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Effect of Conidial Number and Nutrition on the Germination of Conidia in *Septoria glycines*

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分生胞子數 및 營養狀態가 대두갈색무늬병균의 分生胞子 發芽에 미치는 影響

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ABSTRACT

Conidial germination of *Septoria glycines* Hemmi, brown spot fungus of soybean, was studied by slide germination test. Poor conidial germination of *S. glycines* was observed on sterile distilled water, but potato dextrose agar(PDA) and distilled water floated with soybean leaf disc furnished a satisfactory medium for conidial germination. Exogenous supply of carbon source was essential for conidial germination, while phosphorous and potassium were not evident as that for carbon. Soluble starch was the most suitable as a carbon source for conidial germination and followed by D-glucose, D-galactose and lactose in that order. Maximum germination was attained in the 5×10^{-2}mol. concentration of glucose. Germination was decreased with increment of conidial concentration and was almost completely suppressed in the density of 10,000 conidia per mm^2. It suggested existing a self-inhibitor(s). Non-washed conidia germinated more than washed conidia and this was obvious when the conidia density was over 2×10^3 conidia per mm^2 on the dry agar block.

INTRODUCTION

Septoria brown spot caused by *Septoria glycines* Hemmi is one of the most serious fungal disease in soybean. It occurs in the field everywhere the soybean is growing and results in a remarkable reduction of soybean yield.[13,14,19] The disease appears early in the rainy season on the primary leaves of soybean plants. As the soybean plant grows, the pathogen spreads from lower to upper leaves in warm humid weather condition throughout the growing season.[11]

Since the disease infection is initiated in most instances by a conidial germination within or near the phylloplane, conidial germination is a crucial event for pathogenic fungi in particular and it becomes a determining factor in the onset of host colonization. On this account, many studies on nutritional factors and self-inhibition of conidial germination

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have been reported with a number of fungi,[1,3,5,6,9,17] however, little information is available for septoria brown spot fungus. Therefore, our purpose was to determine nutritional effects and self-inhibition for conidial germination of *Septoria glycines* Hemmi.

MATERIALS AND METHODS

Isolation of the causal organism: *S. glycines* isolates used in this study were originally collected in August, 1978 from naturally infected soybean plants in the field. Infected leaves were washed three times under running tap water to reduce number of saprophytic fungi. The leaves were placed on a moist filter paper in petri plates. After 3~5 days at 30°C, pycnidia production was abundant and conidia were oozed out above the pycnidia. Then the conidial mass was transferred to potato dextrose agar plate(PDA) by needle under magnifier. After another 3~5days, uncontaminated colonies were transferred to separate PDA plates and incubated for culture.

Nutritional requirement for conidial germination: Conidia were obtained from 14-day old cultures in petridishes with labroatory PDA media incubated at 25°C in dark condition. Conidial mass oozed out on the top of pycnidia was collected into a few ml. of sterile distilled water. For determination of percent germination, slide germination test[8] was applied in all experiments unless otherwise indicated. Conidia with concentration of 500~1,000 conidia/mm^2 were placed in water drops on sterile glass slide and incubated in moist chamber at 23~25°C for desired time period. The glass slides were acid cleaned and rinsed with several changes of distilled water. Impurities in agar reagent was removed by leaching with pyridine solution by method of Lilly and Barnett.[12] The percentage of germinated conidia was determined microscopically by counting at least 100 conidia from randomly selected fields on each plate. The criterion of germination was the formation of a microscopically visible germ tube which is roughly corresponding to the diameter of the conidia.

Effect of conidia numbers on germination: The conidia collected from 14-day old PDA cultures were suspended with sterile distilled water for desired concentration without agitating. These were considered to be nonwashed conidia. Washed conidia was prepared by centrifuging the conidial suspension at 5,000rpm for 30min and precipitant was resuspended with distilled water. This procedure was repeated three times. Fresh suspension of washed and nonwashed conidia were deposited onto wet and dry agar blocks. Wet agar block was made with 1cm^2 piece of 2% water agar on a glass slide. For dried agar block was air dried at room temperature for 20 hours. The percentage of conidial germination was determined with at least 200 conidia on each agar block.

RESULTS

Since poor conidial germination on distilled water was observed, effect of various media was evaluated for conidial germination of *S. glycines*(Fig.1). Potato dextrose agar(PDA) and distilled water floated with soybean leaf disc could furnish a satisfactory medium for germination. Generally, about 50% of the conidia began to form germ tubes at approximately 12 hours on these media and more than 90% of the conidia were completed germination within 48 hours. Even on the purified water agar plate, germination was as good as other media at 48 hours. Whereas, germination on distilled water was less than 15% after 72 hours. This indicates that exogenous supply of certain nutrients was required for conidial germination of *S. glycines*.

