

## Appendix IV

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

The procedures described below were adapted from those of the Canadian Seed institute (CSI), the Canadian Food Inspection Agency (CFIA) or the United States Department of Agriculture Grain Inspection, Packers and Stockyards Administration (GIPSA).

1. Definitions (Anonymous 2000)
  - 1.1. Primary Sample: A small portion taken from one point in the lot. Each primary sample is obtained by passing the sampling equipment through the seed stream once (including hand grab samples).
  - 1.2. Composite Sample: A sample obtained by combining and mixing all of the primary samples taken from a seed lot.
  - 1.3. Retained Sample: A sample taken from a lot of seed and held for a certain time period by the BSF as backup in case additional testing is required.
  - 1.4. Stream Sample: A sample obtained by moving a container through the entire cross-section of a moving flow of seed.
2. Sampling from carriers
  - 2.1. Equipment (Anonymous 1995)
    - 2.1.1. Probe. Probes are constructed of brass or aluminum and come in various sizes, with standard lengths of 5, 6, 8, 10, and 12 feet. The depth of the carrier or container dictates the length of probe that is used to draw the sample. Probes consist of two tubes, one inside the other. The inner tube is divided into compartments. Depending on its length, a probe may have 11, 12, 16, or 20 compartments. The outer tube has slots that match the compartment openings of the inner tube. When the slots in the tubes are aligned, grain can enter into and be emptied from the compartments.

Carriers	Probe Lengths	Compartments
Hopper Cars	10- or 12-foot	20 compartments
Boxcars	6-foot	12 compartments
Trucks	5- or 6-foot	11 or 12 compartments
Hopper-Bottom Trucks	6-, 8-, or 10-foot	12, 16, or 20 compartments

- 2.1.2. Sampling Canvas or Cloth. Sampling canvases, which are usually made of flat duck cloth or similar material, must be longer than the probe used to draw the sample. This "extra length" is needed so that the grain from the entire length of each probe may be placed on the canvas and examined without being spilled. Always keep sampling canvases clean, dry and free of holes. Half sections of pipe or troughs (e.g., rain gutters) may be used instead of sampling canvases. Troughs must be longer than the probe used to draw the sample.

Other Containers - Use grain probes that will reach the bottom of the container.

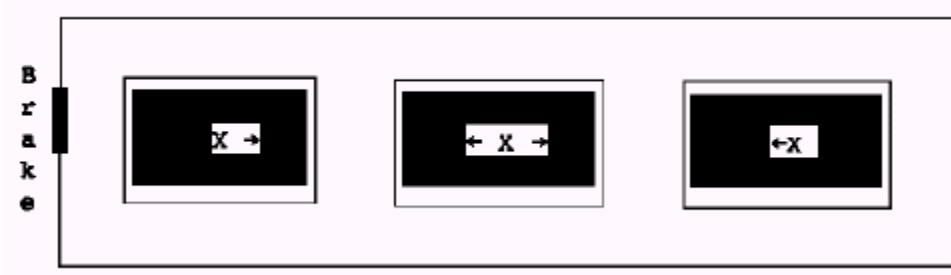
## 2.2. General Procedures

- 2.2.1. Drawing the Primary Sample (Anonymous 1995). Prior to sampling, record the carrier's identification number on the sample label. Next, spread the canvas on a level surface. Make sure the probe and canvas are clean and dry.
- 2.2.2. Sample size. In accordance with CSI procedures (Anonymous 2000), a retained sample of at least 2.0 kg should be retained for appeal and retesting. Since the *Fusarium graminearum* whole seed testing method protocol calls for a 250-500g sample, the composite sample should weigh at least 2.25 kg. The working component used for *Fusarium graminearum* determinations should then be divided from the composite sample using one of the CSI-approved dividing methods: coffee can, mechanical divider, seed pan or quarter down (Anonymous 2000). The size of a seed lot (e.g. truck vs. railcar) has an insignificant impact on the required composite sample size, since sample size will always be very small in relation to the size of the lot. Consequently, a 2.5 kg composite sample can be collected from all carrier or storage types.
- 2.2.3. For each type of carrier, there is an established sampling pattern (see section 2.3). Probe the carrier in the areas identified for the particular type of carrier. There are many techniques for using a probe. Regardless of which technique is used, follow these general rules to obtain a representative sample:
- Insert the probe at a 10-degree angle from the vertical, with the slots facing upward and completely closed. Keep the slots closed until the probe is inserted as deeply as possible into the grain. If the slots are not kept closed, a disproportionate amount of grain from the top of the lot will fall into the probe's compartments as it is being inserted.
  - If the grain contains sand or grit, it is permissible to insert the probe with the slots facing downward to avoid "freezing" the probe. After the probe is inserted, turn the slots upward before opening.
  - After the probe is fully inserted (with the slots facing upward), open the slots and move the probe up-and-down in two quick, short motions. When sampling grains, such as oats and barley, additional up-and-down movements may be necessary to fill the probe.
  - Close the slots completely. Then, grasp the probe by the outer tube and withdraw it from the grain. Do not pull the probe by the wooden handle. This can cause the inner tube to be pulled out of the outer tube. When this occurs, the probe must be emptied, reassembled, and the area reprobated.
  - Empty the probe on the canvas or trough and transfer to the sample bag, taking care not to spill any portion of the sample or allow fine material to be blown away.
  - After placing the sample and completed sample ticket into the sample bag,
  - Tighten the drawstrings at the top of the bag so that it is closed securely.
  - Carefully remove the bag from the carrier so that none of the sample is lost or spilled. Do not throw or drop the sample to the ground.

