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The Nature of Fungistasis in Sterile and Glucose-peptone Amended Soil on *Helminthosporium victoriae* and *Mortierella* n. sp.

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殺菌土壤, 自然土壤 및 Glucose-peptone으로 改良한 土壤條件이 *Helminthosporium victoriae*와 *Mortierella* n. sp.에 미치는 靜菌作用

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Abstract: The characteristics of the six different agricultural soil from Michigan were as fo llows. Colwood and Capac soil were loam, Gilford and Ceresco were sandy clay loam, Sission was clay loam, and Spinks was sandy loam. pH of Gilford sandy clay loam was 6.6 whereas that of the soil ranged 5.4~5.9. Gilford sandy loam found to contain a relatively higher amount of organic matters as compared to other soils. Furthermore, the numbers of bacteria in Gilford sandy clay loam were significantly higher than those in other soils. The populations of fungi in Gilford sandy clay loam and Colwood loam soils were significantly greater than those in other soils. On the other hand, the densitics of actinomycetes in Gilford sandy clay loam and Ceresco sandy clay loam soils were significantly different from those in other soils. The population of anaerobic bacteria varied depending on the soils; Ceresco sandy clay loam, Capac loam, Colwood loam soils have higher numbers of bacteria, whereas Gilford sandy clay loam was very lesser than the other soils. In the ^{14}C-glucose respiration by soil microorganisms after 10 hrs, the respiration rate was decreasing in the order of Ceresco sandy clay loam, Spinks sandy loam, Colwood loam, Sission clay loam, Capac loam and Gilford sandy clay loam. Germination of test propagules on natural soil soil was 0~5%, and it was germinated 90~98% on autoclaved soil and PDA. The propagules differed in thier germination response to nutrients added to the soils. In general, more nutrients were required to promote germination on Capac loam and Gilford sandy loam soil than Spinks sandy loam soil. Especially *Mortierella* n. sp. required more nutrients for germination to obtain the same ratio as *Helminthosporium victoriae*.

Introduction

Ko and Lockwood (1967) proposed that soils are maintained in a fungistatic status by continuous microbial competition for minute qualities of nutrients that may be required for germination by many spores. However spores and sclerotia of certain fungi will germinate in water alone, but only sparesely in soil. Germination of such propagules is also suppr-

essed when incubated on sand undergoing aqueous leaching in a system designed to impose upon the propagules diffusion stress similar to that imposed in soil through microbial activity (Ko & Lockwood, 1967; Hsu & Lockwood, 1973, and Bristow & Lockwood 1975[a,b]). Evidence proided by Hora and Baker (1970) suggested the involvement of a volatile fun gisatic factor in addition to nutrient deprivation. Ammonia has been identified as the volatile fungistatic factor in alkaline soil (Ko *et al.*, 1974), and ethylene was also reported to be the active substance in several Australian soils (Smith, 1973). This research was undertaken to evaluate the roleof the microbial nutrient titration in soil fungistasis in light of nutrition amendments with glucose and peptone.

Materials and Methods

Characteristics of soils. Six different agricultural soils from Michigan were used. Soils were taken from the field, air-died, passed through a 10mm sieve and stored in large plastic bags at 4°C for duration of work. Subsamples were taken from the soil and allowed to equilibrate at room temperature (24°C) for several days before use. Textural analysis were determined by hydrometer method (Day, 1965), and pH of the soils water paste(1:2 W/W) was measured using a glass electrode. Organic matter contents were measured by combustion method.

Maintainance of propagules. *Helminthosporium victoriae* (strain 418) and *Mortierella* n. sp. were maintained on potato dextrose agar. Culture transfer were routinely derived from one parent culture of each fungus.

Preparation of propagules suspension. The following procedure was routinely used to compare propagules suspensions of the fungi. The surface of an agar culture was flooded with cold sterile and dilute Pfeffer's salt solution (Bristow & Lockwood, 1975[a]). Propagules were gently dislodged with a bent glass rod, and the suspension was passed through a 250μm stainless steel sieve into a cold(5°C) centrifuge tube. Propagules in the capped tube were washed 3 times by centrifugation ($10^4 \times g$ for 5 min. at 5°C).

