Bioassay for Quantification of *Pythium aphanidermatum* in Soil

M. E. Stanghellini and W. C. Kronland

Department of Plant Pathology, University of Arizona, Tucson 85721.
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ABSTRACT

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A potato baiting technique was developed that provided a rapid, inexpensive, and quantitative means of assessing the absolute inoculum potential of populations of *Pythium aphanidermatum* in naturally infested field soils.

Avoiding the planting of susceptible crops in known infested fields is one method of reducing crop losses resulting from certain soilborne plant pathogens for which no chemical controls or resistant cultivars are currently available. Avoidance can be based on preplanting prediction of the risk of disease by measuring the population density or absolute inoculum potential of a particular plant pathogen(s). Selective media are commonly employed in estimates of the population densities of particular soilborne plant pathogens. However, such media are generally too expensive for commercial use. Also, selective media do not distinguish between pathogenic and nonpathogenic strains of a particular pathogen or provide data regarding the biological resistance (i.e., suppressiveness) of a soil. However, estimation of the absolute (7) or standard (3) inoculum potential, which involves collection of soil from the field and a host bioassay, provides data on the maximum capacity of a pathogen population to infect a population of fully susceptible host plants under conditions optimum for infection. Prediction of high or low risk of disease is then based upon knowledge of the absolute inoculum potential and its relation to disease incidence. Although plant bioassays have been developed and are currently in use for certain root diseases (5,8,9), these require collection of large quantities of soil, extensive greenhouse space, and often 3–4 wk of cultivation before the degree of injury can be assessed.

In a previous study (11) we developed a potato baiting technique for estimating the absolute inoculum potential of *P. aphanidermatum* in rhizosphere soil. This study was undertaken to develop that technique into a commercially feasible method for quantifiying *P. aphanidermatum* in soil.

MATERIALS AND METHODS

Population density and inoculum potential determinations. Both the population density and inoculum potential of *P. aphanidermatum* were estimated for every soil sample used in our study.

The oospore population density of *P. aphanidermatum* in each soil sample was estimated by using a species-specific isolation medium (4). Each soil sample was air-dried for 24 hr after collection, sieved, and thoroughly mixed before assay. A 10-gram subsample of each soil was diluted 1:10 (10 g of soil in 90 ml of 0.1% water agar) and mixed on a Vortex stirrer for 3 min. Ten 1-ml aliquots were then dispensed evenly across the surface of 10 petri plates containing the solidified selective medium and incubated at 36 C for 48 hr. The soil was then washed from the agar surface and colonies were counted.

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The absolute inoculum potential of *P. aphanidermatum* in each soil sample was assessed by using a previously described potato baiting technique (11). In initial studies, a naturally infested soil containing 40 +5 oospores per gram of air-dried soil was serially diluted with an uninfested soil from the same field. Ten 1-g subsamples of each dilution were then dispensed into separate vessels 2.5 cm in diameter and 1.5 cm tall (Fig. 1A and B). The soil in each vessel was brought to saturation with sterile distilled water and a 1-cm piece of fresh potato tuber tissue, 3-mm thick, was placed on the soil surface. A water agar slice (0.5 cm in diameter and 3 mm thick) was then placed on top of the potato tissue. The 10 vessels were placed in a petri dish and incubated for 48 hr at 27 C. Water agar slices were then removed and placed on the selective medium contained in petri dishes (Fig. 2B). The percent of water agar slices colonized by *P. aphanidermatum*, which reflected the percent of potato slices colonized, was determined after 24 hr of incubation at 37 C. Previous studies (11) showed that saturated soil conditions and incubation temperatures of 27 C for 48 hr provided the optimum environmental conditions for maximum colonization of the bait. Percent colonization data presented are means of four independent trials (each consisting of 10 1-g subsamples) per soil dilution; the population density data (estimated by using the selective medium) represent the means of three separate 10-g subsamples from each soil dilution.