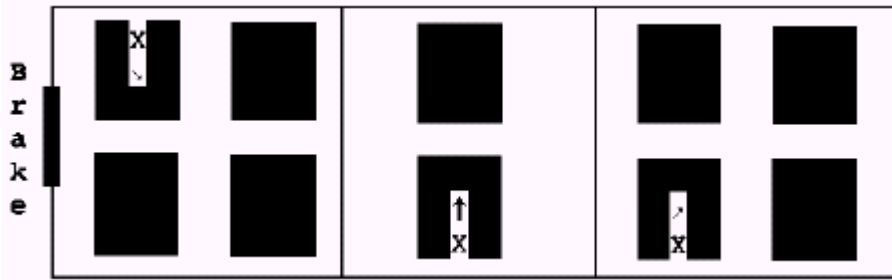
2.3. Sampling patterns (Anonymous 1995). The following diagrams show the standard sampling patterns. Insert the probe at the points marked (X), with the tip of the probe pointed toward the direction of the arrowhead. When two arrowheads are shown, the tip of the probe may be pointed in either direction.

2.3.1. Sampling Patterns for Hopper Cars.

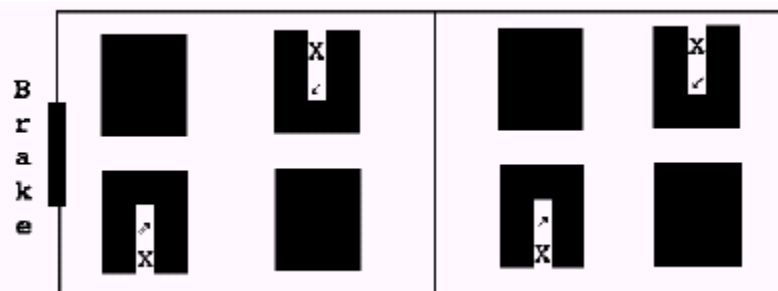
- 3-Compartment, Trough or Door Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10 degree angle, the probe may be inserted either in the center of each hopper or slightly off center in order to miss the crossbeams



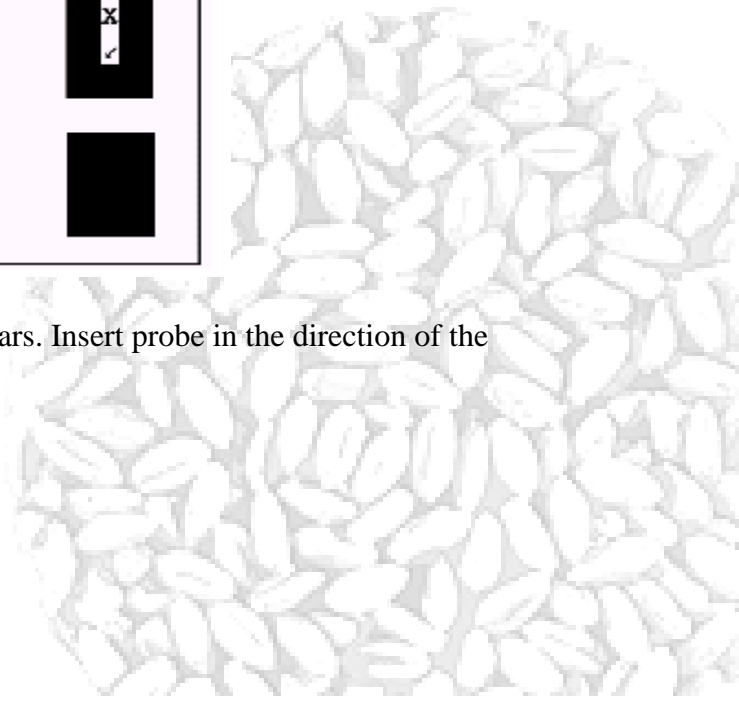
- 3-Compartment, 10-Hatch Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.

