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The Relationships between Inoculum Density and Absolute Inoculum Potential of *Pythium aphanidermatum*

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ABSTRACT

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The percentage of potato baits colonization measured by potato baiting technique as absolute inoculum potential and percentage of cucumber seedlings affected by damping off obtained from pot tests increased with the increase of population densities of *Pythium aphanidermatum* in soils. There is a significant correlation between % potato baits colonization and % damping off of cucumber seedlings. Of the 68 field soils assayed for correlation between inoculum density and absolute inoculum potential, a regression equation of $Y = 41.76 + 6.70 \ln X$ ($r = 0.63$, $P \leqq 0.01$) and ID_{50} of 3.4 oospores/g of soil were obtained. However, when 46 of the 68 soils with pH higher than 5.0 were assayed, the regression equation of $Y = 41.78 + 8.22 \ln X$ ($r = 0.99$, $P \leqq 0.01$) and ID_{50} of 2.7 oospores/g of soil were obtained. For the remaining 22 soils with soil pH lower than 4.99, there were no significant correlation between population densities and % potato baits colonized by *P. aphanidermatum*. In some soils with relatively low values of absolute inoculum potential, a change form suppressive state to conducive occurred when the soils were reinforced to 50 oospores/g of soil and bioassayed by potato baiting technique 3 month after collection. Soil Fy-1 was found to have a high value of ID_{50} (53.6 oospores/g of soil) of *P. aphanidermatum* and capable of reducing the establishment of the pathogen in this soil. It could possibly be characterized as suppressive soil.

Key words: *Pythium aphanidermatum*, inoculum density, absolute inoculum potential, suppressive soil, cucumber.

INTRODUCTION

Recently, there has been a considerable interest in the suppressiveness of certain soils to *Pythium* spp. Techniques have also been developed in laboratory and greenhouse to predict the risk of diseases caused by *Pythium* spp. of a paticular soil by measurring the inoculum density with selective media (6,7,14), soil drop assay method (4,12,18), germination of hyphal swellings (5) or oospores (11), and estimating the absolute (16) or standard (2) inoculum potential with plant bioassay method. However, estimating the inoculum density by using a selective medium is not a good measure for disease forcasting or disease suppression (2) and the plant bioassay method is usually too tedious (19). Stanghellini and Kronland (19) have shown that the potato baiting bioassay technique is simple, inexpensive and fast to provide an efficient and quantitative means of measuring the absolute inoculum potential of *P. aphanidermatum* (Edson) Fitzp. in naturally infested soils. But, its relation to disease incidence is still not known.

In this study, the potato baiting bioassay technique was used to investigate the relationships among inoculum density, absolute inoculum potential and disease incidence of *P. aphanidermatum* in field soils, and tried to select the possible suppressive soils.

MATERIALS AND METHODS

Soil collection

Sixty eight soil samples were collected from vegetable fields located in different parts of central Taiwan. Approximately 20 kg of soil were collected from the top 15-cm layer, air-dried, and passed through a 4-mm-mesh sieve for later use. The soil pH was determined by adding 10 g of soil to 20 ml of $CaCl_2$ solution (0.01 M). The oospore density and the absolute inoculum potential were estimated soon after collection.

Preparation of inoculum

Oospore suspension: The isolate Pa-1 of *P.*

aphanidermatum used in this study was derived from diseased cucumber (9). It was cultured in the oat-water culture (baked oat, 10 pieces; sterilized distilled water, 25 ml) at 28 C for a month. Then, the mycelial mat was removed to feed the apple snails, *Pomacea canalinculata* (Lamarck). The oospores of the *P. aphanidermatum* were collected from snails faeces suspended in 200 ppm of vancomycin solution and centrifuged at 7000 rpm for 5 min. The supernats which contained oospores were kept in 4 C incubator until use (10).

Wheat inoculum: Sterilized wheat kernels were colonized by *P. aphanidermatum* in flasks in the laboratory, dried, and ground for later use.