The above soil bioassay procedure, herein designated method I, was subsequently modified to decrease the labor time and number of vessels employed. This modification, designated method II, was used to assess the absolute inoculum potential of *P. aphandermatum* in soil samples collected from 52 fields. The latter soil samples were obtained as follows: a soil core, 2.5 cm in diameter and 15 cm long, was collected every 20–25 rows across each of 52 fallow or recently planted sugar beet fields (each about 40 ha) and pooled into one composite soil sample (consisting of 20–25 cores) per field. The depth and time of year of soil sample collection was based upon the results of previous studies (10) which showed that highest population densities of oospores of *P. aphanidermatum* occurred in the top 15 cm of the soil profile and that no fluctuations in the population density occurred during the growing season prior to the onset of root rot which occurs about 8–9 mo after planting. A 24-g subsample of soil from each of the 52 composite soil samples was dispensed into a four-sectioned petri dish (Fig. 2A). Each section contained about 6-g of soil. The soil in each section was brought to saturation with sterile distilled water. Twelve pieces of bait (three per section) were then placed on the soil surface and covered with a slice of water agar. The baited soil sample was then processed as described above. The four-sectioned plate was used to reduce the possibility of secondary spread of the fungus subsequent to primary colonization of a single bait. Percent colonization data presented are means of two independent trials (each consisting of a 24-g soil subsample) for each of the 52 composite soil samples and the population density data, which were estimated by use of the selective medium, represent the means

of three separate 10-g subsamples from each of the 52 composite soil samples.

RESULTS

Results from studies employing method I, in which 1-g subsamples of a naturally infested field soil (diluted to known population densities of *P. aphanidermatum*) were individually bioassayed with the potato baiting technique, are presented in Fig. 3A. The percentage of baits colonized increased and the population density increased. Regression analysis of log-probit transformations for dosage response indicated a significant linear relationship between the population density of *P. aphanidermatum* and the percent of baits colonized (Fig. 3B). The population density required for 50% colonization of the baits (ID50) was 6.2 oospores per gram of soil.

Results from studies employing method II, in which a 24-g subsample of soil (containing a known population density of *P. aphanidermatum*) from each of 52 different fields was bioassayed with the potato baiting technique, are presented in Fig. 4A. The percentage of baits colonized increased as the population density increased (Fig. 4B). Regression analysis of log-probit transformations of the dosage response indicated a significant linear relationship between population density and percent of baits colonized (Fig. 4B). The population density required for an ID50 was 7.4 oospores per gram of soil.

DISCUSSION

Forecasting systems have been developed for predicting damage caused by foliar plant pathogens (12) and root diseases caused by nematodes (2). However, relatively few forecast systems exist for diseases caused by soilborne fungal plant pathogens (1,9). The development of a forecast system for soilborne plant pathogens is dependent upon numerous factors among which the more important is the development of a quantitative method of assessing the potential destructiveness of the pathogen. The results of our study have shown that the potato baiting bioassay technique (method I or II) provides an efficient and quantitative means of measuring the absolute inoculum potential of *P. aphanidermatum* in naturally infested field soil. A significant linear relationship was shown to exist between the population density and percent of baits colonized. In the absence of a detectable population of the fungus, little or no colonization occurred. Of the 52 field soils assayed by using a selective medium, 28 contained an estimated population of 0 oospores per gram of soil. When the latter soils were bioassayed with the potato baiting technique (method II), no colonization occurred in 14 of the 28 soils. The remaining 14 soil samples did contain an infective population of the fungus. The population of *P. aphanidermatum* in the latter soils was apparently between 0 and 1 oospore per gram of soil; 1 oospore per gram of soil was the lower limit capable of being detected on the selective medium. In the presence of a detectable population, the percentage of baits colonized increased as population density increased.