The final suspenaion volume ranged from 10~20 ml and held in ice throughout the experiments. The density of conidia in suspension was deertmined by microscopically counting conidia by haemocytometer. In all assays of propagules germination, propagules in suspension were vaccum deposited on 1.5cm × 1.5cm pieces of Nuclepore membrane filters (0.4μm pore dia., Nuclepore Corp., Pleasantan. LA, U.S.A.). Propagules on densities membrane were 10^3 for two fungus

Sensiitivity of propagules to soil fungistasis. Germination of propagules was determined on sterieized soil and nonsterile soil (natural soil) amended with increasing amounts of glucose and peptone(Hsu & Lockwood, 1971) to determine their sensitivities to mycostasis. Fifty gram samples of the soil were wetted to −0.05 bar matric potential, and were equilibrated from 16~24 hr before use. Soils in 9cm dia glass dishes were sterilized by autoclaving for 1hr. Natural soil or amended soil were contained 9cm dia glass dishes. Glucose concentrations ranged from 10^2 to 10^3mg/g of soil and peptone concentrations were 1/5/(W/W) that of glucose. The soil was well mixed with spatula. smoothed, and allowed to equilibrate for 1hr. In each experiment, duplicate Nuclepore membranes bearing fungal propagules wererplaced on duplicate samples of untreated and treat soil. Conidia were incubated on the soils for 12~16hr prior to germination assay. Membranes bearnig propagules were stained with phenolic rose bengal, destained in water, and mounted on glass slide with double sticky tape. Germination was counted microscopically with incident illumination. Three to four experiments were done.

Microbial populations in soils. Microbial populations in soils were estimated by dilution plate counts on the following media: Chitin medium (Hsu & Lockwood, 1975) for actinomycetes; trypticase soy broth (Rhode, 1968) containing 50ppm of PCNB (pentachloronitrobenzene) to inhibit actinomycetes (Farley & Lockwood, 1968) for aerobic bacteria; and PDA supplemented with 250mg of chloramphenicol and 0.5ml of a detergent TMN (Union Carbide,

NY., U.S.A.) for fungi. Plates were streaked with 0.2ml of soil suspension and incubated at 24C for 5 ~7 days for bacteria and fungi and 10~12 days for actinomycetes. Anaerobic bacteria were determined using thioglycolate broth(Difco, Detroit, MI., U.S.A.) and the most probable number technique (Alexander, 1965). Two to three determinations of microbial populations per soil were made.

Soil respiration using ^{14}C-glucose. Four gram of soil in small incubation chambers were wetted to about −0.05 bar matric potential and equilibrated for 16~24hr. Soils were then pulsed with uniformly labelled ^{14}C-glucose (specific radioactivity 3×10^3 dpm). The respiration apparatus and $^{14}CO_2$ collection methods were identical to those used in the ^{14}C exudate method (Filonow & Lockwood, 1979), and radioactivity was measured in a Packard Tri-carb liquid Scintilation counter (Model 578).

Results

The characteristics of the six different agricultural soils from Michigan were as follows: Colwood and Capac soilwere loam, Gilford and Ceresco were sandy clay loam, Sission was clay loam, and Spinks was sandy loam (Table I). pH of Gilford sandy clay loam was found to be 6.6 whereas that of the other soils ranged 5.4~5.9. Gilford sandy clay loam exhibited a higher level of organic matter than the other soils although contents of organic matter in soils varied. Spinks sandy loam showed a lower amounts of organic matter as compared to the others. In populations of microorganisms in soil, bacteria did not differ in Colwood loam, Capac loam, Sission clay loam, Spinks sandy loam and Ceresco sandy clay loam, but it was significantly greater in

Table I. Characteristics of soil.

