An Enrichment Method to Estimate Potential Seedling Disease Caused by Low Densities of *Pythium ultimum* Inocula in Soils

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ABSTRACT

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Inoculum densities of *Pythium ultimum* of about 25 propagules (sporangia and ripened oospores) or less per gram of soil can initiate significant seedling disease of alfalfa or cotton when environmental conditions are favorable. However, inoculum densities of *P. ultimum* are frequently too low to measure directly. Therefore, low densities of inocula were magnified by amending soils with dried cotton leaf fragments (1 mm^2) and measuring population enhancement. A direct correlation was found between final inoculum density and disease severity measured in soils before amendment. This enrichment technique may be used to estimate the inoculum potential of *P. ultimum* in greenhouse or field soils.

Pythium ultimum Trow is a widespread soilborne pathogen in temperate climates, where it causes preemergence and postemergence damping-off and root necrosis in many economic plants. Stanghellini (3) suggested that exogenously dormant propagules (EDP) (sporangia and ripened oospores) are the principal functional inocula of *P. ultimum*. However, the relationship between inoculum density and disease severity is frequently ambiguous. This is particularly true when disease is initiated by low inoculum densities that cannot be measured accurately.

This study sought to determine the relationship between inoculum density of *P. ultimum* and disease severity under optimal conditions for disease development and to evaluate a simple enrichment technique for estimating low inoculum levels of the pathogen in field soils.

MATERIALS AND METHODS

Soil samples taken from the tillage layer (0–15 cm deep) were air-dried, ground, and sieved through a 1-mm^2 mesh screen. Soils from six different sites in Fresno County, CA, were used in this study. They were from West Side Field Station (Panoche sandy loam), Boston (Oxalis clay), Stone (Lethent clay loam), Britz (Oxalis clay), Site 13 (Oxalis silty clay), and Site 26 (Panoche sandy loam). The pH values of these soils ranged from 7.7 to 7.9.

Cultures of *P. ultimum* (ATCC 32939) were grown on rolled-oat agar/water plates (1) at 22–24 C for 7 days under diurnal conditions. Mycelial mats were then removed and placed in soil (Panoche sandy loam, West Side Field Station, Five Points, CA). The infested soil was moistened to about 50% (v/w, air-dried basis) and air-dried at 22–24 C for 48 hr. It was then ground and sieved (1-mm^2 mesh), mixed thoroughly, and stored at 12 C.

We measured EDP density by the soil-drop method (4) 24 hr before the inoculum was used in the disease assay. EDP densities were adjusted to 0, 10, 25, and 50 per gram of air-dried soil by diluting the infested soil with noninfested field soil. Thus, we adjusted the density of *P. ultimum* in the soil by supplementing the natural but low populations with inocula grown from culture.

Thirty-three seeds of alfalfa (*Medicago sativa* L. cv. Moapa 69) were planted on the soil surface in each clay pot (75 mm i.d.). Plantings at each inoculum density were replicated five times. Soil in the pots was covered with a 1-cm layer of sterile vermiculite, irrigated to about 30% (v/w, ca. −0.1 bar matric potential), and incubated at 23 ± 5 C for 14 days in the greenhouse.

We then removed seedlings from soil and measured disease severity on a three-point scale, with 0 = healthy and 3 = dead. The ratings were converted to percentage values, with 1 = 33%, 2 = 67%, and 3 = 100% (Fig. 1).