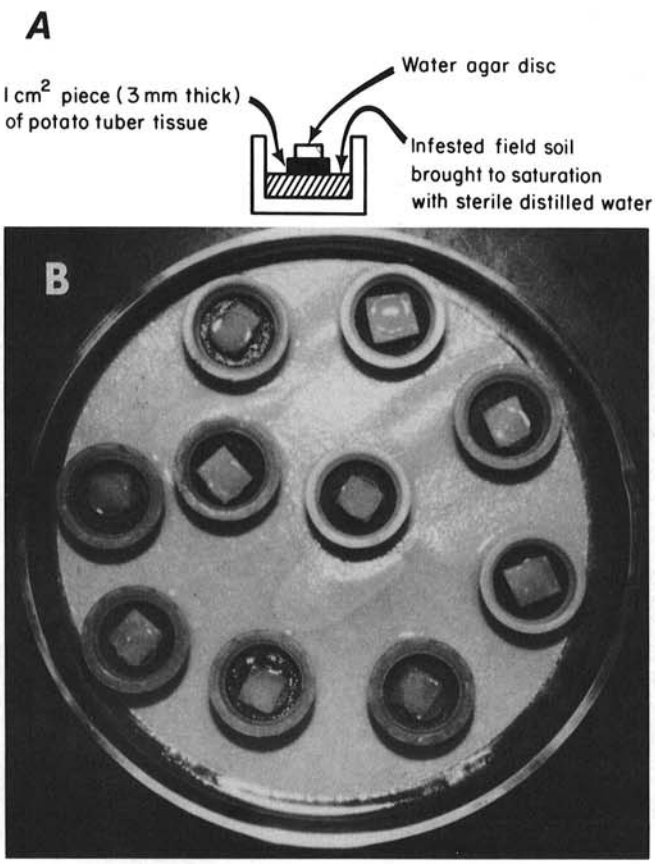

Fig. 1. Potato baiting bioassay method I for detecting *Pythium aphanidermatum* in soil. **A,** Schematic drawing and **B,** a photograph of 1-g soil samples being assayed.

