

bottle to form tetraamminecopper sulfate ($[\text{Cu}(\text{NH}_3)_4]\text{SO}_4$) solution used for this test. After mixing, a light blue precipitate of cupric hydroxide ($\text{Cu}(\text{OH})_2$) should form. If no precipitate forms, add additional CuSO_4 until a precipitate appears. Since the strength of household ammonia can vary, formation of a precipitate indicates that a complete reaction has taken place between CuSO_4 and $\text{NH}_4 \text{OH}$; otherwise fumes from excess ammonium hydroxide may cause eye irritation.

(2) Preparation of seeds: To insure imbibition, scratch, prick, or otherwise scarify the seed coats of the sweetclover seeds being tested. Soak seeds in water for 2 to 5 hours in a glass container.

(3) Chemical reaction: When seeds have imbibed, remove excess water and add enough test solution to cover the seeds. Seeds coats of yellow sweetclover will begin to stain dark brown to black; seed coats of white sweetclover will be olive or yellow-green. Make the separation within 20 minutes, since the seed coats of white sweetclover will eventually turn black also.

(4) Calculation of results: Count the number of seeds which stain dark brown or black and divide by the total number of seeds tested; multiply by the pure seed percentage for *Melilotus* spp.; the result is the percentage of yellow sweetclover in the sample. The percentage of white sweetclover is found by subtracting the percentage of yellow sweetclover from the percentage of *Melilotus* spp. pure seed.

(c) *Wheat*. In determining varietal purity, the phenol test may be used. From the pure seed sample count four replicates of 100 seeds each. Soak the seed in distilled water for 16 hours; then flush with tap water and remove the excess water from the surface of the seeds. Place two layers of filter paper in a container and moisten with a 1 percent phenol ($\text{C}_6 \text{H}_5 \text{OH}$) solution. Place the seed, palea side down, on the two layers of filter paper and cover the container. A preliminary observation may be made at 2 hours. At 4 hours, record the number of seeds in each of the following color categories:

- (1) Ivory.
- (2) Fawn.

(3) Light Brown.

(4) Brown.

(5) Brown Black.

(d) *Soybean*. In determining the varietal purity, the peroxidase test may be used. Remove and place the dry seed coat from seeds into individual test tubes or suitable containers. Add 10 drops (0.5-1.0 ml) of 0.5 percent guaiacol ($\text{C}_7 \text{H}_8 \text{O}_2$) to each test tube. After waiting 10 minutes add one drop (about 0.1 ml) of 0.1 percent hydrogen peroxide ($\text{H}_2 \text{O}_2$). One minute after adding hydrogen peroxide, record the seed coat as peroxidase positive (high peroxidase activity) indicated by a reddish-brown solution or peroxidase negative (low peroxidase activity) indicated by a colorless solution in the test tube. Various sample sizes may be used for this test. Test results shall include the sample size tested.

(e) *Oat*. In determining the varietal purity, the fluorescence test may be used. Place at least 400 seeds on a black background under a F15T8-BLB or comparable ultraviolet tube(s) in an area where light from other sources is excluded. Seeds are considered fluorescent if the lemma or palea fluoresce or appear light in color. "Partially fluorescent" seeds shall be considered fluorescent. Seeds are considered non-fluorescent if the lemma and palea do not fluoresce and appear dark in color under the ultraviolet light.

[59 FR 64514, Dec. 14, 1994]

EDITORIAL NOTE: For Federal Register citations affecting §201.58a, see the List of CFR Sections Affected, which appears in the Finding Aids section of the printed volume and on GPO Access.

§ 201.58b Origin.

The presence of incidental weed seeds, foreign matter, or any other existing circumstances shall be considered in determining the origin of seed.

[5 FR 35, Jan. 4, 1940. Redesignated at 20 FR 7940, Oct. 21, 1955]