Diseases Caused by Nematodes

First Report of a Sheathoid Nematode (*Hemicriconemoides litchi*) on Longan in Taiwan

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Longan (*Dimocarpus longan* Lour.) is a subtropical fruit, widely grown in China, Taiwan, and Southeast Asia. Longan has a high commercial value, and US\$242,200 of dried longan fruits are exported to the United States from Taiwan every year (Wang et al. 2010). A soil sample from a longan orchard located in Changhua County, Taiwan (24.0162657°N, 120.5291457°E) was collected in June 2019. Sheathoid nematodes were the dominant species in this sample and over 40 adult individuals per 100 g of soil were found. In December 2019, sheathoid nematodes were collected again from the same tree and processed for identification by morphological and molecular characteristics. Nematodes were extracted using the modified Baermann funnel method (Wu et al. 2010) for 24 h. The morphometric data from 15 females with variant tail types were taken. All individuals had a close sheath, lip region set off with two annuli, stylet frequently slightly dorsally curved with rounded knobs, no vulval flaps, tail narrowing to a broadly rounded terminus, or tapering to a truncate end, anus situated three to four annuli posterior to the vulva. The body length = 552 µm (483 to 616 µm), body width at mid-body = 34.02 µm (27.75 to 40.03 µm), a = 15.93 (14.73 to 18.87), b = 4.65 (4.04 to 5.06), V% = 91.57 (86.74 to 92.56), stylet length = 66.66 µm (63.51 to 69.6 µm), tail length = 28.48 µm (23.56 to 37.45 µm), ring number = 114 to 130, Rs = 14 to 19, Roes = 22 to 28, RV = 9 to 13, Ran = 5 to 9, and RVan = 3 to 4. Since the stylet length was less than 70 µm, they are more fitted to be *Hemicriconemoides litchi* (Van den Berg et al. 2015). DNA

samples extracted using Viagen DirectPCR lysis buffer from single females ($n = 10$) were processed to amplify the 28S D2-D3 expansion segment and the ITS region using primers sets D2A/D3B and TW81/AB28, respectively (Van den Berg et al. 2014). The D2-D3 region of the nematodes collected in this study (MT-539384) shared 99% similarity with several *H. litchi* sequences deposited in the GenBank database (e.g., KP192481, KF856540), and the ITS region (MT556011) also shared 99% similarity with several *H. litchi* sequences (e.g., KP192482, GQ354786). Therefore, based on morphology, molecular data, and phylogenetic relationship analysis (Nguyen et al. 2020) the nematodes from the longan orchard were determined to be *H. litchi*. To measure the reproductive factor (Rf), 60 sheathoid nematodes (57 females and three males) recovered from Baermann extraction were inoculated onto root systems of a longan tree in a 15-cm-diameter pot filled with sterilized soil. One hundred sixty-eight days after inoculation, three 100-cm³ subsamples of soil from the pot were processed as mentioned previously, and the average number was 48/100 cm³ of soil. The final population was approximately 768 nematodes per 1,600 cm³ of soil, with the Rf value of 12.8 confirming longan as a host, although no symptoms were observed. The DNA from three individuals in the three subsamples with variant tail types was used to obtain D2-D3 and ITS region sequences, and this confirmed the species as *H. litchi*. *Hemicriconemoides* spp. is associated with root malformation and nutrient deficiencies on agricultural fruit trees (McSorley et al. 1980; Milne et al. 1971); however, aboveground evidence of damage may not become immediately obvious, and the importance of sheathoid nematodes is easily overlooked (Chen et al. 2011). This is the first report of *H. litchi* as a parasite of longan tree in Taiwan.

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