Estimation of inoculum density and absolute inoculum potential

For determining inoculum density, 10 g of the tested soil sample were placed in 90 ml of steril water and agitated for 3 min. Then, one-ml aliquot was dispensed to the surface of a 9-cm petri dish containing a selective medium (Difco cornmeal agar, 17 g, pimaricin (Myprozine, potency 92.2%, American Cyanamid) 100 mg, streptomycin sulfate 200 mg, rose bengal 150 mg and benomyl 5 mg in 1 L of water) (3), 10 times replicated. After 48 hr of incubation at 36 C, soil was washed from agar surface and colonies that originated from oospores (examined under compound microscope) were counted.

The absolute inoculum potential (AIP) was determined by (i) Potato baiting bioassay technique: Twenty-five grams of soil sample were placed in a 9-cm petri dish. Enough sterilized distilled water was added to bring the soil moisture to near saturation. Twelve pieces of fresh potato tuber slices (0.3 cm × 1 cm) were then placed on the soil surface. On the top of each potato slice was a water agar slice (0.3 cm × 0.47 cm). The agar slices were removed after 24–36 hr incubation at 28 C for growth on a selective medium (3) at 37 C (19). The pecentage of the water agar slices colonizd with *P. aphanidermatum* was recorded after 24 hr; and (ii) Cucumber seedling bioassay method: Seed of the suceptible cucumber cultivar 'Joy' (produced by Known You Seed Company) (9), were placed on moist filter papers in a petri dish at 28 C. Twelve healthy, germinated seeds were selected and sown in each 4-in pot containing the tested soil. The pots were kept at 28 C in a growth chamber (Coviron SH10). Within 3 weeks, % damping off of cucumber seedlings was recorded and the pathogen was reisolated by using a selective medium (3).

Correlation between the potato baiting and cucumber seedling bioassays

A silt loam soil (pH 7.2) collected from a cucumber field at Chi Hu (Ch-1) had naturally infested with 65 oospores of *P. aphanidermatum* per gram of soil as estimated by a selective medium (3). This soil was serially diluted with an uninfested soil from the same field. Each of the diluted soil sample and fifteen other soil samples that differed by soil texture and soil pH range (3.76–7.85) were tested for oospore density of *P. aphanidermatum*. % potato baits colonized, and % damping-off of cucumber seedlings. The regression were calculated.

ID_{50} of *P. aphanidermatum* in different soils

Two soils of different inoculum potential were tested for ID_{50} (Number of oospores per gram of soil which rendered 50% of the potato slices colonized). Soil Ch-1 (pH 7.2) contained 65 oospores/g of soil, and rendered 100% of potato baits colonized. Soil Fy-1 (pH 4.7) contained 2 in-oospores/g of soil, and produced no colonized potato bait. Oospore suspension was added to each soil sample to bring the population to 100 oospores/g of soil from which a serial dilution to known population density was made. The absolute inoculum potential was determined by potato baiting bioassay technique and the ID_{50} was also estimated from the regression equation.

Suppressive effect of soil

Soils Ch-1 and Fy-1 were mixed with wheat inoculum of *P. aphanidermatum* (sterilized wheat kernels colonized by the pathogen in flasks in the laboratory, dried and ground) at the proportion of 0.1%, w/w. To the mixtures, water was added to bring the soil moisture to near saturation. They were allowed to dry on greenhouse bench for 16 days. The population density, % potato baits colonized, and % damping-off of cucumber seedlings were estimated and recorded.

RESULTS

Correlation between the AIP values estimated by potato baiting and cucumber seedling bioassay methods

Serially diluted subsamples of Ch-1 soil to known population densities of *P. aphanidermatum* and 15 different field soils were individually bioassayed by potato baits and by the cucumber seedling methods. The values of AIP obtained were significantly correlated in both cases, with $Y = 0.78\ X + 20.22$ ($r = 0.89^{**}$, $P \leq 0.01$) (X = % potato baits colonized, Y = % of damping off of cucumber seedlings) (Fig. 1A) and $Y = 0.78\ X + 2.65$ ($r = 0.93^{**}$, $P \leq 0.01$) (Fig. 1B), respectively. Both the % of potato baits colonized and % of cucumber seedlings affected with damping off increased with the increase of population densities of *P. aphanidermatum*.