To determine what element was essentially required for conidial germination, various compounds were prepared individually with one milimole concentration and a drop of the solution was pipetted onto slide glass for germination test(Table 1). There was a good germination on sterile distilled water by adding glucose solution, but a little on the mineral solutions mixed with various compounds without glucose. The need for other elements, such as potassium and phosphorous, was not so evident as that for carbon. While, lack of sulfur and nitrogen caused less germination until 48 hours. Particularly, nitrogen was believed to need not only for condial germination but also germ tube elongation. When nitrogen lacks

Table 1. The essentiality of various ions for the conidial germination of *Septoria glycines* on sterilized distilled water

Treatment[a]	Lack of nutrients	Germination(%)		
		24 hrs	48 hrs	72 hrs
Distilled water	Carbon and minerals	0	5.9	13.2
Glucose	Minerals	13.1	92.1	96.5
Glucose, KNO_3, KH_2PO_4, $MgSO_4$	None	60.0	93.4	100
Glucose, NH_4Cl, KH_2PO_4, $MgSO_4$	None	87.8	99.0	100
Glucose, $NaNO_3$, NaH_2PO_4, $MgSO_4$	Potassium	47.5	97.0	100
Glucose, KCl, KH_2PO_4, $MgSO_4$	Nitrogen	50.0	50.0	65.7
Glucose, KCl, KNO_3, $MgSO_4$	Phosphorus	89.6	98.0	98.0
Glucose, KNO_3, KH_2PO_4, $MgCl_2$	Sulfur	22.1	59.2	80.5
KNO_3, KH_2PO_4, $MgSO_4$	Carbon	0	1.5	11.3

a. In all cases, the concentration of the mineral salts was 1.0millimole

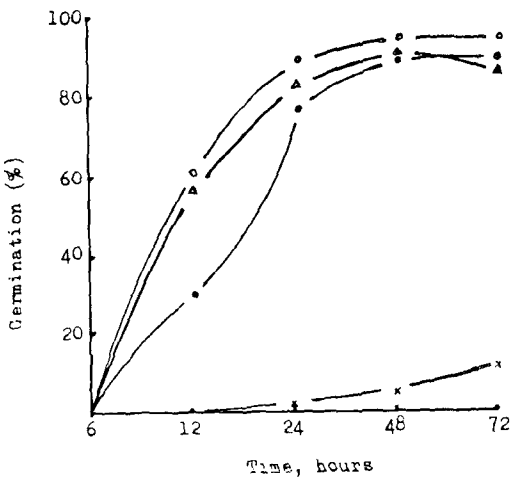


Fig. 1. Conidial germination of *Septoria glycines* with time course on PDA(—○—) distilled water with soybean leaf disc(— · —) purified agar(—△—) and distilled water(—×—)

in media, elongation of germ tubes was very poor. No evidence was found that an exogenous supply of any special amino acids and vitamins was necessary for conidial germination of *S. glycines*. The effect of carbon sources varied with a kind of carbon compounds(Table 2). Of all carbon sources tested, soluble starch was the most suitable and followed by D-glncose and D-galactose in that order. In disaccharides, lactose was good for carbon source, but sucrose was poorly utilized. With starch, germination was 26.5% at 12 hours and reached its maximum 81.6% within 48 hours. D-glucose and D-galactose

Table 2. Effect of carbon sources on conidial germination of *Septoria glycines* in sterilized distilled water[a]

Source	Germination(%)[b]		
	24hrs	48hrs	72hrs
Monosaccharides			
D-Glucose	15.4	75.5	96.5
D-Fructose	—	—	5.5
D-Mannose	—	2.1	19.9
D-Galactose	7.3	75.4	98.7
D-Xylose	—	—	2.5
Disaccharides			
Maltose	—	—	2.1
Lactose	2.5	57.4	95.3
Sucrose	1.7	2.3	3.8
Polysaccharides			
Starch	26.5	81.6	98.3
Inulin	—	—	3.1
Cellulose	—	—	2.7

a. Conidia(10^3/ml) were placed in a water drop with a drop of each carbon source(5×10^{-3} mol.) on sterile glass slide

b. At least 100 conidia were counted on time after incubated at 23~25°C

were much less effective at 24 hours but increased with time course as much as those in soluble starch. While, the least germination occurred with fructose, mannose, xylose and maltose including some polysaccharides other than soluble starch. Concentration of