Soils	%				Texture	pH
	O.M.	Sand	Silt	Clay		
Colwood	5.68	47.1	32.1	20.7	Loam	5.49
Capac	3.33	43.1	32.1	24.7	Loam	5.88
Sission	3.05	43.1	22.1	34.7	Clay loam	5.58
Gilford	11.7	59.8	18.7	21.4	Sandy clay loam	6.58
Spinks	2.35	75.8	8.7	15.4	Sandy loam	5.93
Ceresco	5.42	47.8	28.7	23.4	Sandy clay loam	5.43

Results are means of a duplicate determination. O.M is organic matter contents.

Table II. Populations of microrganisms in soil.

Soils	Colony forming units/g soil			
	Bacteria$\times10^6$	Fungi$\times10^4$	Actinomycetes$\times10^5$	Anaerobes$\times10^5$
Colwood	21a	15bc	11a	110
Capac	18a	13bc	11a	140
Sission	18a	10a	6.6a	3.3
Gilford	33b	16c	12a	13
Spinks	17a	4.1a	15b	4.9
Ceresco	28a	1.3a	24b	280

Numbers within a column followed by the same letter are not significantly different (p=0.05) using Duncan's multiple range test.

numbers present in Gilford sandy clay loam soil (Table II).

Although there no were significantly different in the populations of fungi among Ceresco sandy clay loam, Spinks sandy loam and Sission clay loam, these three soils were significantly different from Gilford sandy clay loam, Colwood loam and Capac loam. In the case of the popul ation densities of actinomycetes Spinks and Ceresco soils were significantly different from that of Colwood loam, Capac loam, Sission clay loam and Gilford sandy clay loam (Table II).

In the ^{14}C-glucose respiration by soil microorganisms after 10 hrs, the respiration rate was decreasing in the order of Ceresco sandy clay loam, Spinks sandy loam, Colwood loam, Sission clay loam, Capac loam and Gilford sandy clay loam (Fig. 1). Germin

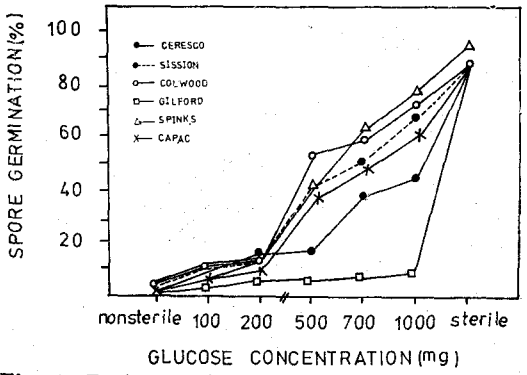

Fig. 1. Evolution of $^{14}CO_2$ from soils following the application of (^{14}C)-glucose to the soil sur face.

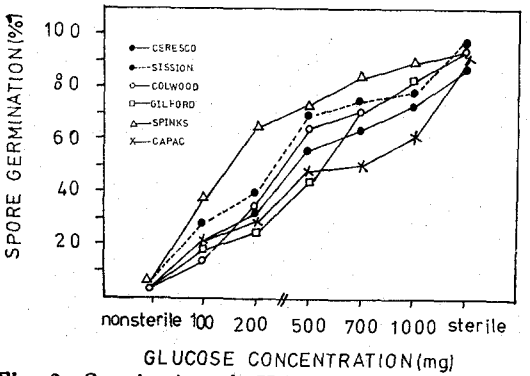

Fig. 2. Germination of ***Helminthosporium victoriae*** conidia on soils supplemented with glucose and peptone and control soils.

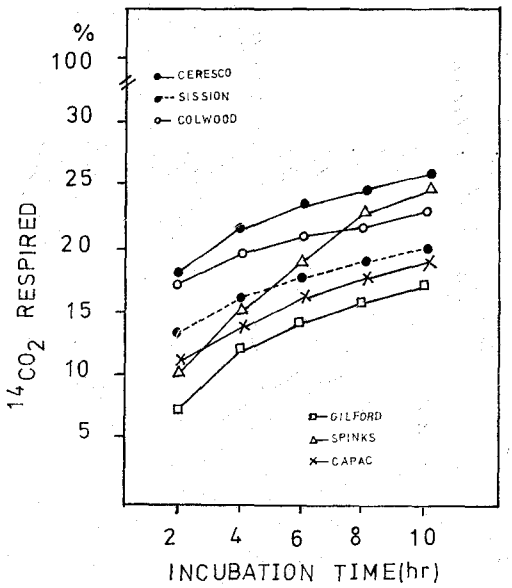

Fig. 3. Germination of ***Mortierella*** n. sp. conidia on soils supplemented with glucose and peptone and control soils.

