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Pythium Population의 생태적 특성 고찰을 위한 Soil Dilution Plating Method의 개발

이윤수*

Development of Soil Dilution Planting Method for Ecological Studies of *Pythium* Populations

Youn Su Lee*

Abstract

The number of colonies often decreased more than would be expected in a dilution from 1:50 to 1:100; however, a lack of agreement between the dilution series and the number of colonies obtained also occurred at higher dilutions. In the experiments with each soil subsample, there was sometimes poor agreement between the number of colonies obtained at the same dilution from the A and B subsamples. However, repeated 1:50 dilutions of soil suspensions of subsamples A and B yielded similar numbers of colonies. In the second experiment series conducted with a second composite soil sample, the number of colonies obtained from each soil subsample decreased following air drying. The results suggest that it was difficult to obtain a uniform distribution of *Pythium* propagules in the two sugarcane field soils tested. The high number of propagules detected at the 1:50 dilution could have been due to hyphal fragments or connected hyphal swellings that separated during the final mixing or during plating.

* 강원대 농과대학 농업과학연구소(Institute of Agricultural Science, College of Agriculture, Kangwon National University).

INTRODUCTION

Pythium species have been recognized as important pathogens affecting seedlings and fine roots of many plants[1]. In addition, many species are common soil inhabitants that survives as saprophytes or facultative parasites.

It is difficult to determine the true numbers of *Pythium* and numerous other fungal propagules in soil, and soil microorganisms present in the soil along with *Pythium* species tend to inhibit and mask the population of *Pythium* in soil dilution platings. Screening experiments with various antibiotics, fungitoxicants, and other chemicals indicated that some compounds might be useful in developing selective media for *Pythium* species[2]. Polyene antibiotics were found to be especially useful for the selective isolation of *Pythium*[3,4]. Other selective media for *Pythium* have been developed and used since then[3,5,6].

Pythium species, particularly *P. arrhenomanes*, cause root rot and significant growth reductions in sugarcane in Louisiana[7,8]. Experiments to develop a soil dilution plating method for use in ecological studies of *Pythium* populations in sugarcane plants and field soil are reported.

MATERIALS AND METHODS

Experiment series one (Experiment 1 through 4). Soil samples were randomly collected from a sugarcane field at the LSU St. Gabriel Branch Experiment Station with a shovel, combined, and thoroughly mixed to form a composite sample. One composite soil sample was sieved with a No. 35 sieve (opening in 0.5 mm) and divided into two equal parts. A soil suspension was prepared from each subsample by adding 50 g of soil to sterile 0.8% water agar to give a total volume of 250 ml. The suspension was them poured into a sterile 500 ml flask and set on a mechanical shaker for 30 min. Next, 10 ml of soil suspenison was withdrawn and put into a sterile 100-ml graduated cylinder. Sterile 0.8% water agar was added to give a total volume of 100 ml (1:50 soil dilution). The suspension was stirred and the dilu-

