Bioassay of Pyrenochaeta terrestris Inoculum in Soil

Sidney R. Siemer and Edward K. Vaughan

Research Assistant and Professor, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331. Present address of senior author: Amdal Company, 6507 North Colonial St., Fresno, California 97704.

Portion of a Ph.D. thesis by the senior author, Oregon State University. Oregon Agricultural Experiment Station Technical Paper No. 2863.

Accepted for publication 24 August 1970.

ABSTRACT

A method is presented for assaying soil for propagules of *Pyrenochaeta terrestris* pathogenic on onion. Disease readings were made 6 weeks after planting susceptible Southport White Globe onion in selected soil:sand dilutions. Results obtained with this bioassay method showed that infective *P. terrestris* propagules were most often associated with soil particles 0.5 to 1.0 mm in diam, and that the propagules survive to a depth of 18 inches in the soil. Large variations in inoculum level existed in the same field as well as between fields. Phytopathology 61:146-148.

Additional key words: onion pink root.

The cause of pink root disease of onions has been known since 1926 when Hansen (3) named the causal organism *Phoma terrestris* Hans. Gorenz et al. (2), after detailed morphological studies, concluded that the organism responsible for the disease was *Pyrenochaeta terrestris* (Hans.) Gorenz, J. C. Walker, & Larson, not a *Phoma* species. Since these definitive studies, knowledge of the biology of *P. terrestris* has been limited by lack of a specific method to study the occurrence of this organism in soil. Watson (10), Hess (4), and Hess et al. (5) have published methods for identification of *P. terrestris,* but these methods are not applicable to direct assay of infective inoculum. A measure of the number of *P. terrestris* propagules in soil would aid in evaluation of resistance to pink root in onion breeding lines and in determining effects of crop rotations on inoculum level. This paper reports a method that allows partial assessment of infective *P. terrestris* inoculum in the soil.

MATERIALS AND METHODS.—The soils assayed were from onion fields in Malheur County, Oregon. These soils are silt loam, but have not been characterized. The samples were air-dried, and weighed portions of sieved soil were diluted with clean, Del Monte El-20 mesh quartz sand to give 500 g of the desired soil:sand mixture. Each dilution level was replicated 5 times. Each sample of sand and soil was tumbled until thoroughly mixed, and placed in a new 10- × 10-cm sq plastic pot. Unless otherwise noted, 20 seeds of the susceptible Southport White Globe onion were planted 0.25-inch deep in each pot, and all pots were watered at the same time with Hoagland's solution (6). The containers were then covered with Kraft paper for 4 days to prevent surface drying and to promote even germination.

Subsequent waterings were made as required, usually every other day, and Hoagland's solution was added at 7-day intervals. Grow-Lux fluorescent tubes were used to increase day length to 14 hr. Ambient temp in the greenhouse were between 24 and 30 C. Evaluations were made 6 weeks after planting, because preliminary tests had shown that this was the shortest possible exposure to infective *P. terrestris* propagules that would allow adequate symptom expression. Any plant having one or more pink roots was rated as infected, and the percentage of infected plants was used to compare the infective levels of the three soils.

The effect of the indicator cultivar was studied using Yellow Sweet Spanish (moderately susceptible) and Southport White Globe (very susceptible), the two cultivars grown most commonly in eastern Oregon. To decrease variation due to treatment, 50 seeds of each cultivar were planted in the same pot using a template to cover half the pot at a time. This allowed planting Yellow Sweet Spanish on one side and Southport White Globe on the other. Dilution levels of one part soil to 10, 100, 1,000, and 10,000 parts sand were used.

RESULTS.—Results of the preliminary experiment were encouraging (Table 1), and it was recognized that a high level of inherent variation would be present in an assay method of this type.

At the 1:10 dilution, the indicated disease level in Southport White Globe was often twice that in Yellow Sweet Spanish. At greater dilution levels, the difference between cultivars was not as evident, which might be expected as the inoculum density decreases (Table 2). For most purposes, the Southport White Globe cultivar is the more desirable indicator.

Soil samples were collected at various depths to 18 inches, and *P. terrestris* was found at all depths but was most abundant in the top 6 inches. Wilhelm (11) found a similar distribution of *Verticillium* in soil.

TABLE 1. Percentage of onion plants with obvious symptoms of infection by *Pyrenochaeta terrestris*. Readings were made after six weeks

Dilution, soil:sand	Field 1	Field 2	Field 3
	% Plants infected[a]		
1:1	83	70	100
1:2	100	83	100
1:5	91	45	88
1:10	80	18	6
1:50	50	37	36
1:100	22	50	37

[a] Based on 5 replicates of 20 plants each.

