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Detection of Fusarium Roseum in Nucleus Blocks

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The eradication of Fusarium roseum f. cerealis from carnation stock has been difficult. As the fungus is not found within cuttings, the usual "culturing" methods are not adequate. In spite of the careful application of sanitation measures the organism has been found repeatedly in nucleus and increase blocks at Colorado State University and in the wholesale group mother blocks. Thus some other means of carry-over has been postulated. Receiving the most attention has been the possibility that spores of the fungus might be carried over on the cuttings. Thus "culturing", which would only detect organisms in the interior tissues of the stem, and the usual sanitation practices such as steaming, would not eradicate the fungus.

In order to eradicate F. roseum f. cerealis from carnation varieties a technique for the detection of this fungus is highly desirable. Cuttings may be washed and the wash-water mounted under the microscope to determine the presence of spores; however, this is only of value when large quantities of inoculum are present. With the advent of periodic sprays, the inoculum potential has been lowered to a considerable extent. Thus spores are seldom detected by this method. A further refinement to the technique has been attempted. This consists of centrifuging the wash water, pouring off the supernatant, and examining the remaining residue. Even this method, which concentrates the material found on the cuttings, has not been dependable.

The need for a sensitive test for the presence of spores has been underscored by recent experiments. Only 1 spore in 10 cc of perlite (bottom heat 60°F) was required by a strain of F. roseum f. cerealis (frequently found in the Denver area) to produce a severe lesion during the rooting period.

The cuttings to be used in the nucleus block are derived from previous nucleus plants or from the healthiest plants obtainable. In any case they are periodically sprayed with Captan while they are still on the mother plant. They are then indexed for disease organisms (5), and only cuttings free of organisms within the stems are retained. These are dipped in Panodrench 4 solution (2) and rooted. Strict sanitation measures are practiced throughout the operation. After rooting, each cutting is placed in an individual pot in the nucleus block. The sprays and dips kill many of the spores on the cuttings. If any inoculum should escape, however, it should subsequently be found in the soil in the pot containing the nucleus plant. Thus a technique which would detect the presence of F roseum in the pot could prove to be a valuable tool.

A very simple and promising technique for the detection of certain fungi found in the soil has recently been developed by Mueller and Durrell(1). This technique

[1]Personal communication with Mr. Kenneth Mueller.

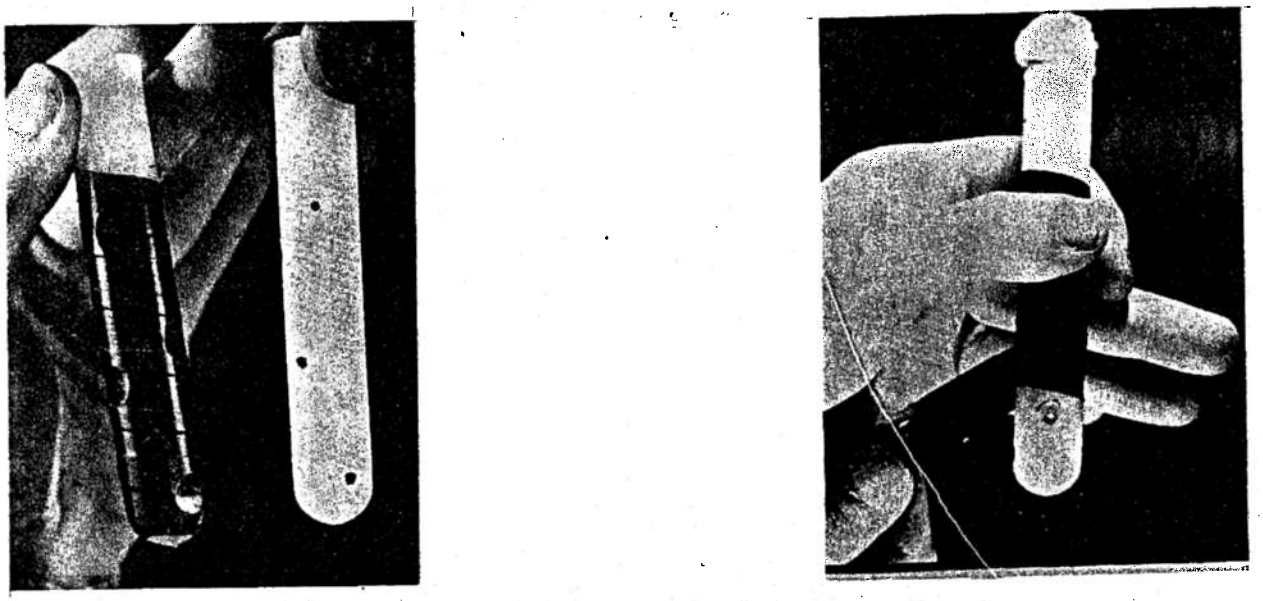

Figure I. Soil microbiological tubes. a) Tubes before and after wrapping with Koroseal electric tape. b) Tube being unwrapped after removal from the soil before transferring agar and fungus to suitable medium for identification. (Courtesy Mueller and Durrell)

with certain modification has been useful in determining the presence of F. roseum in the soil of nucleus blocks. The fungus is "trapped" out of the soil by means of a soil microbiological tube (Fig. 1). The tube is of plastic construction. Holes are bored through the wall. The tube is then wrapped spirally with Koroseal electric tape and filled with nutrient agar. Preliminary studies[1] have indicated that potato dextrose agar at pH 8 is quite effective in trapping out F. roseum. After the tube has been filled with nutrient agar, it is plugged with cotton and autoclaved. Before embedding the tube in the soil, a puncture in the tape over the opening of each hole is made with a hot needle. This provides a means of entrance for the organism. After 3-4 days the tube may be collected. The plastic tape is unwound exposing each hole in turn. A transfer needle is used to transfer the agar and fungus invader to a suitable medium for identification.

Those pots in which F. roseum is detected by this method may then be discarded.

How sensitive is this method of detection? Spore suspensions of the Fusarium stem rot pathogen were thoroughly mixed with steamed soil at the rate of 1, 10, and 100 macroconidia per 10 cc of steamed soil. Soil microbiological sampling tubes were placed in the soils at each of the inoculum densities. They were collected after 87 hours and the fungus invaders in the holes were transferred to acidified potato dextrose agar for identification. The results (Table I) indicated that the sampling tubes were able to detect F. roseum at an inoculum density of 1 spore/cc of soil.

Table I.--Number of holes[a] invaded when soil microbiological sampling tubes were embedded in steamed soils infested with various inoculum densities of F. roseum f. cerealis.

Inoculum density (spores/10 cc)	Number of holes invaded
100	19
10	18
1	1
Control	0

[a]A total of 40 holes in 5 tubes were exposed to each inoculum density.

The 1956 nucleus block at the University was sampled shortly before reindexing for 1957. Tubes were placed in 22 nucleus pots. F. roseum was isolated from 11 of these.

A measure of dependability of the soil microbiological sampling tube for the detection of the Fusarium stem rot pathogen must depend on its performance over a period of time. If it proves to be a sensitive technique for the detection of the pathogen, its simplicity should make it a popular tool in the disease-free stock program.

Literature Cited

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3. Tammen, James, R. R. Baker, and W. D. Holley. 1956. Development and production of pathogen-free propagative material of ornamental plants. V. Control of carnation diseases through the cultured-cutting technique. U. S. Dept. Agr. Pl. Dis. Rptr. Suppl. 238:72-76.