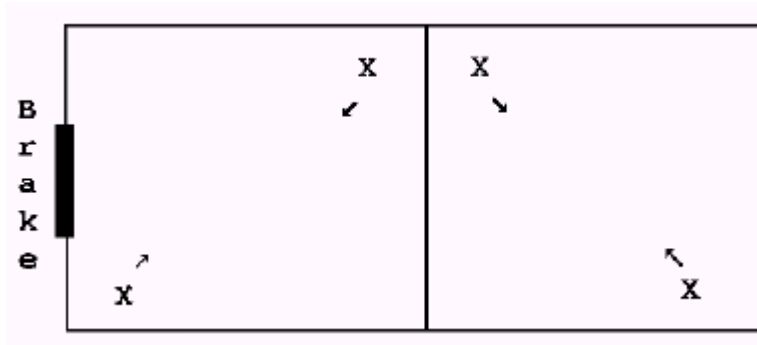


- 2-Compartment, 8-Hatch Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.

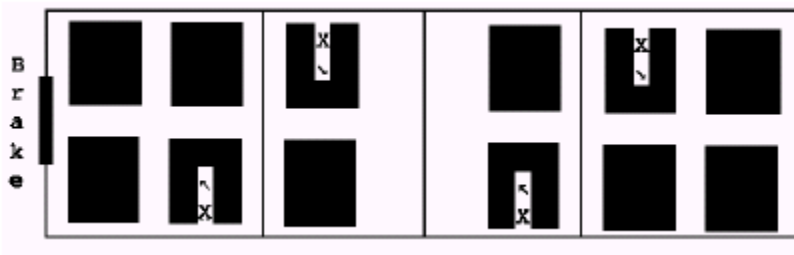


- 2-Compartment, Open Top Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.

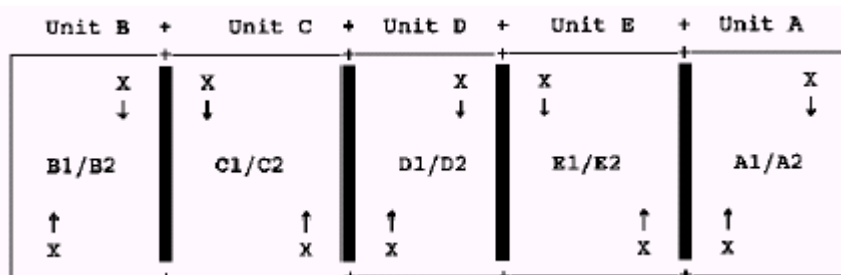




- 4-Compartment, 12-Hatch Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle.

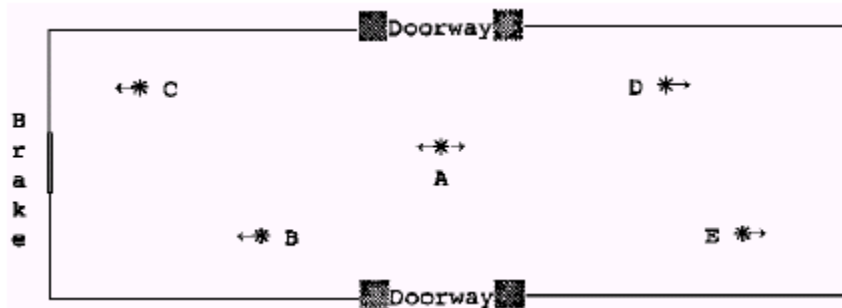


- Articulated Type Hopper Cars. Insert probe in the direction of the arrow at an approximately 10-degree angle. Articulated type hopper cars (e.g. "Super Hoppers") are easily recognized because of their configuration. The cars design permanently mounts five, two-hatch type hopper cars onto six sets of wheels. The five car units carry the equivalent of three jumbo hopper cars. Since articulated hopper cars are unique in design, samplers should also be aware that their identification system is different from that of standard hopper cars. The manufacturer often labels articulated hopper car units. If the units are labelled, use this information for identification. If they are not labeled, identify one end unit of the car as the A unit and identify the other end unit as the B unit. Then, identify the three middle units as C, D, and E, going from unit B to unit A. Each unit has two compartments or hoppers. The B-end compartment within a unit is identified as 1 and the A-end unit within the same compartment is identified as 2.



