

## EXTERNAL FILTH AND INTERNAL INSECT INFESTATION IN WHOLE CORN

### PRINCIPLE

Whole corn is suspended in aqueous borax solution to float insects and insect fragments, which are collected on filter paper for microscopic identification and counting. The remaining rinsed corn is next comminuted in the presence of aqueous hydrochloric acid in a blender, mineral oil is added to the slurry, which is then cooked or autoclaved to allow separation of internal insects or insect fragments at the interface of the 2-phase system upon standing and cooling. The aqueous phase is gradually replaced with clean water until the insects and insect fragments can be filtered in the absence of other suspended solids. The filter residue is also evaluated microscopically and total infestation is reported as the sum total of insect and insect fragments recovered in the two steps.

### SCOPE

This method applies to whole corn. Whole insects and insect fragments are counted regardless of size.

### SPECIAL APPARATUS

1. Blender: Osterizer, 10-speed, or equivalent
2. Separatory Funnel: Kilborn or equivalent (see figure 1)
3. Wrist-Action Shaker

### REAGENTS

1. Borax Solution: 10% in water
2. HCl Solution: HCl/water (7:93 by volume)
3. Mineral Oil: paraffin oil, white, light, 125/135 Saybolt Universal
4. Alcohol Solution: 95% isopropyl alcohol/water (60:40 by volume)

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5. Glycerol/Alcohol Solution: 50:50 by volume

**PROCEDURE**

Weigh 50 g whole corn into 500 mL Erlenmeyer flask. Add 250 mL borax solution on wrist-action shaker at medium speed and mix for 10 mins. Decant liquid through filter paper (S&S No. 8, ruled) on Büchner funnel. Rinse remaining corn in flask with alcohol solution and filter washings through same filter paper. Transfer filter paper to Petri dish, apply glycerol/alcohol solution and examine microscopically (Note 1). Count all insect fragments and whole insects.

Transfer rinsed corn left in Erlenmeyer flask to pint blender jar, add about 150 mL HCl solution and blend at high speed for 1 minute. Transfer blender contents quantitatively to 1.5 L beaker. Rinse blender head and jar with HCl solution and combine rinsings in beaker. Add 30 mL mineral oil to beaker. Antifoam agent may be added if necessary.

Place beaker on preheated hot plate, bring to rolling boil, and hold at boiling for about 15 mins., or digest in autoclave (Note 2). Transfer beaker contents quantitatively to Kilborn funnel by rinsing beaker and stirring rod with 60% alcohol solution. Add rinsings to Kilborn funnel. Fill funnel to about 2.5 cm from top with cold water and let stand 5 mins. Slowly drain and discard lower layer until interface is about 5 cm above constriction (Note 3). Rinse sides of funnel with 60% alcohol solution, then fill funnel with about 10 cm alcohol solution; let oil layer separate thoroughly and drain slowly. Discard lower layer until interface is about 5 cm above constriction. Rinse sides of funnel with alcohol solution. Let oil layer separate and drain slowly, discarding lower layer. Repeat this step, if necessary, to obtain clear lower layer.

Remove clamp and empty funnel into 250 mL beaker. Thoroughly rinse funnel with alcohol solution, collecting rinsings into the 250 mL beaker. Filter mineral oil and rinsings through ruled filter paper using Büchner funnel. Rinse beaker thoroughly with alcohol solution onto same paper. Transfer paper to Petri dish and add glycerol/alcohol solution. Examine microscopically and count whole insects, cast skins and head capsules of primary infestation insects.

**CALCULATION**

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Contamination of 50 g sample of corn shall be total of both counts.

### NOTES AND PRECAUTIONS

1. Make microscopic examinations at 30× using widefield stereoscopic microscope on properly cleared paper. Filth or infestation may be made to stand out in contrast to white background of filter paper that has been properly cleared. Paper may be cleared by wetting with enough glycerin/alcohol solution to fill fibers but not enough to cause extracted material to float.  
  
