June 24

Technology of the month – June 2024

A new and effective non-contact soil application method for basal infection by Sclerotinia sclerotiorum

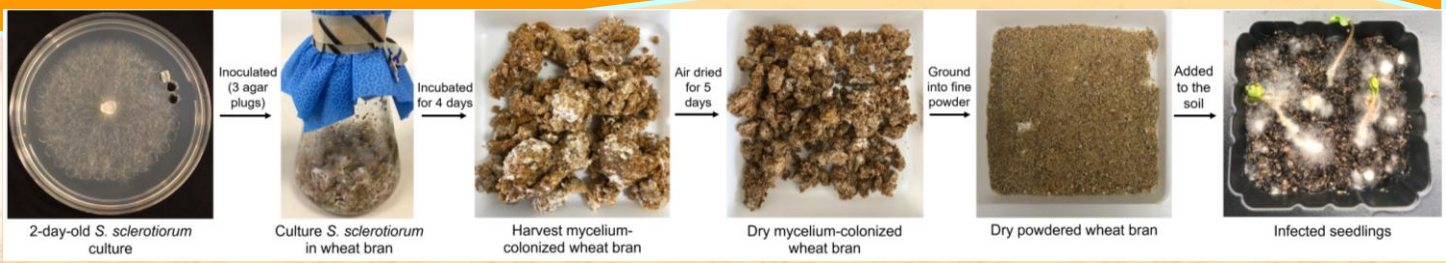

Fig. 1. *Dry ground inoculum carriers (using wheat bran as an example) prepared from Sclerotinia sclerotiorum incorporated into soil for basal infection*

Background

Sclerotinia sclerotiorum is a widespread and economically devastating soil-borne fungal pathogen known to infect and cause disease in a range of plants. This pathogen produces long-term inoculum called sclerotia that can either germinate carpogenically by ascospores infecting above-ground plant parts or myceliogenically to infect stem base and roots. Standard inoculation methods that aim to reproduce basal infection for research purposes either rely on direct contact between the pathogen and host, using *S. sclerotiorum* mycelium agar plugs wrapped around the stem or sclerotia placed directly beneath root mass. Here, we describe our newly developed non-contact method that enables consistent infection of plant basal stem with *S. sclerotiorum* through soil inoculation.

Recommendation

This non-contact method can be used for:

i. testing effectiveness of soil application, fumigation of fungicides or biocontrol agents against basal infection by *S. sclerotiorum*;
ii. testing disease-suppressive activity of soils to basal infection of *S. sclerotiorum*, thought to be conferred by soil microbiome;
iii. measuring disease progression;
iv. screening of basal stem infection in disease-resistant host genotypes.

Method

Substrate preparation: We used 4 organic substrates as carriers of *S. sclerotiorum* mycelia; wheat grain, millet, wheat bran, and red rice. Equal quantities of each organic substrate by volume (~100 mL) were placed in individual 250 mL conical flasks, following an addition of 20 ml distilled water or half-strength potato dextrose broth before being autoclaved.

Inoculum: Agar cultures of *S. sclerotiorum* (isolates CU11.19 or CU8.24) were prepared by bisecting a single *S. sclerotiorum* sclerotium and incubating on potato dextrose agar (PDA). Three agar plugs taken from the margin of 2-day-old *S. sclerotiorum* colonies grown on PDA were inoculated into each flask. After 4 days of incubation at 20°C in darkness, the inoculum was extracted from flasks prior to sclerotia formation and air-dried in a laminar flow at room temperature (~20°C) for 3–5 days, then ground for 10 seconds using a blender to obtain dry ground inoculum. A mixture of dry ground inoculum and potting mix or field soils (1:10, w/w) was prepared. Reduced quantities (1:20 or 1:30, w/w) of the dry inoculum slow down the infection process.

Host plants and inoculation: Seedlings of canola, lupin, and lettuce were grown in a mini greenhouse with a tray containing 24 pots. Two application methods were used: (1) four 4-day-old seedlings were transplanted equidistantly to a new pot that was previously filled with a mixture of inoculum and soil as described above; (2) inoculum was applied directly to a pot with 4-day-old seedlings. In method (2), a mixture of dry inoculum and soil was added as a top layer in pots with seedlings. Method (2) is an optimised protocol based on the first method, which requires less inoculum/soil mixture. Distilled water was sprayed to moisten the soil surface in both approaches, and high humidity (>90% RH) was maintained. All mini greenhouses were placed in a plant growth chamber at 20°C and 12-hr light/ dark photoperiod.

Fig. 2. Four-day-old seedlings were grown or transplanted equidistantly in each pot

Canola Lettuce Lupin

Further Reading: (1) Han, V.C., Michael, P.J., Crockett, R., Swift, B., & Bennett, S.J., 2024. Plant Dis. (in press, https://doi.org/10.1094/pdis-11-23-2412-sc); (2) Han, V.C., Michael, P.J., Swift, B., & Bennett, S.J., 2023. Biol. Control 186:105346. https://doi.org/10.1016/j.biocontrol.2023.105346; (3) Underwood, W., Gilley, M., Misar, C.G., Gulya, T.J., Seiler, G.J., & Markell, S.G. 2022. Plant Dis. 106:1366-1373. https://doi.org/10.1094/pdis-06-21-1314-re.

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