TABLE 2. Percentage of infection of Southport White Globe and Yellow Sweet Spanish onions by *Pyrenochaeta terrestris* in seven soil samples from three fields

Field no.	Onion variety and soil:sand dilution level							
	Southport White Globe				Yellow Sweet Spanish			
	1:10	1:100	1:1,000	1:10,000	1:10	1:100	1:1,000	1:10,000
	% Plants infected[a]							
1	77	25	19	9	39	24	14	1
2	83	46	38	9	48	47	25	2
3	53	36	11	2	6	35	9	2
4	36	30	8	2	16	17	4	0
5	43	19	12	0	23	22	18	0
6	53	32	16	2	38	19	24	4
7	37	29	15	1	46	19	13	0

[a] Based on 5 replicates of 50 plants each.

Examination of soil particles of various sizes showed that *P. terrestris* inoculum was most often associated with the large soil particles (Table 3). This could be expected because the larger particles are agglomerates of smaller soil particles and fungal propagules. Also, *P. terrestris* is associated with undecomposed organic debris which would naturally be in the larger soil particles. Percentages of infection decreased sharply with soil particles smaller than 0.147 mm. This suggests that single spores or small mycelial fragments are seldom responsible for infection and that the infective units are pycnidia, microsclerotia, or mycelium and/or spore agglomerates.

This method was compared with a disease rating system to evaluate fumigation treatments of field soils. Agreement between evaluation methods was excellent. Both showed which treatment gave best disease control and showed gradations of disease control due to differences in rates of fumigant application.

DISCUSSION.—Research studies of *P. terrestris* have been impeded by the difficulty of determining populations of the fungus in soil. Andersen & Huber (1), using a modification of the soil sampling tube, were able to approach some quantification. Direct isolation procedures using various modifications of the soil dilution technique were frustrated by lack of precision and by inability to identify *P. terrestris* isolates on the plate. Identification of pure cultures required excessive time and labor. An attractive alternative is a biological assay.

A soil:sand dilution method, using onion seedlings as biological indicators, seems to have at least limited applicability. This method, like other biological assays, suffers from lack of precision, but does offer a usable research tool. Sensitivity may be increased by using a soil fraction having an average particle size between 0.5 and 1.0 mm.

TABLE 3. Influence of soil particle size on infection of Southport White Globe onion by *Pyrenochaeta terrestris* at 1:10 soil:sand dilution

Particle size (mm)	Avg % infection[a]
1.168	8.6
1.168	10.3
0.589	13.0
0.295	7.6
0.147	8.6
0.074	1.6
0.038	2.0

[a] Based on 5 replicates of 20 plants each and on soil from 2 different locations.

It was obvious that dilutions greater than 1:2 were needed, since onion plants were fewer and less vigorous at low levels of soil dilution. This was due at least partly to soil compaction, and probably also to the activity of microorganisms other than *P. terrestris*. The number of seeds planted yielded too few plants even at higher soil dilution levels. A better reading can be obtained with 50 seeds/pot. After 6 weeks, only a few diseased roots showed a bright pink color, while many roots were yellow or brown. In contrast, onions grown in pure sand had clean, white roots. These observations in conjunction with earlier ones of root sections devoid of pink color but clearly parasitized by *P. terrestris* mycelium made it clear that reading pink color for indication of disease gives a conservative measure of actual infection.

The method indicates only those propagules of *P. terrestris* pathogenic to onions. Substitution of another biological indicator, such as tomato, might add to its usefulness. An interesting and potentially profitable line of research would be an attempt to determine host specificity of *P. terrestris*.

This method may have application in studies not approached in the current work. A study of the fluctuation of inoculum levels of pathogenic lines of *P. terrestris* from year to year, combined with observation of an onion cultivar's disease susceptibility, may show why a resistant onion cultivar is not always resistant when grown in the same field during successive years. Possibly the inoculum level becomes so high that the cultivar's ability to resist pathogen entry is overcome, as suggested by Kreutzer (7).

Kulik & Tims (8) did extensive work which indicated that isolates vary in their ability to attack onion, but all of their isolates came from a field cropped to shallots, a very close relative of onion. Taubenhaus (9), working in Texas, showed that short-term crop rotations were of little value. A very natural extension of these studies would be to determine what crops, if any, increase or decrease *P. terrestris* inoculum pathogenic

to onions. A system is needed whereby the level of pathogenic propagules can be predetermined to direct fumigant application to those fields most likely to be benefited.

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