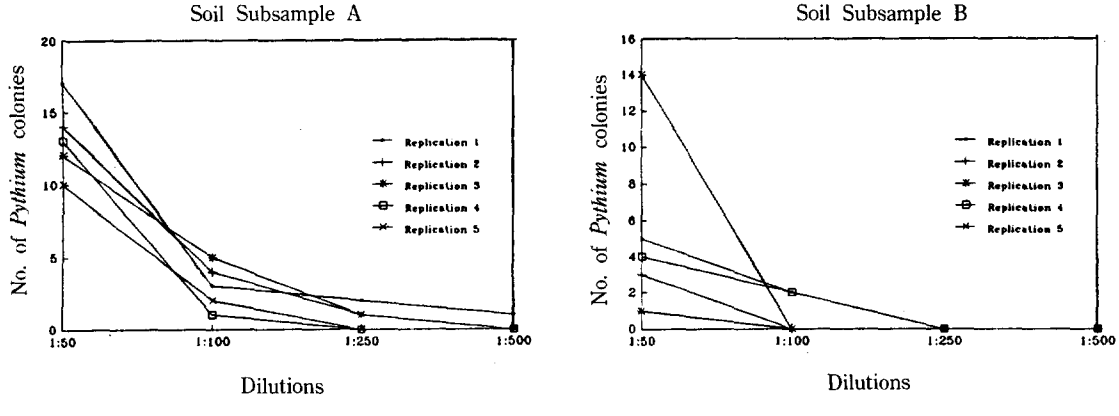

Fig. 1. Comparison of numbers of *Pythium* colonies in different replicates in a soil dilution plating with four dilutions in first experiment. Composite soil sample was mixed and divided into two equal parts, Soil A and Soil B.

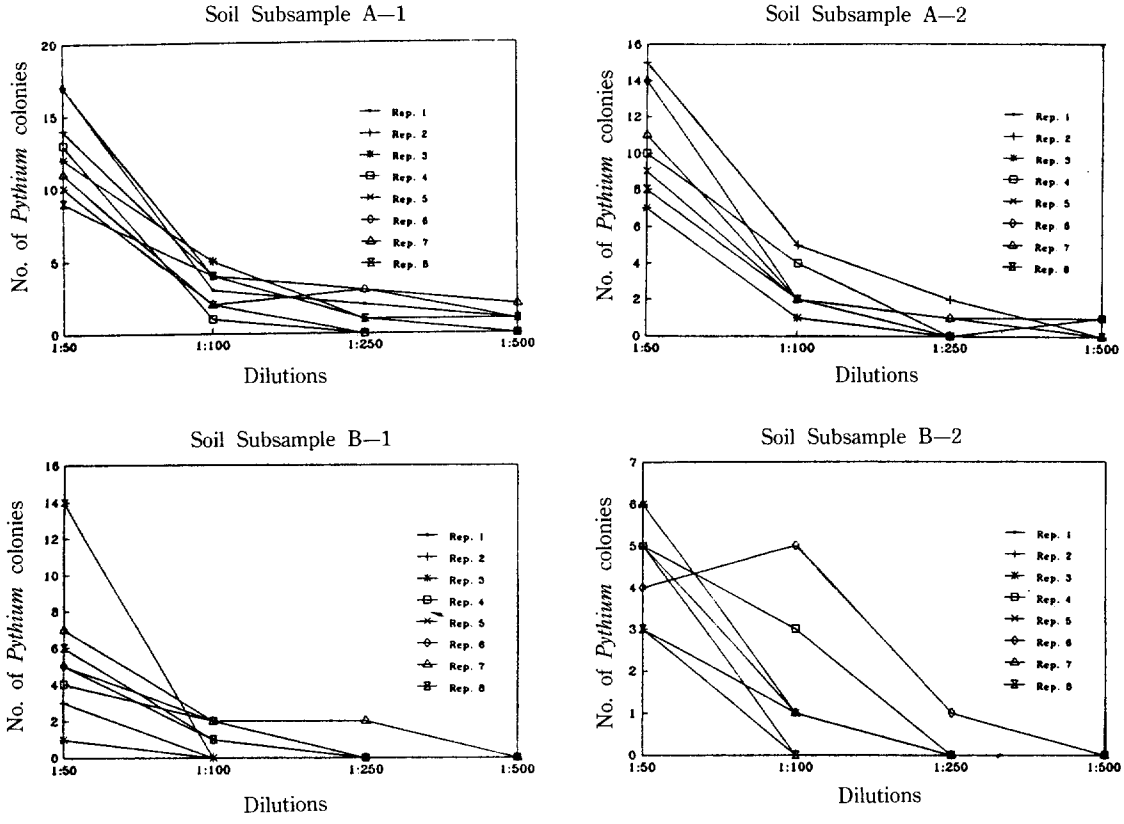

Fig. 2. Comparison of numbers of Pythium colonies in different replicates in a soil dilution plating with four dilutions in second experiment. Composite soil sample was mixed and divided into two equal parts for both Soil A and Soil B.