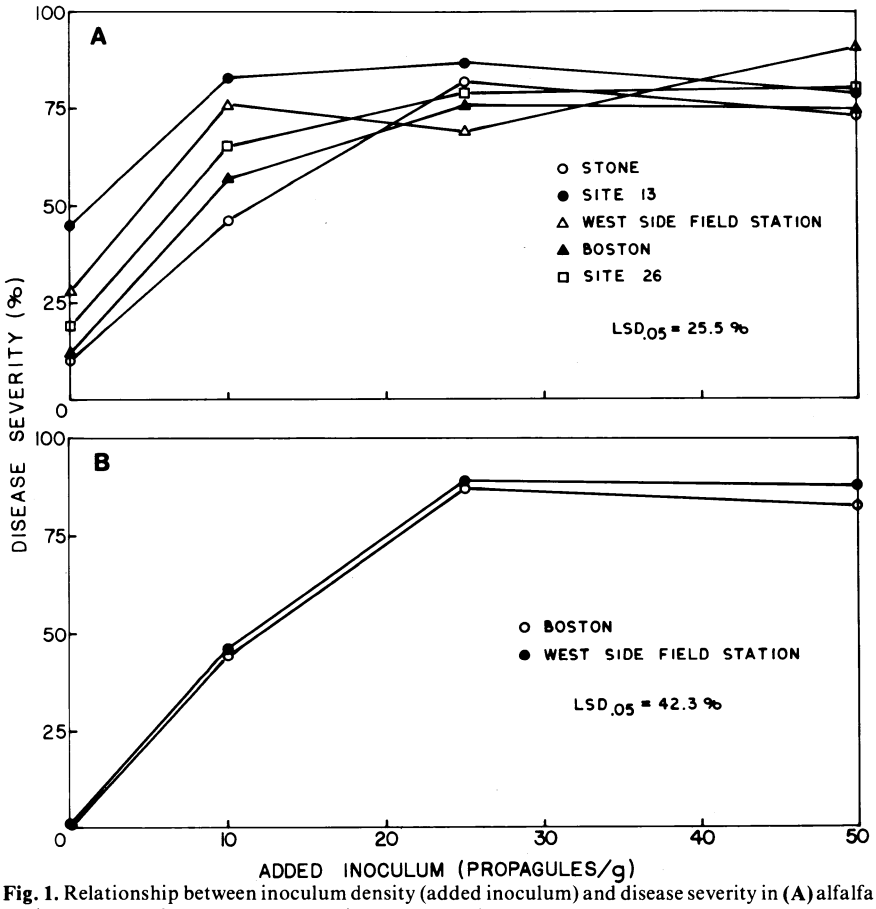

Fig. 1. Relationship between inoculum density (added inoculum) and disease severity in **(A)** alfalfa cultivar Moapa 69 and **(B)** cotton cultivar Acala SJ-2.

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Ten seeds of cotton (*Gossypium hirsutum* L. cv. Acala SJ-2) were planted 1 cm deep in clay pots (10 cm i.d.) in infested soil with a range of EDP densities. Each treatment had six replicates. Soil was covered with vermiculite and irrigated as with alfalfa, but incubation lasted 21 days. Disease severity was expressed as the percentage of seedlings showing symptoms in relation to uninoculated controls.

Dry, mature, green cotton leaves were crushed, sieved (1-mm^2 mesh), and incorporated into soils at 0.5 g of leaves per 150 g of air-dried soil). Soils were then moistened up to 20% (v/w, air-dry basis) and held within a constant moisture apparatus (1) at 21–23 C for 7 days. Densities of *P. ultimum* EDP were measured by the soil-drop method (4).

We identified *Pythium* spp. by culturing the isolates as for inoculum preparation and viewing sexual structures with a phase-contrast microscope.

Table 1. Relationship between density of *Pythium ultimum* propagules, alfalfa seedling disease, and propagule density after enrichment[x]

Soil	Date collected	Seedlings diseased (%)	*Pythium ultimum* (EDP/g of soil)	
			Natural	Enriched
Britz	3 May 1979	82.6 a[y]	16 a	433 a
West Side Field Station	19 Sept. 1979	75.9 ab	16 a	360 b
Site 13	15 Nov. 1979	44.6 b	n.d. a[z]	213 c
Britz	14 July 1978	30.2 bc	n.d. a	17 e
West Side Field Station	15 Nov. 1979	27.0 bc	n.d. a	n.d. e
Site 13	19 Sept. 1979	24.0 bc	n.d. a	83 d
Boston	15 Nov. 1979	19.2 c	n.d. a	n.d. e
Site 26	15 Nov. 1979	18.2 c	n.d. a	58 d
Boston	19 Sept. 1979	18.0 c	n.d. a	4 e
Stone	19 Sept. 1979	17.8 c	n.d. a	n.d. e
Britz	15 Nov. 1979	16.0 c	n.d. a	n.d. e
Stone	15 Nov. 1979	14.4 c	n.d. a	n.d. e

[x] Densities of exogenously dormant propagules (EDP) of *Pythium ultimum* were measured 7 days after soils were amended with ground cotton leaves (0.5 g of dry, mature, green leaves in 150 g of air-dried soil).

[y] Within each column, means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] *Pythium ultimum* was not detectable in these soils.

RESULTS

A *P. ultimum* density of 25 EDP/g of soil had the potential to incite the maximum amount of disease in alfalfa or cotton seedlings (Fig. 1). We found no significant evidence of disease suppression among soil samples Stone, Site 13, West Side Field Station, Boston, and Site 26 infested with inocula of *P. ultimum* grown from culture.

Significant seedling damping-off was measured in trials where EDP densities were very low or undetectable (Fig. 1A; Table 1). Yet *P. ultimum* was responsible, as determined by isolation frequency, for 96% of the damping-off. Because the build-up of *P. ultimum* on organic substrates is directly related to the original EDP levels in soil (Lifshitz, *unpublished data*), we evaluated the relationship between EDP buildup and disease severity in soils with low populations of *P. ultimum*. The results showed a positive correlation between enrichment densities of EDP and disease severity ($r = 0.733$, $P < 0.01$; Table 1).

DISCUSSION

The significance of *P. ultimum* inoculum density in soil was shown by the relationship between EDP density and disease severity. We attributed differences in alfalfa disease index values of non-infested soils to differences of low natural densities of *P. ultimum* inoculum per gram of soil. When environmental conditions were suitable, inoculum densities of 10–25 EDP or less per gram of soil initiated significant infection levels. Pieczarka and Abawi (2) found similar results with bean.

Using indicator plants to measure the disease potential of a field soil is laborious. Further, low population densities of *P. ultimum* in field soils are difficult to measure directly. However, natural inoculum densities can be magnified by an EDP enrichment assay that in our tests showed a direct correlation between final EDP density and disease severity measured in soils before amendment. Because enrichment is a simple and sensitive technique, we suggest its usefulness in estimating the inoculum potential of *P. ultimum* in greenhouse or field soils.

LITERATURE CITED

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