

TABLE 2—GERMINATION REQUIREMENTS FOR INDICATED KINDS—Continued

Name of seed	Substrata	Temperature (°C)	First count days	Final count days	Additional directions	
					Specific requirements	Fresh and dormant seed
Squash	B, T, S	20–30	4	7	Keep substratum on dry side; see ¶(a)(3).	
Tomato	B, P, RB, T	20–30	5	14	Light; KNO ₃ .
Tomato, husk	P, TB	20–30	7	28	Light; KNO ₃ .	
Turnip	B, T	20–30	3	7		
Watermelon	B, T, S	20–30; 25	4	14	Keep substratum on dry side; see ¶(a)(3).	Test at 30 °C.

¹ Hard seeds may be present. (See §201.57)

² Rhizomatous derivatives of a johnsongrass sorghum cross or a johnsongrass sudangrass cross.

[20 FR 7928, Oct. 21, 1955]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting §201.58, see the List of CFR Sections Affected, which appears in the Finding Aids section of the printed volume and at www.fdsys.gov.

EXAMINATIONS IN THE ADMINISTRATION OF THE ACT

§ 201.58a Indistinguishable seeds.

When the identification of the kind, variety, or type of seed or determination that seed is hybrid is not possible by seed characteristics, identification

may be based upon the seedling, growing plant or mature plant characteristics according to such authentic information as is available.

(a) *Ryegrass*. In determining the pure seed percentage of perennial ryegrass and annual ryegrass, 400 seeds shall be grown on white filter paper and the number of fluorescent seedlings determined under ultraviolet light at the end of the germination period (see §201.58(b)(10)).

(1) Fluorescence results are to be determined as test fluorescence level (TFL) to two decimal places as follows:

$$\% \text{ TFL} = \frac{\text{Number of normal fluorescent seedlings}}{\text{Total number of normal seedlings}} \times 100$$

(2) The percentage of perennial ryegrass is calculated as follows:

$$\% \text{ Perennial ryegrass} = \frac{\% \text{ VFL (annual)} - \% \text{ TFL}}{\% \text{ VFL (annual)} - \% \text{ VFL (perennial)}} \times \% \text{ Pure ryegrass}$$

where VFL=Variety fluorescence level.

(3) Using results from the above formula, the percentage of annual ryegrass is calculated as follows:

$$\% \text{ Annual Ryegrass} = \% \text{ Pure Ryegrass} - \% \text{ Perennial Ryegrass}$$

(4) If the test fluorescence level (TFL) of a perennial ryegrass is equal to or less than the variety fluorescence level (VFL) described for the variety, all pure ryegrass is considered to be perennial ryegrass and the formula is not applied.

(5) If the test fluorescence level (TFL) of an annual ryegrass is equal to or greater than the variety fluorescence level (VFL) described for the variety, all pure ryegrass is considered to be annual ryegrass and the formula is not applied.

(6) A list of variety fluorescence level (VFL) descriptions for perennial ryegrass varieties which are more than 0 percent fluorescent and annual ryegrass varieties which are less than 100 percent fluorescent is maintained and

published by the National Grass Variety Review Board of the Association of Official Seed Certifying Agencies (AOSCA). If the variety being tested is not stated or the fluorescence level has not been described, the fluorescence level shall be considered to be 0 percent for perennial ryegrass and 100 percent for annual ryegrass. Both VFL (annual) and VFL (perennial) values must always be entered in the formula. If a perennial ryegrass variety is being tested, the VFL (annual) value is 100 percent. If an annual ryegrass variety is being tested, the VFL (perennial) value is 0 percent. For blends the fluorescence level shall be interpolated according to the portion of each variety claimed to be present.

(b) *Sweetclover*. To determine the presence of yellow sweetclover in samples of white sweetclover, at least 400 seeds shall be subjected to the chemical test as follows:

(1) Preparation of test solution: Add 3 grams of cupric sulfate (CuSO_4) to 30 ml of household ammonia ($\text{NH}_4 \text{OH}$, approximately 4.8 percent) in a stoppered 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

Agricultural Marketing Service, USDA

§ 201.58d

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.

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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 at www.fdsys.gov.

§ 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]

§ 201.58c Detection of captan, mercury, or thiram on seed.

The bioassay method may be used according to the procedure given in Association of Official Seed Analysts, Handbook No. 26, "Microbiological Assay of Fungicide-treated Seeds", May 1964.

[38 FR 12733, May 15, 1973]

§ 201.58d Fungal endophyte test.

A fungal endophyte test may be used to determine the amount of fungal endophyte (*Acremonium* spp.) in certain grasses.

(a) Method of preparation of aniline blue stain for use in testing grass seed and plant material for the presence of fungal endophyte:

(1) Prepare a 1 percent aqueous aniline blue solution by dissolving 1 gram aniline blue in 100 ml distilled water.

(2) Prepare the endophyte staining solution of one part of 1 percent aniline blue solution with 2 parts of 85 percent lactic acid (C₃H₆O₃).

(3) Use stain as-is or dilute with water if staining is too dark.

(b) Procedure for determining levels of fungal endophyte in grass seed:

(1) Take a sub-sample of seed (1 gram is sufficient) from the pure seed portion of the kind under consideration.

(2) Digest seed at room temperature for 12-16 hours in a 5 percent sodium hydroxide (NaOH) solution or other temperature/time combination resulting in adequate seed softening.

(3) Rinse thoroughly in running tap water.

(4) De-glume seeds and place on a microscope slide in a drop of endophyte staining solution. Slightly crush the seeds. Use caution to prevent carryover hyphae of fungal endophyte from one seed to another.

(5) Place coverglass on seed and apply gentle pressure.

(6) Examine with compound microscope at 100-400x magnification, scoring a seed as positive if any identifiable hyphae are present.

(7) Various sample sizes may be used for this test. Precision changes with sample size; therefore, the test results must include the sample size tested.

(c) Procedure for determining levels of fungal endophyte in seedlings from seed samples suspected to contain fungal endophyte:

(1) Select seeds at random and germinate.

(2) Examine seedlings from the sample germinated after growing for a minimum of 48 days.

(3) Remove the outermost sheath from the seedling. Tissue should have no obvious discoloration from saprophytes and should have as little chlorophyll as possible.

(4) Isolate a longitudinal section of leaf sheath approximately 3-5 mm in width.

(5) Place the section on a microscope slide with the epidermis side down.

(6) Stain immediately with the endophyte staining solution as prepared in paragraph (a) (2) and (3) of this section. Allow dye to remain at least 15 seconds but no more than one minute.

(7) Blot off the excess dye with tissue paper. Sections should remain on the slide, but may adhere to the tissue paper; if so, remove and place in proper position on the slide.

(8) Place a coverglass on the sections and flood with water.

(9) Proceed with evaluation as described in paragraph (b) (6) and (7) of this section.

[59 FR 64515, Dec. 14, 1994]