreased competition with other pests. This shift in the farming system has meant that application of Aldicarb at sowing is now commonplace and is likely to be responsible for the overall decline in nematode populations and their isolation only in the latter part of the growing season (i.e. 146 day sampling).

Our observations of *T. ewingi* represent the first record of this nematode in association with cotton farming systems in Australia. Previously *T. ewingi* has only been observed in association with wheat in Australia (McLeod *et al*. 1994). The system we investigated had been under monocultures of cotton for up to 28 months since the haying-off of the previous wheat crop, suggesting that *T. ewingi* is surviving on cotton despite not being observed within the recovered root material. In the USA, stunt nematodes have been found in association with cotton (Gazaway and Mclean 2003), but are considered to be either weak or non-pathogenic. They have, however, been implicated in cereal crop losses, particularly in the low rainfall regions of Oregon. The results reported in this study neither indicated the existence of an interaction between nematodes and Verticillium wilt nor the occurrence of large populations of potentially cotton pathogenic nematodes. While Australian cropping systems and soils might differ from those in other countries, observations of previously unrecognised plant parasitic nematodes serve as a reminder that we still know very little about our soil microfauna and continue to highlight the importance of farm hygiene practices to restrict the spread of potential pathogens.

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