

Appendix 3A: Field Inoculum Production

Detailed instructions for inoculum production were obtained from the soil-borne diseases section of the LRC with technical assistance provided by Cassandra Malligan and Damian Herde.

Subculture of isolates

A hyphal agar plug of each *Fusarium pseudograminearum* isolate was placed into the centre of CZA₁₀₊ (Appendix 2B) plates. Two plates were prepared for each isolate and incubated at 25°C for 7 days.

Grain Preparation

300 mL of millet grain was placed in 1L flasks using a funnel. 1L of deionised water was put into each flask to cover grain. Flasks were stoppered and shaken well and placed in the coldroom overnight. The following morning the stoppers were removed and the bulk of the water poured off. A piece of muslin was secured over the top of each flask with a rubber band and flasks were turned upside down to continue draining in the sink. Flasks were then righted and restoppered, once the grain was shaken to the bottom. Flasks were autoclaved using a grain cycle which ensures grain remains above 70°C for 8 mins. Flasks were removed immediately to prevent caramelisation of grain, cooled and left in a cold room overnight.

Inoculation

The following morning flasks were autoclaved for a further 20 mins on a general sterilisation cycle and placed inside a laminar flow unit to cool. To inoculate, ½ a plate of mycelium was scraped from the agar and dropped onto grain. Each flask was shaken to distribute inoculum around the grain and incubated at 23°C.

Fungal Growth

After 7 days incubation the clumps formed in each flask were broken by a combination of shaking and tapping. This procedure was repeated daily for 11 days.

Grain Drying and Grinding

Each flask was emptied into an individual tray lined with blotting paper and covered with a second sheet of paper. Only one flask of each isolate was selected based on the extent of visual colonisation.

Trays were placed in a growth cabinet in the dark at 25°C. After 4 days clumps in grain were broken up with gloved hands and each tray was shaken every 2nd day thereafter for seventeen days. Inoculated grain was ground in a mill at LRC using a 2mm plate. The mill was cleaned with an air gun between isolates and major components sprayed with 70 % Ethanol. Each isolate was stored in plastic bags in a cold room until required.