tion procedure was repeated to form additional soil dilutions. From each dilution, single 1-ml aliquots were transferred onto plates of VP_3 medium[5] in 9-cm-diameter petridishes prepared within 36 hr. The VP_3 medium was prepared as follows: 20 g sucrose, 10 mg $CaCl_2$, 10 mg $MgSO_4$ $7H_2O$, 1 mg $ZnCl_2$, 0.02 mg $CuSO_4$ $5H_2O$, 0.02 mg MoO_3, 0.02 mg $MnCl_2$, 0.02 mg $FeSO_4$ $7H_2O$, 100 μg Thiamine HCl, 17 g Cornmeal agar, and 23 g Difco agar was added into 990 ml of deionized water in a 2 L flask, and autoclaved for 20 min. When the autoclaved basal medium was cooled to 50−55°C, 75 mg vancomycin, 100 mg PCNB, 50 mg penicillin, 5 mg pimaricin, and 2.5 mg rose bengal, mixed to a paste in 5 ml sterile distilled water. The paste residue was rinsed and added to the medium with another 5 ml of sterile distilled water. The medium was mixed with magnetic stirrer.

An aliquot was spread over the surface of a plate using a sterile, bent glass rod while the dish was rotated. For the first experiment, five replicates, for the second and third experiments, 16 replicates for each dilution of each soil subsa-

