

PROPOSED RULES FOR TESTING PRE-INOCULATED LEGUME SEED

(Pre-inoculated seed is legume seed that has been treated with a culture of proper rhizobia to produce nodulation of the legume plants and efficient nitrogen fixation; such treatment having been applied in advance of seeding the treated seed.)

a) Sampling shall be done in accordance with Section 1.1 to 1.5 of the "Rules for Testing Seeds 1960" (Proc. Ass'n. Off. Seed Anal. 49(2); 1-2, 1960), and as subsequently amended.

b) The sample when drawn shall be placed in a clean container. Precautions shall be taken to prevent contamination of the sample with other rhizobia and other organisms. (A clean envelope, or paper bag is preferred container. Cloth bags present a possibility of contamination. Bottles or cans with thigh lids will prevent air movement through the container, thus endangering viability of the rhizobia).

c) The sample shall be kept as cool as possible from the time it is drawn until it reaches the seed laboratory. As soon as the sample reaches the seed laboratory building, it shall be placed under refrigeration until it can be tested. (The inspector should be furnished with an insulated carton or cooler during warm or hot weather to prevent the samples from getting too hot before they are delivered to the seed laboratory building).

d) The working sample to be used in planting shall be taken from the composite official sample without discrimination after thorough mixing in the sample container. The working sample for seeds the size of alfalfa and red clover shall consist of at least 50 seeds, which shall be planted in at least two replicates. For large seeds, such as soybeans, the working sample shall consist of at least 20 seeds, which shall be planted in a minimum of two replicates.

The seeds shall be planted and grown in a suitable nitrogen free sterile substratum, which may consist of sand, vermiculite or a combination of these.

Earthenware jars or bottomless bottles, equipped and arranged in such a way that nutrient solution and water can be added to the substratum as needed, shall be provided. The substratum and its container shall be covered, then sterilized by using steam under pressure sufficient to destroy living organisms in the substratum. If the earthenware jar one-half gallon capacity, is used, a large bore glass tube should be placed vertically in the pot and approximately $\frac{1}{2}$ inch of solution should be kept in the bottom of this tube at all times the test is in progress to insure good growing conditions. If the bottomless bottle is used, (Leonard method), it should be equipped with a screw cap with a small hole. The level of the nutrient solution, in this instance, shall be kept above the level of the bottle cap. An alternative method would be a cap containing a small bore glass tube with a wick.

e) After sterilization is completed, the substratum in the container shall be kept covered until the seeds are planted. After the seeds are planted, the cover should be replaced until the seeds germinate and seedlings start to emerge, whereupon, the covering shall be removed to permit seedling development and aeration.

f) Bryan's modified Crone's Nitrogen-free solution, or an equivalent nitrogen-free solution shall be used to moisten the substratum to the proper moisture level. Bryan's modified Crone's Nitrogen solution is made as follows:

KCl -----	10.0 gm.
CaSO ₄ -----	2.5 gm.
MgSO ₄ · 7H ₂ O -----	2.5 gm.
Ca ₃ (PO ₄) ₂ -----	2.5 gm.
FeO ₄ -----	2.5 gm.

1.5 grams of this mixture which has been ground is dissolved in 1,000 cc. of water.

g) Both positive and negative controls shall be planted at the same time the official sample is planted. A negative control shall consist of surface sterilized seed of the kind being tested. (A number of methods for surface sterilizing seed may be used, such as hydrogen peroxide, calcium hypochlorite, hot water at 60° to 65°C. for three minutes. Compounds of mercury or other heavy metals should not be used to sterilize seed in this instance, because these will leave a residue on the seed).

A positive control shall be planted consisting of a sample of surface sterilized seed to the kind being tested, which has been inoculated with an effective culture of rhizobia for the kind being tested.

h) The plants shall be grown under conditions of light, humidity, aeration, and temperature favorable to optimum growth for the kind of seed being tested. After the seeds have been planted in the test containers, they shall be placed in a greenhouse or growth chamber.

i) The minimum length of time for the tests should be not less than 4 weeks, or until such time as there is significant differences in response as between the positive and negative controls.

Evaluation of Results

Evaluation of results consists of comparing the responses of the seedlings resulting from the planting of the pre-inoculated seed sample with the seedlings in the negative and positive control samples. The following criteria shall be used: 1. General appearance -- size, vigor and color of seedlings. 2. Nodulation of the plants -- placement, color, size, and number of the nodules on the root stem of each seedling. If desired, dry weights of the plants and a nitrogen determination on the plants may be made. Plants containing 1.7 per cent or more of nitrogen usually have benefited from the treatment. --(continued)

A sample of pre-inoculated seed shall be considered satisfactory if 90% of the plants have nodules of good placement, size, and color, and if the plant shows good size, vigor, and color of seedlings. If less than 67% of the seedlings fail to show response, the sample shall be reported as unsatisfactory. Samples having 67% or more, but less than 90% responding plants shall be reported as fair.

LUCILLE BAILEY TO RETIRE

Lucille Bailey, congenial secretary in the Michigan Laboratory plans to retire on March 24. She has been in the lab for the last ten years and served other state departments since World War II.

In recent years, two major operations have been her lot. Between times a number of falls have been rather crippling. So she says she'll be glad to divide her time between her home in Lansing and Livingston, Montana, where her sister lives.

BEST OF LUCK, LUCILLE!

MICHIGAN --- WINTER WONDERLAND

Ed Johnson, Lansing

At Christmas time we sang about Frosty the Snowman. And we in Michigan have found him around ever since. January set records for continued cold -- no one dared to think about a January thaw, and February didn't do much better.

The snowman put his heart in the business and had more snow every time one turned around. The heating oil man is waxing fat and the merchant with snow shovels hasn't had time to think about seeds and spring business. All the rest of us have, though.