

Appendix III

Protocol for Testing Cereal Seed for *Fusarium graminearum* Using the Whole Seed Testing Method

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The following is a protocol of the Mycology lab of the Grain Research Laboratory. The authors recommend that one also know the regional source of the seed to be tested. This knowledge can be used to assess the likelihood of finding infected seeds in a seed lot. The farther west the source of the seed, the less likely one will find detectable levels of *Fusarium graminearum*.

Obtain a representative sample of the lot to be tested. A subsample of 250g to 500g is sufficient.

1. Store the subsample at -15°C.
2. Mix the subsample then remove a random selection of 200+ seeds.
3. Examine the remainder of the subsample and select those seeds with a shriveled, chalky white appearance (applicable to wheat only).
4. Surface disinfect the random and selected seeds in steps 3 and 4 by soaking them in a solution of 25ml Javex bleach and 500 ml H₂O. Swirl the seeds in the solution for 1 minute, pour off the liquid through a strainer, and place the seeds onto filter paper in an open petri dish. Dry the seeds using a laminar flow hood. (Note: Failure to completely dry the seeds can result in bacterial problems.)
5. Place 200 randomly selected seeds plus the selected seeds onto Potato Dextrose agar (Difco) in 100mm X 15mm plastic petri plates, Plate a maximum of 10 seeds per plate. (Note: Agar plates should have been poured 2 to 3 days earlier and allowed to dry before being used. Using wet plates can cause bacterial problems. One should get about 60 plates per liter of agar, with each plate having an agar thickness of about 4mm. Avoid using plates that are too thick or thin.)
6. Incubate the plates at 23°C for 5 days. Plates are exposed to a daily cycle of 12 hrs of light, consisting of 1 long wave UV light and 4 fluorescent lights 50cm above the plates and 12hrs of darkness.
7. Examine the cultures with a dissecting microscope. Make slides of the spores and examine under the compound microscope. *Fusarium graminearum* should sporulate under the above conditions. The most diagnostic spores will be found in the aerial mycelium around the seed (if there are any spores there) or in wet sporodochia (occasionally formed by this species). Dry sporodochia are more typically formed than wet sporodochia on the agar around the seed. However, diagnostic spores are infrequent in the dry sporodochia. Both wet and dry sporodochia formed by *Fusarium graminearum* are exclusively or predominantly red. On rare occasions, one can find perithecia forming on the seed or on the agar after 5 days. Slashing the culture and returning it to the incubation chamber for another few days often results in perithecial production.