Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Leptosphaeria biglobosa* ‘brassicae’ Causing Blackleg on *Brassica rapa* subsp. *pekinensis* in China

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Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis* [Lour.] Hanelt) is a major leafy vegetable crop grown in China and eastern Asia (Fordham and Hadley 2003). In December 2018, black leg symptoms were observed on ‘Qingza No.3’ of Chinese cabbage during harvest, Chibi (29°46′37.38″N, 114°05′6.88″E), Hubei, China. Symptoms were first noted in late November as black spots on leaf petioles and basal stems. Then, black spots enlarged as oval or irregular-shaped grayish lesions. Finally, lesions enlarged and coalesced with black pycnidia observed, and some diseased leaves became blighted. The disease incidence was about 80% in three fields surveyed (~2 ha in total). Diseased plant tissues were surface sterilized and incubated on potato dextrose agar (PDA) plates at 20°C for 4 days. Three fungal isolates (EP9-19, EP9-22, and EP9-26) were obtained from five of the diseased samples; all produced fluffy, white aerial mycelia and a yellow pigment on PDA. After 14 days, black-brown and globose pycnidia were produced, approximately 150 µm in diameter ($n = 50$). In addition, pink pycnidiospore ooze was observed on the top of pycnidium after 20-day culturing on a V8 juice (20%) agar. Conidia were cylindrical and hyaline, with the mean size of 4.6×2.7 µm ($n = 50$). Two fungal species have been reported to cause blackleg on *Brassica* crops (Williams and Fitt 1999): *Leptosphaeria maculans* and *L. biglobosa*. The former is much more destructive but is not present in China.

These isolates had morphological characteristics matching those of *L. biglobosa* (Williams and Fitt 1999). The genomic DNA of isolate EP9-22 was extracted and sequenced for its actin, β -tubulin, and the internal transcribed spacer (ITS) region of ribosomal DNA as described by Vincenot et al. (2008). Sequences of ITS (GenBank accession no. MN238766), actin (MN242213) and β -tubulin (MN242214) for isolate EP9-22 showed 100, 99.67, and 97.93% identity to the corresponding regions of *L. biglobosa* ‘brassicae’ strain IBCN89 (Vincenot et al. 2008). In addition, the phylogenetic analysis also indicated that isolate EP9-22 belonged to *L. biglobosa* ‘brassicae’. The pathogenicity test was performed according to established protocols (Balesdent et al. 2005). Cotyledons of the 15-day-old Chinese cabbage seedlings (cultivars Xiaozha No.55 and Hualiangzao No.5) were wound inoculated with 10 µl of pycnidiospore suspension (1×10^7 conidia/ml) of the three isolates, with 20 cotyledons per isolate, respectively, and 20 cotyledons wound inoculated with sterile water served as a control group. The treated seedlings were maintained at 20°C and 100% relative humidity with a 12-h photoperiod. The experiment was repeated twice. At 7 days after inoculation, necrotic lesions became visible surrounding inoculation sites for the three isolates, whereas the control group remained healthy. Fungal isolates showing a similar colony morphology to the originals were reisolated from 10 diseased cotyledons but not from the control cotyledons. Based on these results, *L. biglobosa* ‘brassicae’ was shown to be the causal agent of blackleg on Chinese cabbage in China. We believe that this disease has historically often been misidentified as anthracnose by local farmers. The threat from *L. biglobosa* to the production of Chinese cabbage has been assessed. This accurate identification of the causal pathogen is a critical first step toward the development of disease management strategies.

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