Revised Proposed Rule Amendment

James F. Schoen
Beltsville, Maryland

We have received approval from the Rules Committee to propose the following rule/amendments. Additions to section 6.1 provide general information about bio-assays, summarize pertinent references and include a list of six(6) recommended assay organisms. Section 6.2 is added to provide a procedure for a quantitative determination of effectiveness of a treatment by comparison with a check sample.

We should like to have as many laboratories as possible utilize the latter procedure before it is considered for adoption at the next meeting.

Revised proposed rule amendment.
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6. Detection of Seed Treatments

6.1 Detection of treated seeds by bio-assay.

a. General -- The standard laboratory bio-assay for the detection of fungicides on seeds involves placing the seeds on a substratum previously inoculated with the spores of a sensitive fungus. The spores in the substratum quickly germinate and the resulting mycelia begin to spread rapidly. If a bacterium instead of a fungus is used as the test organism, bacterial cells multiply rapidly throughout the substratum. While this growth is occurring, and independent of it, the fungicide on the treated seeds begins to diffuse. After a suitable incubation time, usually 20-48 hours, a clear zone will be seen surrounding each seed that had been treated. The clear zone is the area where the growth of the test organism has been inhibited. Generally, the greater the amount of fungicide on the seed, the larger the area of inhibition. Seed treated with various fungicides usually produce different amounts of inhibition depending on the chemical involved.

b. References -- Contribution No. 26 to the AOSA Handbook discusses the preparation of agar media, agar slants, and spore suspensions; the maintenance of stock cultures, the inoculation of the test medium and the evaluation of seed treatments. The test organisms; (3), (4) and (5) listed below; are specifically mentioned but the procedures are applicalbe to other organisms also.

A report in the AOSA Newsletter 39(3):18-19 details a test that may be performed without maintenance of cultures or preparation of agar media. Preserved spores of Aspergillus niger are added to a nutrient solution and blotters or filter papers saturated with this mixture are utilized as the test substratum. This test is slightly less sensitive than the agar tests discussed in the Handbook.

The use of a commercially available bio-assay kit is described in the Plant Disease Reporter 47(5): 374-377. Small filter paper discs impregnated with spores of Bacillus subtilis and a reducible blue dye are utilized to test small quantities of seed in bulk. If a disc is mois moistened with water the bacterial spores germinate and the disc loses color in contrast to a disc moistened with a pesticide solution washed from seeds. The latter disc retains the original blue color for a time because of inhibition of bacterial growth.

c. Procedure -- It is recommended that at least 100 seeds of each kind, except 50 seeds for bean, corn, pea, and other kinds of similar or greater size be tested against one of the organisms listed below. After the appropriate incubation period, the inhibition of bacterial or fungal growths shall be evidence that a seed treatment sub substance was present on the sample.

d. Recommended assay organisms.

(1) Aspergillus niger.
(2) Bacillus subtilis.
(3) Glomerella cingulata.
(4) myrothecium verrucaria.
(5) Sarcina lutea.
(6) stemphylium consortiale.

6.2 Determination of effectiveness of treatment.

The effectiveness of a seed treatment material containing captan, mercury or thiram present on seed lot shall be determined by a test in which the number of zones of inhibition and the total area of inhibition by seeds of a test sample is compared with the number of zones of inhibition and total area of inhibition produced by the same number of seeds of a check sample. The check sample shall consist of the same species as the test sample, have a similar average seed-size, and shall be treated at the recommended rate with the same seed treatment substance as the test sample. Check samples will be supplied by the Association of Official Seed Analysts.

The test sample shall be considered effectively treated when tested in accordance with section 6.1 and found to produce not less than 90 percent of the number of inhibition zones produced by the check sample, provided the total area of inhibition produced by the test sample is not less than 1/3 of that produced by the check sample.