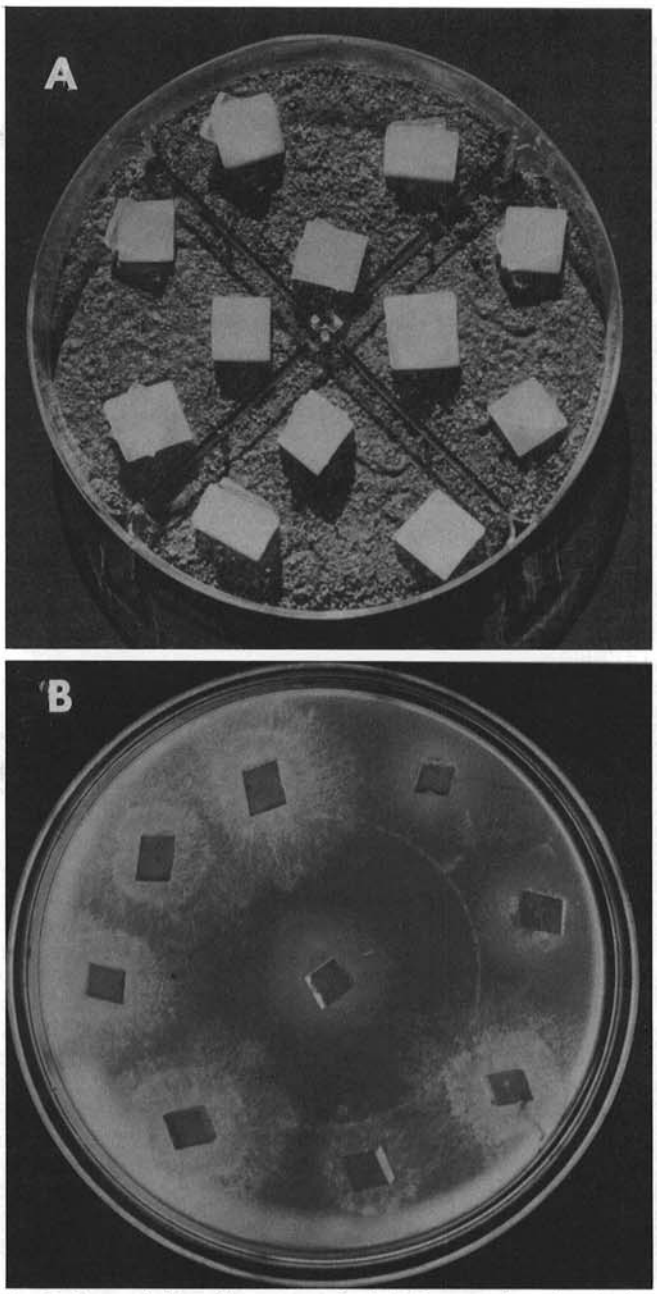

Fig. 2. Potato baiting bioassay method II for assessing the absolute inoculum potential of *Pythium aphanidermatum* in soil. **A,** Photograph of a soil being bioassayed and **B,** growth of *P. aphanidermatum* from the water agar slices on a selective medium.

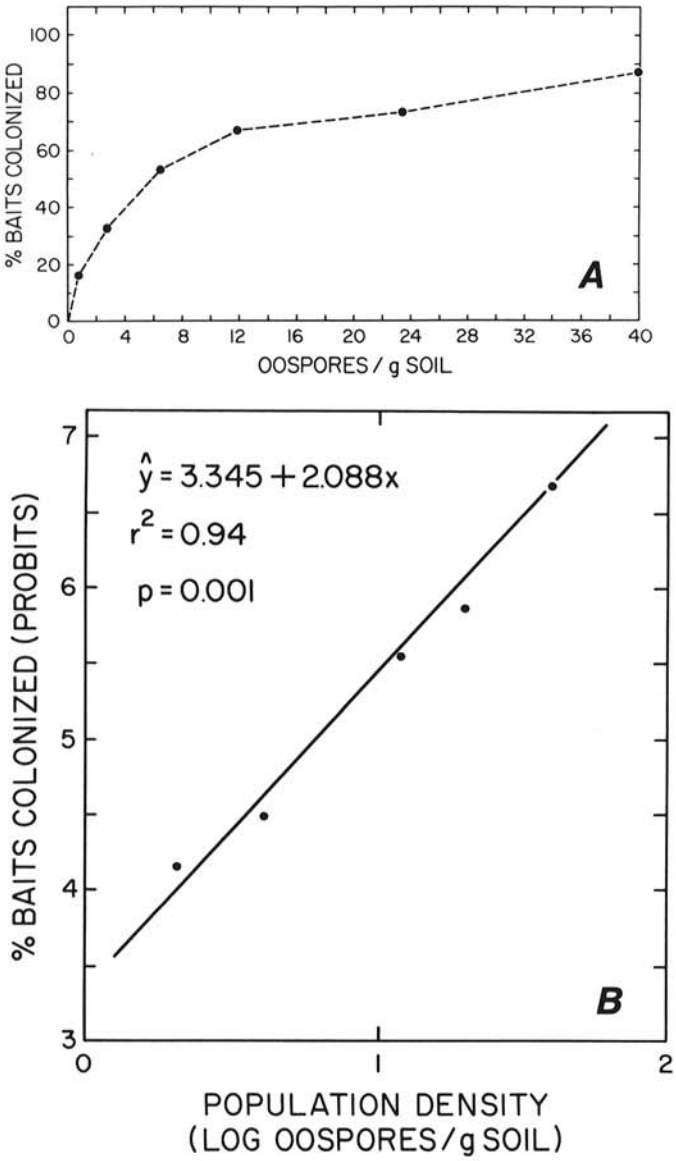

Fig. 3. Relationship between percent of potato baits colonized by *Pythium aphanidermatum* (bioassay method I) and population densities (direct isolation on a selective medium) of oospores in serial dilutions of a naturally infested soil. **A,** Population density (arithmetic) and percent colonization (arithmetic) and **B,** population density (logarithmic) and percent colonization (probit).