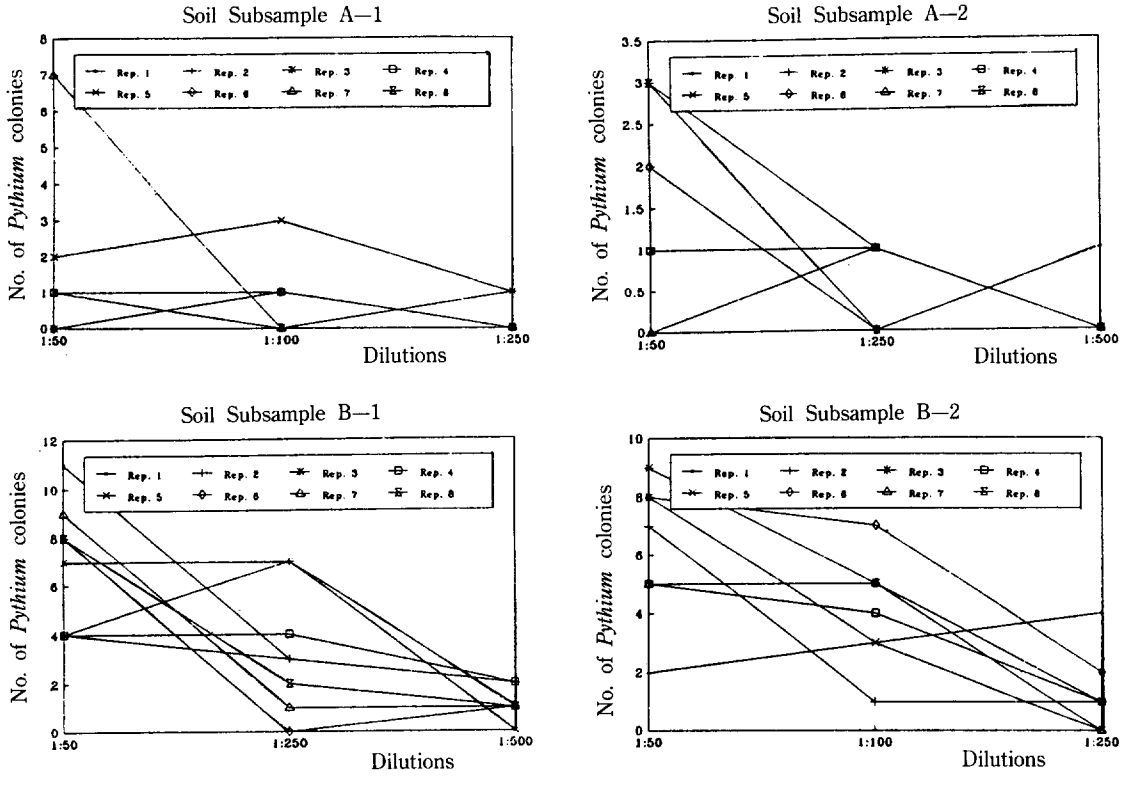

Fig. 3. Comparison of numbers of Pythium colonies in different replicates in a soil dilution plating with four dilutions in third experiment. Composite soil sample was mixed and divided into two equal parts for both Soil A and Soil B.

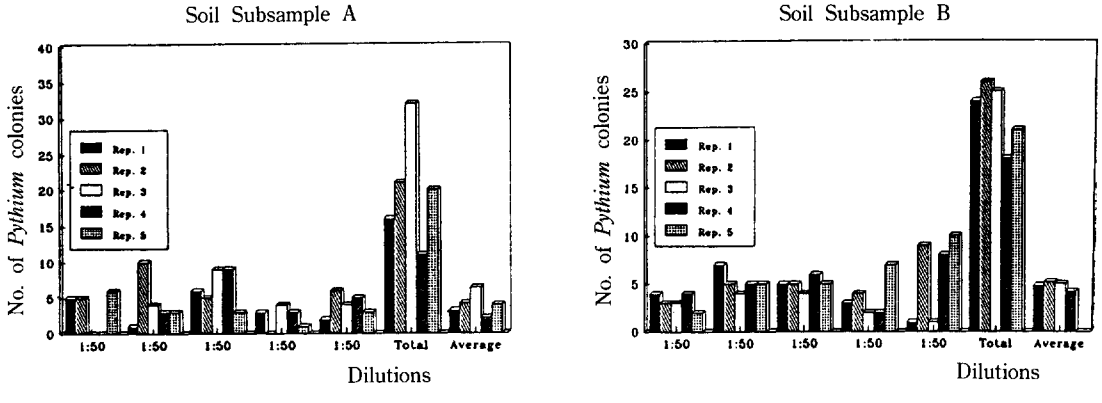

Fig. 4. Comparison of numbers of Pythium colonies obtained in soil dilution plating of repeated 1:50 dilutions made form two soil suspensions in fourth experiment. Composite soil sample was mixed and divided into two equal parts, Soil A and Soil B.

