New Disease Reports

First report of *Clonostachys rosea* causing root rot of *Beta vulgaris* in North Dakota, USA

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In August 2018, sugar beet plants with dull green and chlorotic foliage were observed in Hickson (46.6694°N, 96.8104°W), North Dakota. The taproots were found to have several circular brown to black necrotic lesions (Fig. 1) and the disease incidence was about 5%.

Beet roots were washed to remove soil particles, surface-sterilised in a 10% NaOCl solution for 1 minute, and dipped twice in sterile water. Isolations were done on potato dextrose agar (72 hr at 25 ±2°C). All colonies were white and the surface was feathery (Fig. 2). Ten isolates were examined and these consistently had verticillate and penicillate conidiophores (primary and secondary) similar to those illustrated by Afshari & Hemmati (2017). Conidia were 6.2 to 9.5 µm × 5.9 to 8.9 µm (Fig. 3). Five pure cultures were prepared by single spore isolation. The morphology of isolates was consistent with *Clonostachys rosea* (Moreira *et al*., 2016; Sun *et al*., 2020). DNA was extracted from four isolates using a Norgen Biotek Corp. protocol (Canada, Cat.27300). Isolates were confirmed via sequencing (GenScript, Piscataway, USA) using the internal transcribed spacer (ITS1F/ITS4). A BLAST search demonstrated that the 539 bp sequence was 100% identical to *C. rosea* (GenBank Accession No. KM519669.1). An annotated DNA sequence was deposited into GenBank as MN186772.1.

Greenhouse pathogenicity tests were undertaken on sugar beet using the sequenced isolate. Three-week-old *C. rosea* cultures were mixed with vermiculite and perlite mixer (PRO-MIX FLX, USA) in plastic trays (61 × 38 × 25 cm). For the control treatment no inoculum was added. Sterile water (500 ml/ tray) was added to the mixer to maintain sufficient moisture. Ten seeds of sugar beet cv. Crystal 101 were sown per tray, and the trays replicated thrice with inoculated and control treatments and maintained at 22°C, 75% relative humidity. Plants were watered as needed to maintain adequate soil moisture conducive for plant growth and disease development. After eight weeks, plants were harvested and root rot assessed. Taproots of 16 of the 30 inoculated plants had similar root rot symptoms as described previously (Fig. 4). No disease was observed in control plants. *Clonostachys rosea* was consistently reisolated from the diseased taproots and its identity confirmed using morphological and molecular methods, thus fulfilling Koch's postulates.

Clonostachys rosea has been reported commonly as a mycoparasite or saprotrophic species from soil and various plant materials (Schroers *et al*., 1999). However, there are a few reports of *C. rosea* causing root rots in soybean in Minnesota (Bienapfl *et al*., 2012) and in faba bean in Iran (Afshari & Hemmati, 2017). To our best knowledge, this is the first report of *C. rosea* causing root rot of sugar beet in the USA, or worldwide.

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Figure 1

Figure 2

Figure 3

Figure 4

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