The relationship of population density and absolute inoculum potatial of *P. aphanidermatum* in field soils

The population densities of *P. aphanidermatum* in 68 field soils measured by a selective medium (3) ranged from 0 to 114 oospores/g of soil. Sixty-one of them were

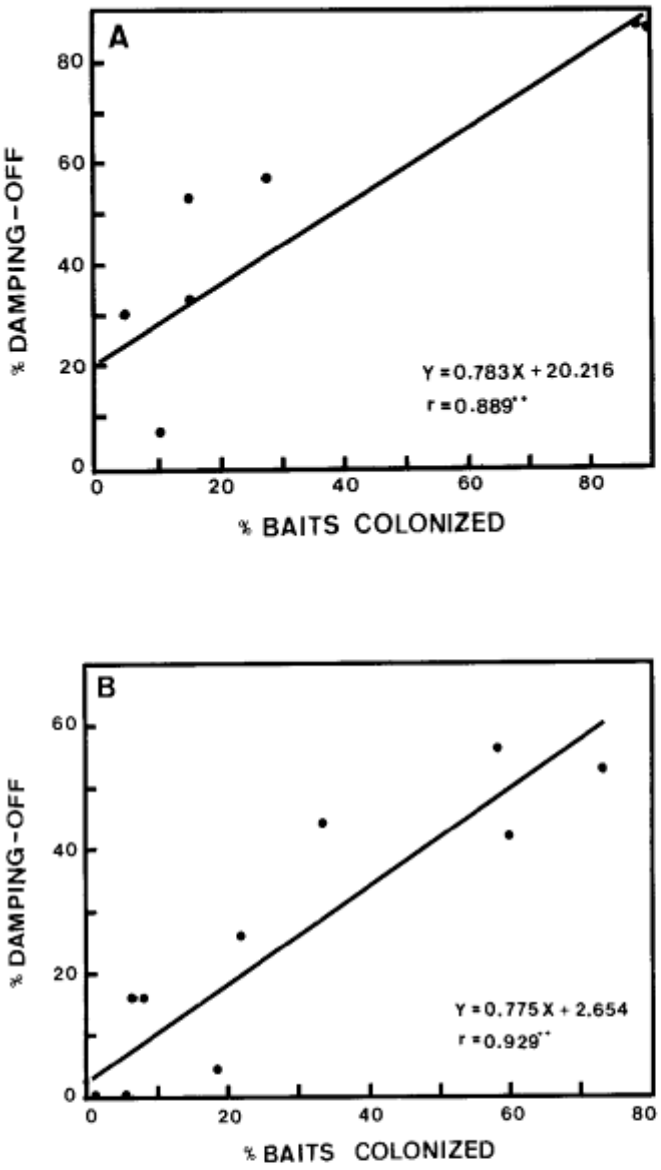

Fig. 1. Relationships between % damping off of cucumber seedlings and % of potato baits colonized by *Pythium aphanidermatum* in serial dilutions of a naturally infested soil sample Ch-1 (A), and in 15 soils naturally infested with different population densities (B).

less than 20 oospores/g of soil, and 33 soils had none. However, by using potato baiting bioassay, *P. aphanidermatum* was still detectable in 17 out of the 33 soil samples negative on using selective medium. The regression equation between the pupulation density and % potato baits colonized was $Y = 41.76 + 6.70 \ln X$, ($r = 0.63^{**}$, $P \leqq 0.01$), $ID_{50} = 3.4$ oospores/g of soil ($X =$ oospore/g of soil, $Y =$ % baits colonized) based on the measurement of 68 soil samples. When the 68 soil samples were seperated into two groups according to their pH (3.56–4.99 and 5.00–7.85), and bioassayed by potato baiting technique, the results showed that there was no significant correlation between population density and % baits colonized by *P. aphanidermatum* ($Y = 38.17 + 2.50 \ln X$, $r = 0.21$, $P > 0.05$) for the group of 22 soil samples with a pH value lower than 4.99. By contrast, the remaining 46 soil samples with a pH value above 5.0 had significant correlation between the the oospore density and % baits colonized ($Y = 41.78 + 8.22 \ln X$, $r = 0.79^{**}$, $P \leqq 0.01$, $ID_{50} = 2.7$ oospores/g of soil) (Fig. 2A).