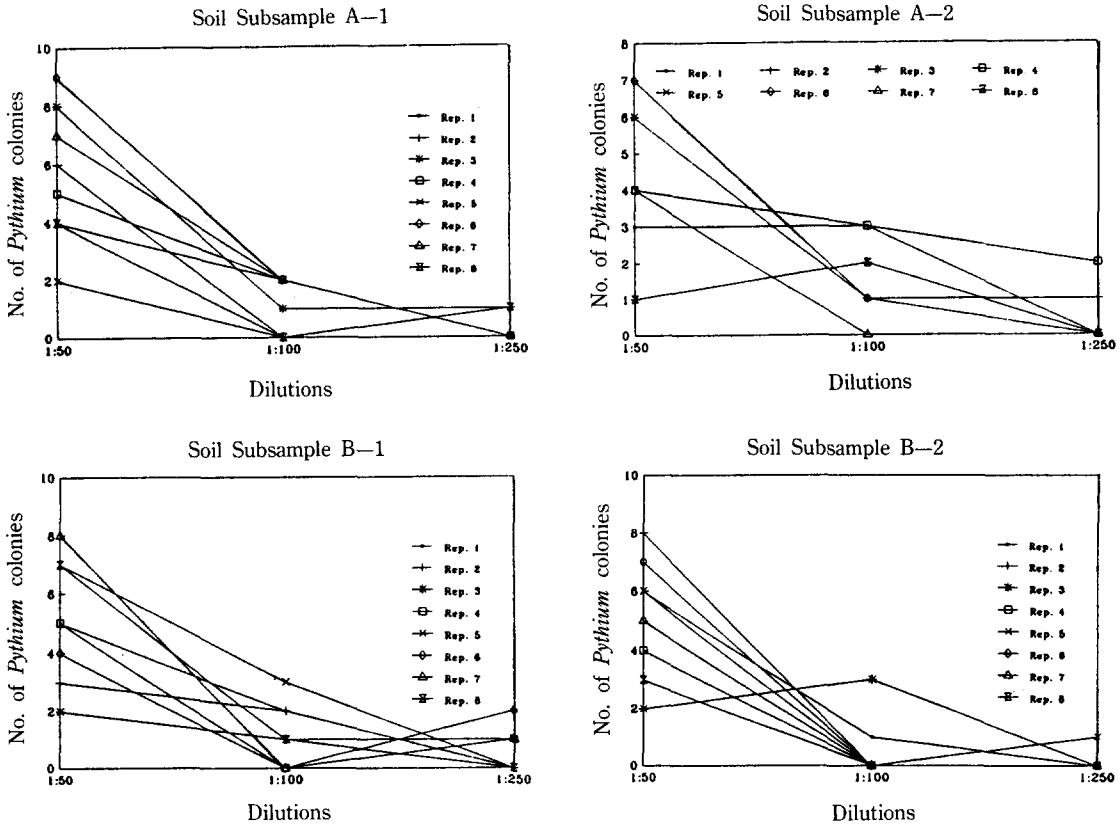

Fig. 5. Comparison of numbers of Pythium colonies in different replicates in a soil dilution plating with three dilutions in fifth experiment. Composite soil sample was mixed and divided into two equal parts, Soil A and Soil B.

mple, and for the fourth experiment, 25 replicates were prepared for each dilution of each soil subsample. For the first and second experiment, soil dilution series of 1:50, 1:100, 1:250 and 1:500 were used; for the third experiment, dilution series of 1:50, 1:100 and 1:250 were used; and for the fourth experiment, dilutions of 1:50 only were used.

Experiment series two (Experiment 5 and 6). For experiment series two, the methods were the same as for experiment series one except for the following: soil dilution series of 1:50, 1:100 and 1:250 were prepared without using 0.8% water agar, and 16 replicates for each dilution of each soil subsample were prepared. For experiment series one, fresh field soil was used, and for experiment series two, soil air-dried for 7 days was used.

RESULTS AND DISCUSSION

The results from a series of soil dilution platings of two subsamples from two composite sugarcane field soil samples from the same location are