- Other Types of Hopper Cars. When sampling other types of hopper cars, use the sampling pattern that will provide the most representative sample.

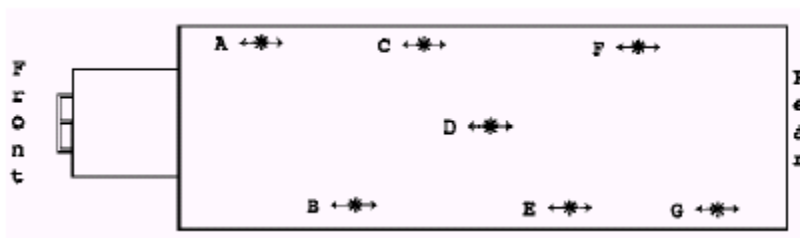
- Sampling Pattern for Box Cars. Insert the probe at an approximately 10-degree angle in the direction of the arrows shown in the diagram. The probe pattern shown may also be used in reverse of the one shown.



- Site A - Draw a sample from the center of the car. The probe may be taken with the slots facing toward either end of the car.
- Site B - Draw a sample approximately 3 - 5 feet back from the doorpost and approximately 2 - 4 feet out from the side of car. The slots in the probe should face toward the end of the car.
- Site C - Draw a sample approximately 3 - 5 feet from the same end of the car and approximately 2 - 4 feet from the opposite side of the car from site B. The slots in the probe face toward the end of the car.
- Site D - Draw a sample approximately 3 - 5 feet back from the doorpost and approximately 2 - 4 feet out from the side of car opposite of site B. The slots in the probe face toward the end of the car.
- Site E - Draw a sample approximately 3 - 5 feet from the same end of the car and approximately 2 - 4 feet from the opposite side of the car from site D. The slots in the probe face toward the end of the car.

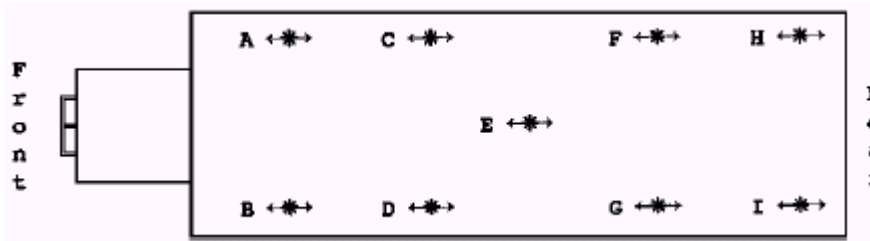
2.3.2. Sampling Patterns for trucks. Insert the probe at an approximately 10-degree angle in the direction of the arrows shown in the diagram. The probe pattern shown may also be used in reverse of the one shown.

- Flat-Bottom Trucks or Trailers Containing Grain More than 4-Feet Deep or 8 Filled Probe Compartments.

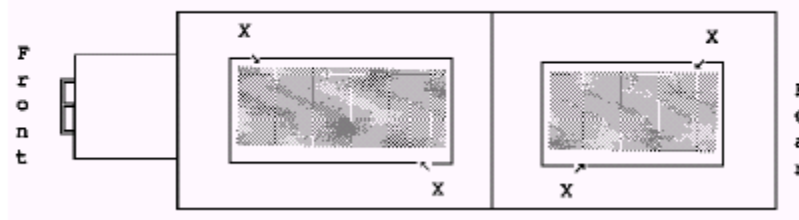
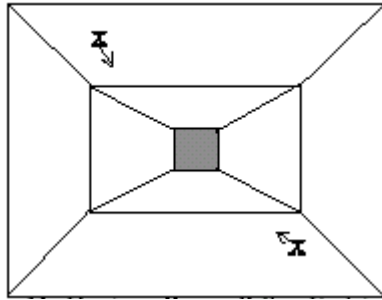


- Site A - Draw a sample approximately 2 feet from the front and side.
- Site B - Draw a sample from the opposite side of site A, approximately halfway between the front and center of the carrier, and approximately 2 feet from the side.
- Site C - Draw a sample from the same side as site A, approximately: (three-fourths) of the distance between the front and center of the truck and approximately 2 feet from the side.

- Site D - Draw a sample from the center of the carrier.
  - Site E - Draw a sample from the side opposite site C, approximately: (three-fourths) of the distance between the rear and center, approximately 2 feet from the side.
  - Site F - Draw a sample from the side opposite site E, approximately one-half the distance between the rear and center, approximately 2 feet from the side.
  - Site G - Draw a sample from the same side as site E, approximately 2 feet from the rear and side of the carrier.
- Flat-Bottom Trucks or Trailers Containing Grain Less than 4 Feet Deep or Fewer than 8 Filled Probe Compartments.