Suppressive effect to *P. aphanidermatum* of soil Fy-1

Of the total soil samples tested, several had a value of % potato baits colonized that departed greatly significantly ($p = 0.05$) from the value estimated by the regression line obtained from 46 soil samples (Table 1). After adding 50 oospores/g of soil into these 'abnormal' soil samples (Table 1), soils Fy-1 and Ln-2 still showed less than 50% baits colonized. This suggested a suppressive phenomenon to *P. aphanidermatum.* Because the collection site of soil Ln-2 was not traceable, soil Fy-1 (pH 4.7) was chosen for further studies of its possible suppressive effect on *P. aphanidermatum.* Soils Ch-1 and Fy-1 were reinforced to 100 oospores/g of soil. Then a serial dilutions was made for each. The popluation density and % baits colonized were determined. The regression equations were shown in Fig. 2B. The ID_{50} of *P. aphanidermatum* in soils Ch-1 and Fy-1 was 1.2 and 53.6 oospores/g of soil, respectively. In another experiment, soils Ch-1 and Fy-1 were infested with wheat inoculum of *P. aphanidermatum* at 0.1%, w/w for 16 days. The results showed that population density and % damping off of cucumber seedlings of *P. aphanidermatum* were high in soil Ch-1 and were low or not detectable in soil Fy-1, respectively (Table 2).

DISCUSSION

Among the 68 field soils assayed by using a selective medium and the potato baiting technique in this study, sixty-one of them had the population densities of *P. aphanidermatum* less than 20 oospores/g of soil, and 33 soils had none. It indicated that *P. aphanidermatum* did not widely distribute in vegetable fields. However, when assayed by using a more sensitive potato baiting technique, *P. aphanidermatum* was detected in 17 out of the 33 soil samples. Stanghellini and Kronland (19) also reported that for 52 soil samples tested, *P. aphanidermatum* was still detectable by potato baiting technique in half of 28 soils which were negative on using selective medium, and concluded that the population densities in these soils were apparently between 0–1 oospores/g of soil, beyond the lower limited capability of the selective medium to detect the presence of *P. aphanidermatum.* There was no significantly correlation between population densities and % potato baits colonized by *P. aphanidermatum* in soils with a pH lower than 4.99 in this study. Soils with a low pH might have dramatic effect on the behavior of *P. aphanidermatum* to grow on selective medium and to colonize the potato baits. In other words, *P.*

TABLE 1. The population densities and absolute inoculum potentials of *Pythium aphanidermatum* in different field soils

Soil	pH	Population density (oospores/g of soil)[1]	Potato baits colonized (%)		Estimated Y value[3]	% colon. (I) minus Est. Y
			(I)[1]	(II)[2]		
Ln-1	5.5	3	1	94	51	−50
Fy-1	4.7	2	0	44	48	−48
Lg-2	6.9	2	4	83	48	−44
Yc-1	4.3	2	6	64	48	−42
El-2	5.4	5	15	50	55	−40
Ln-2	4.4	1	4	44	42	−38
Tn-4	4.8	2	14	100	48	−34
Ch-4	7.1	2	14	97	48	−34
El-1	5.4	0	3	94	−1	4
Ch-1	7.4	65	89	89	76	13
Wp-1	5.1	2	63	75	48	15
Tn-5	6.1	0	31	100	−1	32
DS-1	5.3	1	83	89	42	41

1. Population densities and potato baits colonized by *P. aphanidermatum* in each soil sample were measured soon after collection.
2. Each soil sample was reinforced to 50 oospores/g of soil of *P. aphanidermatum* three months after collection, then the % potato baits colonized was measured.
3. $Y = 4.178 + 8.22 \ln X$ (Y = an estimated value of % potato baits colonized, X = oospores/g of soil).

