



Outbreak of leafspot on blackberry (*Rubus fruticosus*) caused by *Gnomoniopsis* sp. in Iran

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In October 2012, noticeable leaf spots were found on blackberry plants (*Rubus fruticosus*) cultivated in several cities of Mazandaran, a province in northern Iran. Typical lesions observed were light brown, regularly globose and variable in size (Fig. 1). Over time, the lesions enlarged and coalesced, extending until the entire leaf withered (Fig. 2). The incidence of diseased plants was approximately 80% in Sari with 30% of the plants extremely blighted, and yield losses ranging from 50 to 80%. Ten leaves with symptoms were collected from different blackberry plants from several fields in Mazandaran. Infected leaf tissues taken from the margins of the leaf spot were cut into small pieces (5 x 3 mm) and plated onto potato dextrose agar (PDA) after surface sterilisation in 1.5% NaOCl for three minutes. The plates were incubated at 24°C for a week. Hyphae growing out from the tissue pieces were sub-cultured onto PDA. Ten isolates were obtained and studied for their cultural and morphological characteristics. Colony colour varied from whitish to grey, olive and dark brown with moderate to fast growth usually producing abundant conidiomata exuding a slimy conidial mass (Fig. 3). Conidia were fusiform to slightly obovoid, straight to slightly curved, one-septate, 5.5-9 x 2-3.5 μ m (n=50), which is a characteristic feature of *Gnomoniopsis* species (Sogonov *et al.*, 2008).

Total genomic DNA of a representative isolate (Gim68) was extracted using the CTAB extraction method (Groppe & Boller, 1997) and used for PCR sequencing of rDNA genes by using universal primers for ITS 1 and ITS 4 (White *et al.*, 1990) and primers T1/T2 for β -tubulin (TUB2) (O'Donnell & Cigelnik, 1997). The ITS-rDNA and β -tubulin sequence data were deposited in GenBank (Accession Nos. KJ563296 and KJ994800, respectively). A BLAST search in the NCBI GenBank database resulted in close similarity (98% for ITS-rDNA and 95% for β -tubulin) with *G. idaeicola* (KC145891, KC145872, GU320820 for ITS and GU320781 and GU320782 for β -tubulin). A phylogram was constructed comparing the ITS and TUB2 sequences of Gim68 with other homologous sequences from the NCBI GenBank database (Fig. 4). A multiple sequence alignment was performed using Clustal W and a maximum-likelihood phylogenetic tree was constructed from Kimura 2-parameter pairwise distances using MEGA 6 (Tamura *et al.*, 2013). Based upon the molecular data and morphological characteristics, the causal agent was identified as *Gnomoniopsis* sp., with a high probability of it being *G. idaeicola*. Further studies will be conducted to confirm the species.

A pathogenicity test was conducted on detached, green blackberry leaves. Fifteen leaves were inoculated by placing a plug (5-mm-diameter) from the margin of seven-day-old colonies of each of the 10 isolates grown on PDA on a fresh wound site made with a sterilised scalpel. Each wound was covered with moisturised cotton and sealed with Parafilm. Control leaves were inoculated using sterile PDA plugs. The leaves were kept in plastic bags at ambient temperature (25-30°C). After seven days, all inoculated leaves with isolates showed symptoms similar to those observed in the field, whereas the controls remained asymptomatic. The fungus was re-isolated from the inoculated leaves, fulfilling Koch's postulates. The results of this study demonstrated *Gnomoniopsis* sp. as the causal agent of blackberry leaf spot in Iran for the first time. Previously, *G. idaeicola* has been reported on overwintered canes of *Rubus* spp. in France and USA (Walker, 2012).

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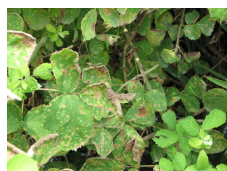


Figure 1



Figure 2

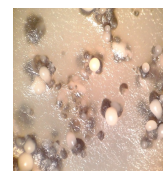


Figure 3

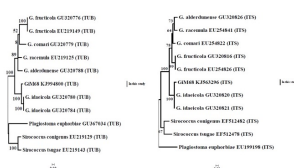


Figure 4

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