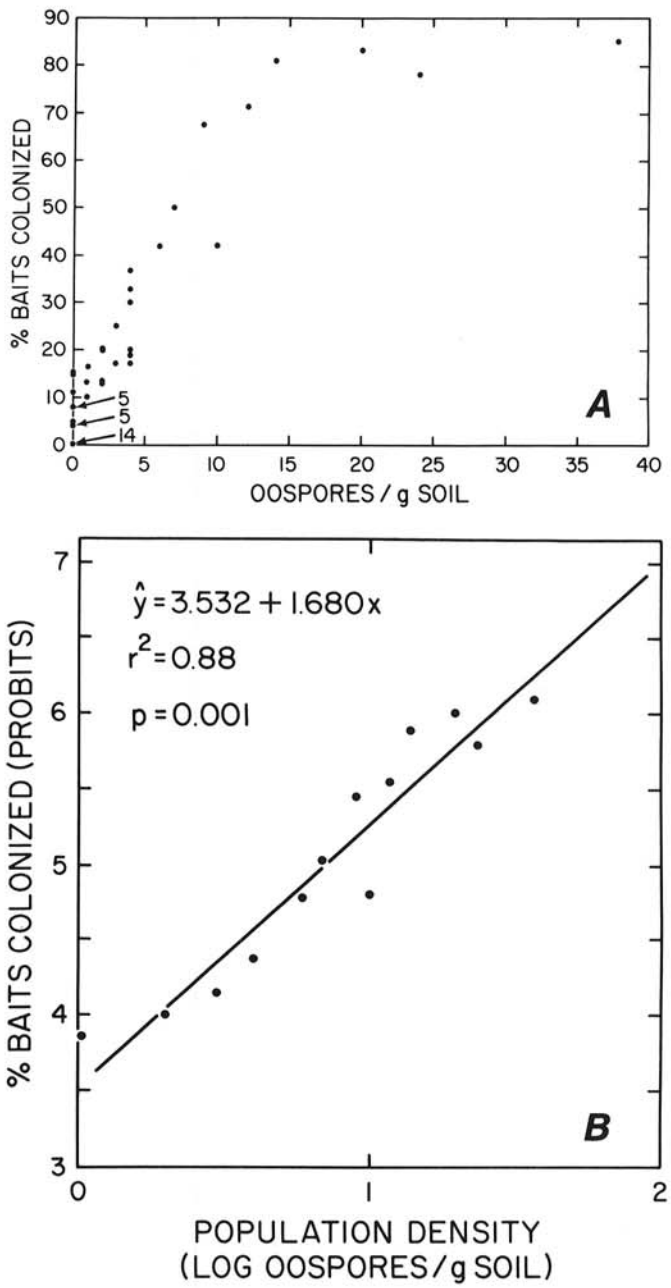

Fig. 4. Relationship between percent of potato baits colonized by *Pythium aphanidermatum* (bioassay method II) and population densities (direct isolation on a selective medium) of oospores in soil samples from 52 different fields. **A,** Population density (arithmetic) and percent colonization (arithmetic). **B,** Population density (logarithmic) and percent colonization (probit).

In addition to being quantitative, the potato baiting technique (in particular, method II) is simple and inexpensive, provides data within 72 hr after collection of soil samples, and eliminates the need for collection of large quantities of soil and extensive greenhouse space necessary for pot culture bioassays.

The potato baiting technique, which provides inoculum potential data on a "worst scenario" basis, may serve as a guide to estimate the maximum potential risk involved in growing a particular crop in a particular field. For example, if each piece of potato bait represented a plant, a population density of 7.4 oospores per gram of soil would, under optimum environmental conditions, result in a loss of 50% of the plants (assuming that a single infection results in plant death). However, the actual risk of disease in a particular crop, which would always be lower than the predicted risk, would be dependent upon the damage threshold of that crop and the occurrence of optimum environmental conditions for manifestation of the absolute inoculum potential. With regard to *P. aphanidermatum*, the relationship between population density and damage threshold to any specific crop grown under field conditions have not yet been established. However, Mitchell (6) reported that the population density of *P. aphanidermatum* required for 50% infection (ID50) of roots of various greenhouse-grown plants ranged from 15 to 24 oospores per gram of soil. Soils containing population densities of 15–24 oospores per gram of soil would have been identified as potentially high disease risk soils based upon results from the potato baiting technique. The utility of the potato baiting technique in predicting disease incidence under field conditions is currently being evaluated.

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