TABLE 2. The inoculum densities and absolute inoculum potentials measured by potato baiting and cucumber seedling bioassays of soils infested with wheat inoculum[1] of *Pythium aphanidermatum* at 0.1% (w/w) for 16 days

Soil	Inoculum density (oospores/ g of soil)	% potato baits colonized	% damping off of cucumber seedlings
Ch-1	458	97	100
Fy-1	0	0	25

1. Wheat inoculum was made by sterilized wheat kernels colonized by *P. aphanidermatum*, dried and ground.

aphanidermatum was more aggressive in soils with soil pH value higer than 5.0. The soil with low pH may contain propagules of *P. aphanidermatum,* but not detectable by using selective medium or potato baiting technique. Lifshitz et al (6,7) and Paulitz and Baker (17) also observed an inverse relationship for saprophytic inoculum increase between two *Pythium* spp. mediated by temperature and pH. However, Martin, and Hancock (13) showed that there was no consistent relationship between soil pH and suppression to *P. ultimum.*

Absolute inoculum potential proposed by Mitchell (16) provided a measurement for the maximum capacity of a pathogen population to infect a population of fully susceptible host plants under conditions optimum for infection. It may serve as a guide to estimate the maximum risk involved in growing a particular crop in a particular soil. Our results demonstrated that the absolute inoculum potentials of soils bioassayed by potato baiting and cucumber seedling methods were highly correlated (Fig. 1A, 1B). The values of % potato baits colonized and % damping off of cucumber seedlings increased as the population densities of *P. aphanidermatum* increased. The potato baiting bioassay technique is a simple, efficient way to demonstrate the suppressive phenomeanon of a soil, which has a low % of potato baits colonization compared to a conducive soil. However, the question whether soil with a low population density and a low AIP value of the pathogen can be considered suppressive or not should be answered with caution because nonsoil factors may have dramatic transitory effects on the behavior of the pathogen. For example, some soil samples *e.g.* Ln-1, Lg-2, Yc-1, E1-2, Tn-4, and Ch-4 (Table 1) had relatively lower AIP values detected soon after collection by potato baiting bioassay technique and might be considered as suppressive soils, but their AIP values assayed by the same method increased to 50% or above when these soils were reinforced to 50 oospores/g of soil later.

Soil Fy-1 was particularly of interest. *Pythium aphanidermatum* had a low value of ID_{50}, *i.e.* 53.6 oospores per gram of soil (fig. 2B), and had difficulty to establish in this soil (Table 2). One may characterize soil Fy-1 as suppressive soil from its absolute inoculum potential. However, caution must be exercised in drawing this conclusion because the soil pH was low (4.7) and the oospores and wheat inoculum used in the experiments

