

16.5.03

AOAC Official Method 985.36 Insect Infestation (Internal) of Oats

Cracking Flotation Method

First Action 1985

Final Action 1996

A. Reagent

Tween 80–Na₄EDTA [(ethylenedinitrilo)tetraacetic acid tetrasodium salt] premix solution.—Measure 420 mL 40% isopropanol in 500 mL graduate. Add 80 mL Tween 80 (polysorbate 80) to 100 mL glass-stoppered graduate. Invert 100 mL graduate over 2 L glass beaker and drain briefly. Rinse 100 mL graduate with several portions of the 420 mL 40% isopropanol, pouring each rinse into beaker. Add rest of 40% isopropanol to beaker, add magnetic stirring bar, **945.75B(n)** (see 16.1.01), and start magnetic stirring. Add 10 g Na₄EDTA to beaker while stirring rapidly. Add 500 mL 40% isopropanol and stir until uniform. Mixed reagent stored in glass-stoppered flask is stable 1 week.

B. Preparation of Test Sample

Mix grain by passing 6 times through Jones sampler, recombining separations before each pass. Separate slightly >25 g and weigh 25 g. Transfer weighed test portion, small amount at a time, to 5 (12.7 cm) or 8 in. (20 cm) No. 2 sieve, and with stiff bristle brush, work insects through sieve as completely as possible.

Grind screened test portion in cutting-type mill set at 0.061 in. (1.5 mm) (Electric coffee grinder can be used.) Particle size of cracked oats, ca 0.061 in. (1.5 mm), can be checked by passing the 25 g cracked oats through No. 14 sieve, **945.75B(r)** (see 16.1.01). Amount of cracked oats retained on sieve should range from 75 to 80% by weight and amount passing through sieve should range from 20 to 25% by weight. When using Labconco mill, rotate blade adjusting wheel counterclockwise until cutting blades are touching. Then rotate adjusting wheel clockwise $\frac{3}{4}$ revolution. This setting should give cracked oats within particle size range mentioned above. Dry damp or tempered grain in forced-draft oven 1 h at 70 –80°C or 2 h in oven without draft.

C. Isolation

Transfer cracked grain, including any residue in mill, to 2 L glass beaker containing magnetic stirring bar, **945.75B(n)** (see 16.1.01), and add 600 mL isopropanol. Cover beaker and stir gently while boiling 5 min on hot plate.

Transfer test portion to No. 100 sieve, **945.75B(r)** (see 16.1.01), with gentle stream of hot tap water. Wash material on sieve with very gentle stream of hot (55 –70°C) tap water to remove isopropanol. Quantitatively transfer material on sieve to original 2 L beaker with H₂O. Add magnetic stirring bar, **945.75B(n)** (see 16.1.01), and mixture of 600 mL H₂O + 50 mL HCl. Cover beaker and magnetically stir contents gently while boiling 15 min on hot plate. Transfer material to No. 100 sieve, **945.75B(r)** (see 16.1.01), with gentle stream of hot tap water. Wash material on sieve with very gentle stream of hot (55 –70°C) tap water until washings show no acidity when tested with blue litmus paper.

Add magnetic stirring bar, **945.75B(n)** (see 16.1.01), to 2 L trap flask, **945.75B(h)(4)** (see 16.1.01). Place wide-stem funnel in flask opening and quantitatively transfer material on sieve to flask with 40% isopropanol. Add 40% isopropanol to total volume of 800 mL.

Clamp stirring rod so stopper or wafer is above liquid in flask. (Trap flask may stand overnight at this point.) Stir gently while boiling 7 min 10 s on magnetic stirring hot plate. Remove flask from hot plate and wash down sides with minimum of 40% isopropanol and immediately add 100 mL Tween 80–Na₄EDTA solution slowly down rod. Hand-stir gently 1 min and let stand 3 min.

Add 50 mL mineral oil, **945.75C(p)** (see 16.1.01), down stirring rod. Stir magnetically, **970.66B(c)** (see 16.1.02), 5 min on cool magnetic stirrer, and let stand 3 min.

Fill flask with 40% isopropanol, added slowly down stirring rod to avoid mixing or agitation of flask contents, and let stand 20 min undisturbed. Trap off into beaker, rinsing neck of flask with 40% isopropanol, and add rinse to trapping in beaker.

Transfer trapping to ruled filter paper, rinsing beaker well with isopropanol. Add 35 mL mineral oil, **945.75C(p)** (see 16.1.01), to flask and hand-stir 1 min. Clamp stirring rod so stopper or wafer is at midpoint of flask. Let stand 5 min, spin stirring rod to free settleings from stopper or wafer, and adjust oil level with 40% isopropanol to ca 1 cm above fully raised stopper. Let stand undisturbed 15 min. Trap off into beaker, rinsing neck of flask well with isopropanol and adding rinsings to trapping in beaker. Transfer trapping to ruled filter paper, rinsing beaker well with isopropanol. Examine papers at 15 \times , counting only whole or equivalent insects and cast skins.

Reference: *JAOAC* **68**, 699(1985).