An Improved Bioassay for Pyrenochaeta terrestris in Soil

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Oregon Agricultural Experiment Station Technical Paper No. 3070.

ABSTRACT

An improved method is described for assaying soil for propagules of *Pyrenochaeta terrestris* pathogenic on onion. Soil samples are tested immediately, without drying or sieving, and the possibility of spread of infection from one test plant to another during the test period is eliminated. Quicker and more satisfactory readings of *P. terrestris* propagules can be obtained with the new method. Phytopathology 61:1299-1300.

Additional key words: onion pink root.

A method for the rapid identification of the onion pink root fungus, *Pyrenochaeta terrestris* (Hans.) Gorenz, Walker & Larson, was described by Watson (5); Hess et al. (1) used near-ultraviolet light to induce sporulation in pure culture, thereby facilitating identification of the fungus. While these procedures are of value in identifying isolates of the fungus, a method was needed for determining the level of *P. terrestris* inoculum in soil.

A bioassay method developed by Siemer & Vaughan (4) followed a procedure similar to that described by Schmitthenner & Williams (3). Soil samples from the field were air-dried and passed through a 2-mm screen, diluted with quartz sand, and planted to the pink root-susceptible onion cultivar Southport White Globe. After 6 weeks, the onions were lifted and the per cent infection determined. While the method is useful, it has two serious disadvantages. The soil samples must be air-dried and pulverized, and changes in the microflora undoubtedly occur while the soil is drying. If no propagules of *P. terrestris* are present, this is not serious, although much time can be wasted. Also, with numerous plants in a pot, it is not possible to tell whether the obvious symptoms resulted from infection of one or of many individual test plants. The modifications described here were intended to speed the process by eliminating the lengthy drying period, and to increase the precision of the assay by eliminating the possibility of spread of the fungus from one test plant to another.

MATERIALS AND METHODS.—Six weeks before soil samples were collected, 200-ml plastic cups were filled to a depth of 8 cm with pure quartz sand and planted to Southport White Globe onions. The sand was saturated with Hoagland's solution (2), and the cups were covered with paper for 6 days to retard evaporative water loss. The day length was increased to 15 hr with Gro-Lux fluorescent tubes. Greenhouse temperatures varied from 24 to 30 C. Six weeks after planting, the plants were thinned to one plant/cup.

Soil samples from the field were prepared for testing immediately, without drying. A 1:10 soil:water suspension was stirred with a magnetic stirrer to break up soil particles. Time required for thorough homogenization was about 10 min. Prior to inoculation, a small glass rod 6 mm in diam was inserted into the sand near the root system of each test plant to a depth of about 7 cm. A 10-ml aliquot of the soil:water suspension was pipetted into the depression when the glass rod was removed. Per cent of plants infected was determined 6 weeks after inoculation. Any plant with one or more pink roots was counted as infected. As a further check on the accuracy of the assay, soil from a heavily infested field was diluted 1:10, 1:100, 1:500, 1:1,000, and the percentages of infected test plants were determined.

RESULTS.—When the two assay methods were compared, the percentage of infected plants was almost always higher with the older method, indicating spread of infection from test plant to test plant, as suspected. Results obtained with the new method were much more consistent and subject to much less fluctuation (Fig. 1). The test of various soil dilutions indicated an acceptable degree of accuracy (Table 1).

DISCUSSION.—Numerous field observations have shown that pink root of onion is not severe early in the season when soil temperatures are comparatively low, but becomes progressively more severe as the season advances and the soil temperature rises. A similar progression in the percentage of test plants infected was detected by the new technique, indicating that the increase in disease severity is paralleled by an increase in the number of propagules of *P. terrestris*. Alternatively, it may indicate an increase in germinability or decrease in dormancy of existing

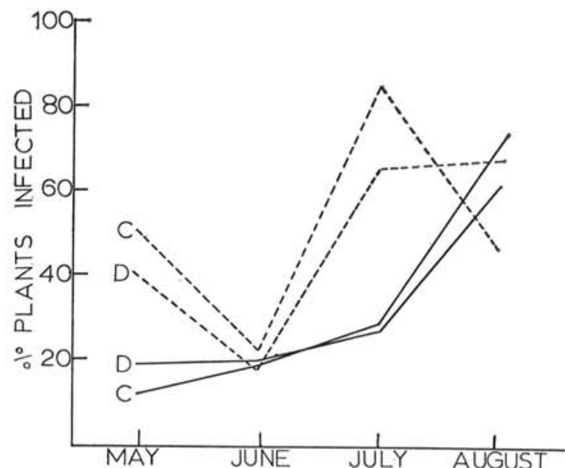

Fig. 1. Comparison of the old and new assay methods for determining the level of *Pyrenochaeta terrestris* propagules in soil. Solid line = new method; broken line = old method. Note the higher percentage of test plants infected and the fluctuations associated with the older method.

TABLE 1. Percentage of onion plants with symptoms of infection by *Pyrenochaeta terrestris*

Dilution, soil: water	% Plants infected[a]
1:10	84
1:100	68
1:500	24
1:1,000	0

[a] Readings made after 6 weeks.

propagules because of changes in the soil microflora or physiological changes within the propagules themselves. The older method, with its wide fluctuations in percentage of test plants affected, hinted at, but did not clearly indicate, such a steady change in the number of detectable propagules.

No bioassay gives a truly quantitative measurement of the number of propagules of a fungus in soil. These modifications of the technique described by Siemer & Vaughan (4) eliminate two important sources of error; the changes in relative numbers of propagules of the various components of the soil microflora during the sometimes long period of drying, and the spread of infection from one test plant to another during the test period. Our results indicate that it also gives a more satisfactory reading of the population of *P. terrestris* propagules in the soil.

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