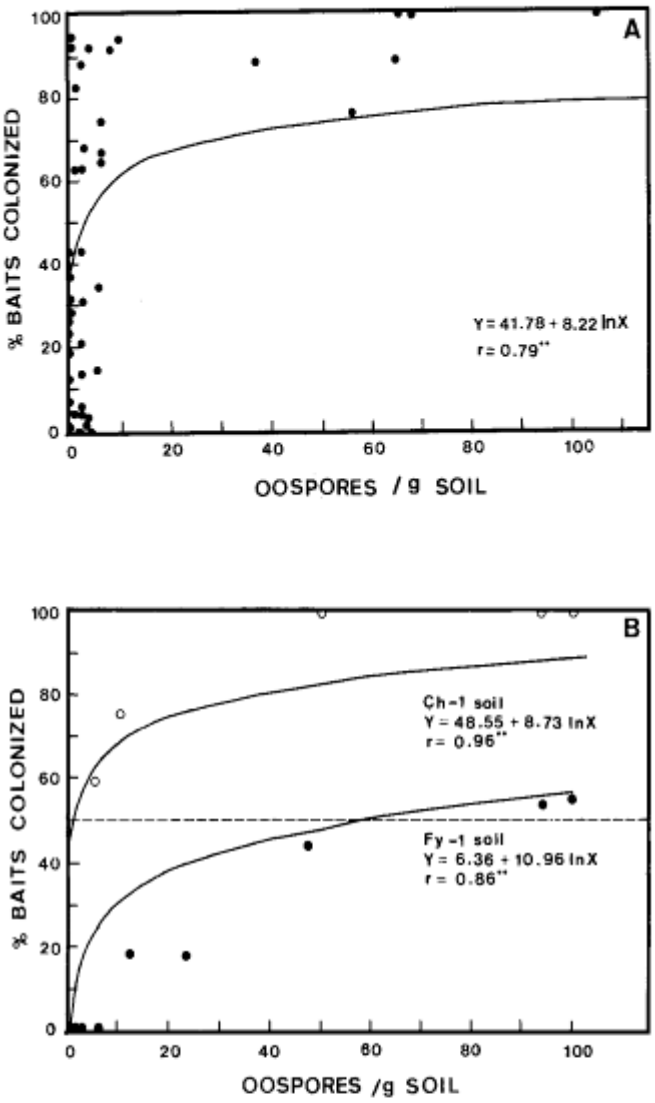

Fig. 2. Relationships between % of potato baits colonization and population densities of *Pythium aphanidermatum* in 46 naturally infested soils which had soil pH values above 5.0 (A), and in a serial dilutions of soils Ch-1 and Fy-1 infested with different population densities (B).

were artificial. Oospores obtained from laboratory may have different physiology from the naturally formed ones and wheat inoculum may serve as substrate for the other soil microorganisms which may compete with *P. aphanidermatum,* and reduce the inoculum potential of the pathogen (1,8,20). More studies on the influence of soil pH on the behavior of naturally formed oospores of *P. aphanidermatum* in Fy-1 soil are needed.

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摘　　要

林益昇[1]、楊慶鴻[1]、M. E. Stanghellini.[2] 1992. 腐敗病菌接種源濃度與絕對接種源潛勢的關係. 植病會刊 1:13-18. (1. 台中市　國立中興大學植物病理學病，2. 美國亞里桑納大學植病系)

利用馬鈴薯誘釣法量取之馬鈴薯塊纏繞率和盆栽胡瓜幼苗之猝倒率皆隨腐敗病菌 (*Pythium aphanidermatum*) 在土壤中的族群密度的提高而增加，馬鈴薯誘釣率和胡瓜幼苗猝倒率呈顯著相關，測定68個田間土壤中的腐敗病菌族群密度與絕對接種源潛勢的相關性，它的迴歸方程式爲 $Y = 41.76 + 6.70 \ln X$ ($r = 0.63$, $P \leq 0.01$)，ID_{50} (馬鈴薯誘釣率50%時土壤中所含腐敗病菌的數目) = 3.4 卵胞子 / 克土。測定其中46個土壤酸鹼度高於5.0的土壤，其迴歸方程式爲 $Y = 41.78 + 8.22 \ln X$ ($r = 0.99$, $P \leq 0.01$)，ID_{50} = 2.7 卵胞子 / 克土。然而測定剩下的22個土壤酸鹼度低於4.99的土壤，其族群密度與絕對接種源潛勢的相關性並不顯著。一些土壤樣本，從田間採集之後，隨即測定並獲得很低的絕對接種源潛勢。但是放置3個月後，將土壤混雜腐敗病菌至50卵胞子 / 克土，則獲得很高的絕對接種源潛勢。換句話說，這些土壤可由抑病變爲導病性質。芬園土壤 (Fy-1) 的 ID_{50} 是53.6卵胞子 / 克土，而且可阻止腐敗病菌在土壤中的建立，可能是抑病土。

關鍵字：腐敗病菌、接種源濃度、絕對接種源潛勢、抑病土、胡瓜。