ation of test propagules on natural soil was 0~5%, indicating that all the soils were mycostatic against the fungi. The two fungi germinated from 90~98 percent on autoclaved sterile soils. The propagules differed in their germination response to nutrient added to the soils. In general, more nutrients were required to promote germination on Capac loam and Gilford sandy clay loam soil than Spinks sandy loam soil. Especially ***Mortierella*** n. sp. required more nutrients for germination to obtain the same ratio as *H. victoriae* (Fig. 2, .3)

Discussion

Mycostasis in soil has been attributed to the inherently low concentration status of soil and rapid soid loss of spore nutrients essential for germination of competing microorganisms in soil(Ko & Lockwood, 1967; Yoder & Lockwood, 1973; and Lockwood 1977). Filonow and Lockwood (1979) reported that microbial nutrient sinks of the coarse textured soils were more active withrawing exudate from the propagules than those of the fine textured soils. Bristow and Lockwo

od (1975) suggested that respiration of ^{14}C-glucose by soil microorganisms may provide an alternative method for comparing the relative efficiency of soil nutrient sinks, since the order of spore exudation on soils was similar to that obtained with ^{14}C-glucose.

In this experiments, mycostasis in light textured Spinks sandy loam, having small numbers of microbes and higher $^{14}CO_2$ evolvement, was largely annulled at the lowest concentrations of glucose and peptone, whereas that in heavier Capac loam and Gilford clay loam soils required more nutrients before substantial germination stimulation occurred. These results were very similar to other reports (Filonow & Lockwood, 1979; Lingapa & Lockwood, 1974; and Griffin 1973). The greater microbial sinks in Gilford sandy clay loam soil may be attributed to the higher numbers of microorganisms as well as to greater microbial activity.

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摘 要

供試한 6個地域의 土性은 Colwood와 Capac이 良質土이고 Gilford와 Ceresco는 砂質埴壤土이며 Sission은 埴壤土이고 Spinke는 砂質土이었다.

土壤酸度는 Gilford砂質埴壤土가 6.6이고 기타는 5.4~5.9의 범위로 나타났으며 有機物含量은 Gilford土壤이 제일 많이 함유하고 있었다. 一般細菌數는 Gilford 토양에서는 다른 土壤에서 보다 통계적으로 많았고 絲狀菌數에서도 Gilford와 Colwood土壤이 다른 토양보다 통계적으로 더 많았다. 放線菌의 密度를 보면 Gilford와 Ceresco의 土壤에서 많았고 嫌氣性 細菌의 數는 Ceresco, Capac 및 Colwood에서 많았으며 반대로 砂質埴壤土인 Gilford에서는 적었다.

^{14}C-glucose를 첨가한 土壤에서의 微生物의 呼吸量은 Ceresco, Spinks, Colwood, Sission, Capac 및 Gilford의 順으로 적었다.

供試菌株 *Helminthosporium victoriae*(#418)와 *Mortierella* n. sp.에 대한 自然土壤, 殺菌土壤및 glucose와 peptone을 濃度別(mg/g 土壤)로 첨가한 土壤에서의 發芽率을 보면 自然土壤에서는 0~5%이고 殺菌土壤에서는 90~98%이었다. 土壤에 添加된 營養物의 濃度에 따라 供試菌의 發芽정도는 달랐다. 일반적으로 Capac과 Gilford에서는 Spinks土壤 보다 發芽에 필요한 營養物을 더 많이 要求하였으며 특히 *Mortierella*는 *H. victoriae*보다 發芽에 있어서 더 많은 量의 營養物을要求하였다.

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