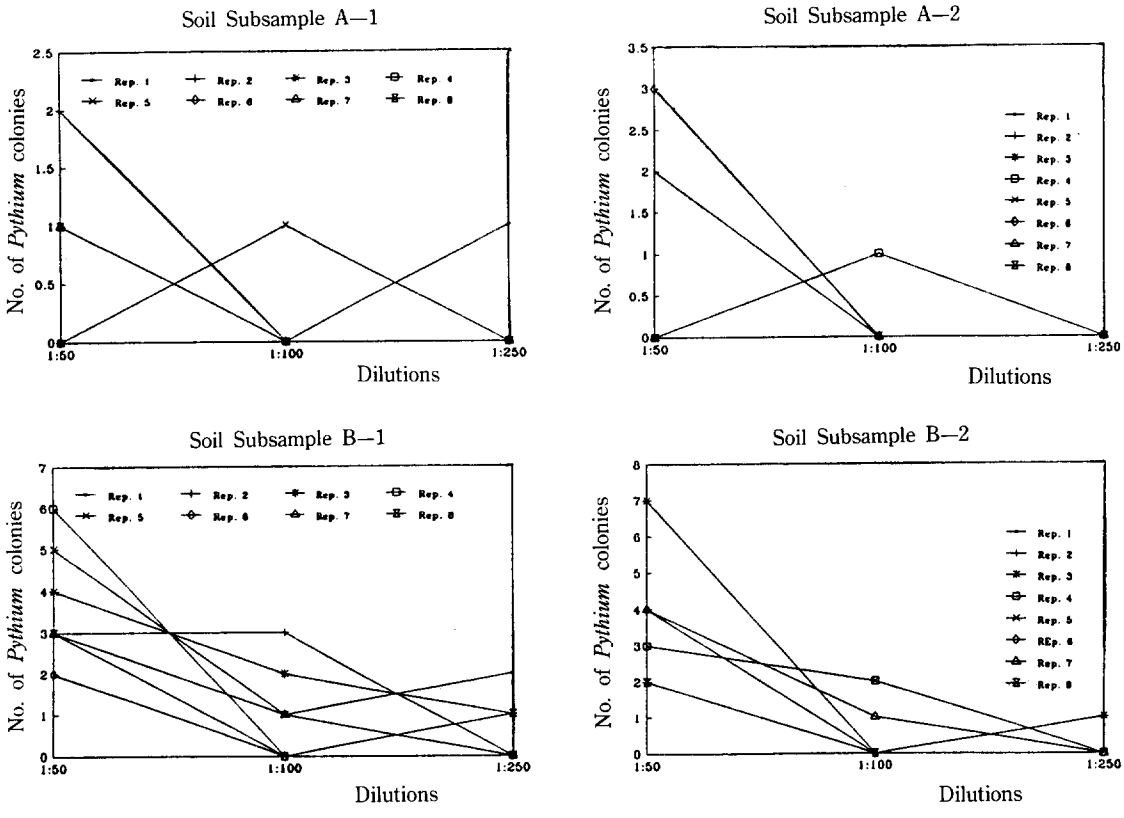

Fig. 6. Comparison of numbers of Phthium colonies in different replicates in a soil dilution plating with three dilutions in sixth experiment. Composite soil sample was mixed and divided into two equal parts for both Soil A and Soil B. The soil was dried for seven days before the experiment was conducted.

presented in Figures 1-6. The number of *Pythium* colonies obtained approximated the dilution series in only 3 of 10 dilution platings (Fig. 3 and 5). The number of colonies often decreased more than would be expected in a dilution from 1:50 to 1:100 (Fig. 1, 2, 5 and 6); however, a lack of agreemet between the dilution series and the number of colonies obtained also occurred at higher dilutions. In the experiments with each soil subsample, there was sometimes poor agreement between the number of colonies obtained at the same dilution from the A and B subsamples (Fig. 1, 2, 3 and 6). However, repeated 1:50 dilutions of soil suspensions of subsamples A and B yielded similar numbers of colonies (Fig. 4). In the second experiment series conducted with a second composite soil sample (Fig. 5 and 6), the number of colonies obtained from each soil subsample decreased following air drying.

The results suggest that it was difficult to obtain a uniform distribution of *Pythium* propagules in the two sugarcane field soils tested. The high number of propagules detected at the 1:50 dilution could have been due to hyphal fragments or

connected hyphal swellings that separated during the final mixing or during plating.

요 약

토양중 *Pythium*속 사상균의 취락조사시 희석평판을 위한 희석배수가 균수에 미치는 영향을 조사한 결과 다음과 같은 결과를 얻었다.

1. 희석배수 1:50은 1:100 보다 기대했던 것 보다 *Pythium*속 취락수가 감소했으며 높은 희석배수에서도 뚜렷한 상관관계를 보이지 않음.
2. 토양시료를 이용한 희석배수실험에서도 유사한 결과를 보였으나 1:50의 희석반복실험결과는 상관관계가 관찰되었음. 그러나 토양을 풍건시켰을 경우 취락의 감소경향을 발견하였음.
3. 본 시험에 사용한 두개의 사탕수수포장에서 채취한 토양에서 *Pythium*번식체가 균일하게 존재하지 않는 것을 확인할 수 있었으며 희석배수 1:50 일때 상관관계를 보인 것은 토양중 균사파편 혹은 분주과정에서 균사위조현상에 기인된 것으로 생각됨.

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