The use of 30× should not preclude use of higher magnification, especially on suspect or doubtful matter. At least twice magnification used in original examination is necessary to show new details. Exercise care to ensure "turning over" of all pieces of material, such as bran, which might obscure filth elements. If suspicion still remains, mount piece, clear thoroughly, and examine under compound scope. A thorough understanding of appearance of authentic materials is assumed.
2. Add 50 mL light mineral oil, cover beaker with watchglass, and autoclave at 121 °C for 15 mins. Remove from autoclave and stir with magnetic stirrer, building up stirring speed until vortex is formed without visible splashing. Stir 5 mins.
3. Operation of separatory funnel: Cool digested material to 40 °C or less. Place about 25 mL light hydrocarbon (heptane) in separatory funnel and transfer digested material to it quantitatively. Let stand about 30 mins. and draw off lower portion until upper layer is within 1½ to 2 inches from tip. Wash sides with boiling or nearly boiling water, let stand about 15 mins., and withdraw lower portion until upper layer is within 1 to 1½ inch from tip. Wash second time if necessary; draw off and discard lower layer again and filter remainder, washing sides of funnel with boiling or nearly boiling water. Do not withdraw solution from funnel so rapidly that vortex will form at oil-water interface. This is sure to cause loss of fragments. Be careful to disturb interfacial layer as little as possible during washing.

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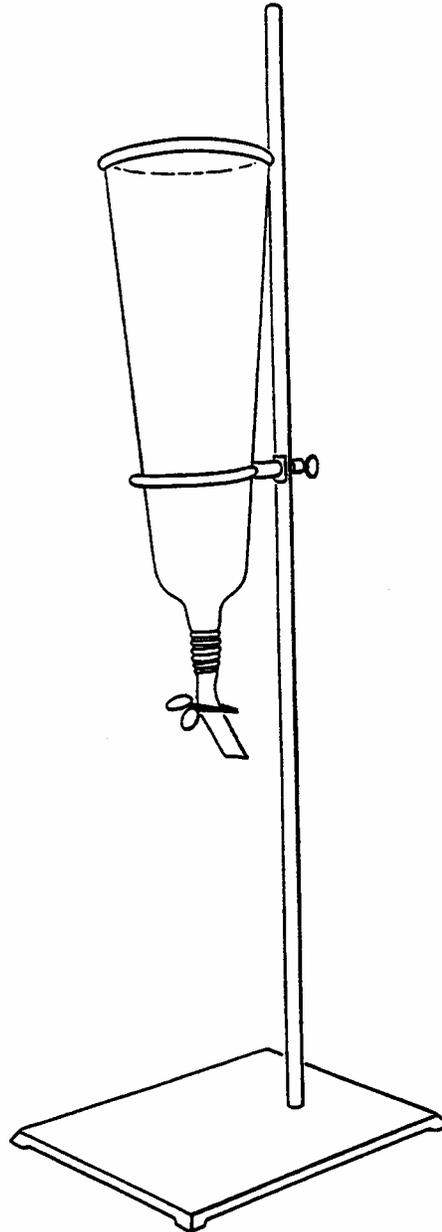
**REFERENCES**

1. *Approved Methods of the American Association of Cereal Chemists*, 9th Edition, 1995, Vol. 1, 28-03, "Special Techniques for Extraneous Matter Methods" (Oct. 26, 1994).
2. *Approved Methods of the American Association of Cereal Chemists*, 9th Edition, 1995, Vol. 1, 28-19 "External Filth and Internal Insect Infestation in Whole Corn" (Oct. 26, 1994).
2. *Official Methods of Analysis of AOAC International*, 16th Edition, (1995), Vol. 1, Ch. 16, p. 4, Method 970.66 "Light and Heavy Filth, General."

**METHOD HISTORY**

Corn, External Filth and Internal Insect Infestation in Whole Corn (A-10), Date of Acceptance 2-27-1996.

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**FIGURE 1**