

Ag 20

Filed October 29, 1975
9:45 am Cary L. Poulson

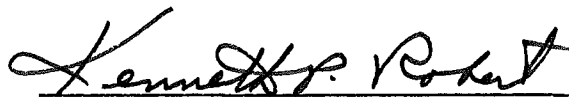
STATE OF WISCONSIN)
)SS
DEPARTMENT OF AGRICULTURE)

TO ALL TO WHOM THESE PRESENTS SHALL COME, GREETINGS:

I, Kenneth P. Robert, Administrator, Plant Industry Division, and custodian of the official records of said Division do hereby certify that the annexed order adopting rules relating to standards for preinoculated legume seed, Wis. Adm. Code Section Ag 20.12, was duly adopted by the department on October 29, 1975.

I further certify that said copy has been compared by me with the original on file in the department and that the same is a true copy thereof, and of the whole of such original.

IN WITNESS WHEREOF, I have hereunto set my hand at Madison, Wisconsin, this 29th day of October, 1975.



Administrator
Plant Industry Division

STATE OF WISCONSIN DEPARTMENT OF AGRICULTURE
ADOPTING RULES

Pursuant to authority vested in the State of Wisconsin Department of Agriculture by section 94.41(1)(j), Wis. Stats., the State of Wisconsin Department of Agriculture hereby adopts rules as follows:

Section Ag 20.12 of the Wis. Adm. Code is created to read:

Ag 20.12 Preinoculated Legume Seeds; Standards of Effectiveness. (1) Test Standards. The effectiveness of inoculum in or on legume seeds when preinoculated with rhizobial inoculum shall be determined in accordance with test methods, standards and procedures prescribed in this section.

(2) Quality Standards. (a) Rhizobial inoculum applied to small seeded legume seed shall be considered ineffective unless 90 percent of the seedlings are found to be satisfactorily nodulated under laboratory tests as prescribed under this section.

(b) A seedling to be considered satisfactorily nodulated shall show evidence of vigorous growth and green color almost equal to positive check seedlings after 21 days, and the formation of at least one pink nodule on the roots.

(c) A positive test in the acetylene-reduction method will be determined by the formation of ethylene in the test.

(3) Test Methods and Procedures. (a) Tests to determine satisfactory seedling nodulation shall be conducted as prescribed in Preinoculated Testing Handbook, revised 1974 edition, published by the Association of Official Seed Analysts, P. O. Box 1705, North Brunswick, New Jersey 08902, as Contribution No. 30 to the Handbook on Seed Testing (Growth Performance Tests for the Presence of Rhizobia on Preinoculated Seed), except as modified under subsection (b). Copies may be obtained from the Association and are also on file in offices of the department, the secretary of state and the revisor of statutes.

(b) Department modifications of the above test are: the test tubes containing media are autoclaved at 121 degrees C for 30 minutes; two seeds are placed in each tube, 35 tubes used per test; one ml of sterile distilled water is added to each tube after approximately 1/4 inch of sterile media or fine gravel is placed upon the seed; and the planted tubes are placed in a dark room or chamber where the temperature is maintained at 20 degrees C for 2 days.

(c) Acetylene-reduction tests to determine nitrogen fixation shall be conducted as prescribed under section IV(37) "Nitrogen Fixation--Assay Methods and Techniques," in Methods of Enzymology, Vol. XXIV, Photosynthesis and Nitrogen Fixation, Part B, edited by Anthony San Pietro, Department of Plant Sciences, Indiana University, Bloomington, Indiana, 1972 edition, except as modified under subsection (d). Copies may be obtained from Academic Press, Inc., 111 Fifth Avenue, New York, New York 10003, and are on file in offices of the department, the secretary of state and the revisor of statutes.

(d) Preparation of the test seedlings prior to the standard acetylene-reduction test is as follows: place the root systems of test seedlings to be analyzed in a 20 ml vial. Remove 1 ml of air, add 1 ml of 1 atmosphere acetylene and allow to react at room temperature for 24 hours. Place the root systems of nodulated control seedlings and the root systems of non-nodulated control seedlings in 20 ml vials. Remove 1 ml of air, add 1 ml of 1 atmosphere acetylene to each and allow to react for 24 hours at room temperature. Add 0.3 ml of air, withdraw 0.3 ml of gas from the test seedling vial and inject into a gas chromatograph packed with separating material that can distinguish ethylene from acetylene. Inject 0.3 ml of gas from the non-nodulated control vial into the gas chromatograph. Inject standards of ethylene and acetylene for comparison of retention times.

(e) Methods of sampling seed to determine compliance with this section shall be those specified in the Rules for Testing Seed described in Wis. Adm. Code Section Ag 20.01.

(4) Test Evaluation: (a) All seedlings produced from each test will be used for nodule evaluation.

(b) Failure in the performance of positive or negative controls will eliminate, from evaluation, those tests established on the same planting date.

(c) The department shall use the acetylene-reduction test to determine the nitrogen-fixing ability of test seedlings prior to initiating enforcement action.

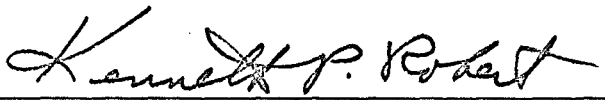
(5) Compliance. The sale or distribution of legume seed labeled or represented as having been preinoculated is prohibited

under section 94.41(1)(j) of the statutes unless the inoculum applied to such seed meets standards of effectiveness as prescribed in this section.

The rules contained herein shall take effect as provided in section 227.026, Wis. Stats.

Dated: October 29, 1975.

STATE OF WISCONSIN
DEPARTMENT OF AGRICULTURE

By 

Kenneth P. Robert, Administrator
Plant Industry Division