- Site A - Draw a sample approximately 2 feet from the front and side.
  - Site B - Draw a sample from the opposite side of site A, approximately 2 feet from the side.
  - Site C - Draw a sample from the same side as site A, approximately: (three-fourths) of the distance between the front and center of the truck and approximately 2 feet from the side.
  - Site D - Draw a sample from the same side as site B, and opposite of site C, approximately: (three-fourths) of the distance between the front and center, approximately 2 feet from the side.
  - Site E - Draw a sample from the center.
  - Site F - Draw a sample from the same side as site C, approximately: (three-fourths) of the distance between the center and rear of the truck and approximately 2 feet from the side.
  - Site G - Draw a sample from the same side as site D, approximately: (three-fourths) of the distance between the center and rear of the truck and approximately 2 feet from the side.
  - Site H - Draw a sample from the same side as site F, approximately 2 feet from the rear and side of the carrier.
  - Site I - Draw a sample from the same side as site G, approximately 2 feet from the rear and side of the carrier.
- Sampling Pattern for Hopper-Bottom Containers, Trucks, and Trailers. Insert the probe at an approximately 10-degree angle in the direction of the arrows shown in the diagram.



### 3. Grain stored in bins (Anonymous 2000)

A minimum composite sample size of at 2.5 kg is required. It is recommended that composite be taken from the stream of a seed lot whenever possible, as opposed to using a bin probe to obtain a sample. Stream samples may be taken as the seed is transferred into the storage bin or as it is being transferred from bin to bin. To obtain a stream sample, the sample container may be moved through the entire seed stream either by hand or automatically.

#### 3.1. Stream samples

3.1.1. Manual sampling. An appropriate container such as a grain scoop, pan stream handler or sample tray may be used. It is run through the entire cross-section of the seed stream so that the sample is uniform, and the process is carried out at least seven times for a lot of seed.

3.1.2. Automatic sampler. This type of sampler is frequently mounted at the discharge of a receiving leg. It allows for the interval of sampling and sample size to be regulated much more precisely than when sampling by hand. The sampler is also placed in a position where there is no de-mixing of seed.

### 4. Drop samples(Anonymous 1999)

A tray (e.g. a metal pan large enough to collect a 1 kg sample and that will not break under pressure), small plastic bags (with approximately 2.5 kg capacity and measuring approximately 43 cm long by 13 cm wide by 0.15 cm thick) and big bags to transport many of the samples enclosed in the small bags will be required. All equipment shall be clean, i.e., free of products and organisms previously sampled. For each bin selected, do as follows.

- Locate the bottom valve (or try hole) of the bin.
- Ensure that the respective belt is locked before sampling.
- If sampling from the valve, position the tray on the belt under the spout, open the valve, let the bin content flow onto the tray, and, when at least 2.5 kg is on the tray, close the valve.

- If sampling from the try hole, position the tray against the try hole, open the valve, let the bin content flow onto the tray and, when at least 2.5 kg is on the tray, close the valve.
- Put at least 2.5 kg of the bin content in a plastic bag. Close and label the bag.

#### 4.3 Top Samples (Anonymous 1999)

A cylindrical sampler (divided bulk probe) (approximately 1 to 3 m long by 4 cm in diameter, with about 3 slots of each approximately 8 cm long by 2 cm large) or an electrically operated suction sampler\*, small plastic bags and big bags to transport many of the samples enclosed in the small bags are required. All equipment shall be clean, i.e. free of products and organisms previously sampled. Locate the bin top. Have the top cover of the bin removed, and probe the bin content. The sampling pattern can be adapted to the most relevant carrier sampling pattern (2.3). Put at least 2.5 kilogram of the bin content in a plastic bag. Close and label the bag.

\* The Probovac probe draws a grain sample from the bin through suction in a double walled pipe; this unit is effective to a depth of about 40 feet, and a representative sample can be obtained from the center of the grain mass.

#### Reference List

1. Anonymous. 1995. *Grain Inspection Handbook, Book 1*. Washington, D.C.: United States Department of Agriculture.
2. \_\_\_\_\_. 1999. *Procedure PI-002: Sampling Grains and Field Crops, their Residues, and Associated Small Organisms*. Nepean, ON: Plant Products Directorate, Plant Health and Products Division, Canadian Food Inspection Agency.
3. \_\_\_\_\_. 2000. *CSI Technical Manual For Bulk Storage Facilities*. Ottawa, ON: Canadian Seed Institute.