Table 3. Effect of different concentrations[a] of D-glucose on conidial germination of *Septoria glycines* on sterile glass slide

Conc.(Mol.)	Germination(%)[b]		
	24hrs	48hrs	72hrs
1	0	1.5	45.0
5×10^{-1}	2.5	52.5	87.0
5×10^{-2}	48.2	86.0	96.5
5×10^{-3}	17.2	50.0	88.0
5×10^{-4}	10.4	45.0	75.5
5×10^{-5}	0	2	3.2
5×10^{-6}	0	2	2.5

a. Conidia(10^3/ml) were placed in a drop of each concentration of D-glucose
b. At least 100 conidia were counted on time after incubated at 23~25°C

D-glucose in media influenced the amounts of conidial germination(Table 3). Germination was markedly stimulated in the concentration of D-glucose as low as 5×10^{-4}mol compared to sterile distilled water, but maximum percentage of germination was attained in the 5×10^{-2}mol concentration. Germination was rather decreased with increment of D-glucose concentration over 5×10^{-1}mol.

Conidial germination was evidently affected by concentration of conidia deposited on the medium. Crowded conidia did not germinate and the failure was suspected due to a substance produced by the conidia themselves, or the matrix in which the conidia were carried from the pycnidium. In this respect, germination per cent was measured with washed or non-washed spores on wet agar plate(Fig. 2). Germination per cent was decreased with increment of conidial concentration. Increasing the conidial density from 5×10^3 conidia/mm^2 to 10^3/mm^2 almost completely supressed germination. Consistently the non-washed conidia germinated more than did non-washed conidia and this was obvious when the conidial density was from 2×10^3 conidia/mm^2 to 3×10^3 conidia/mm^2. At the concentration of 5×10^3 conidia/mm^2, elongation of the germ tubes were markedly reduced about half as long as those of lower conidial concentration. On dry agar blocks, germination per cent was drastically reduced with increment of conidial concentration regardless of washed or non-washed conidia as compared to those at wet agar blocks(Fig. 3). Germination of non-washed conidia was increased slightly as the time goes on, whereas almost did not the washed conidia regardless of agar plate deposited.

DISCUSSION

Although, there are many reports that certain species of fungus require an exogenous carbon, nitro-

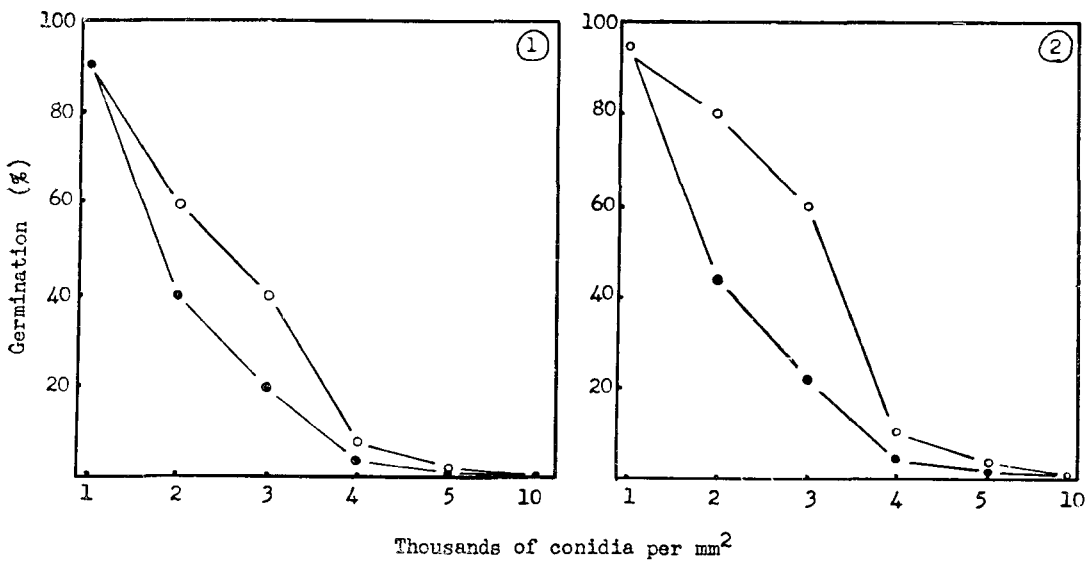
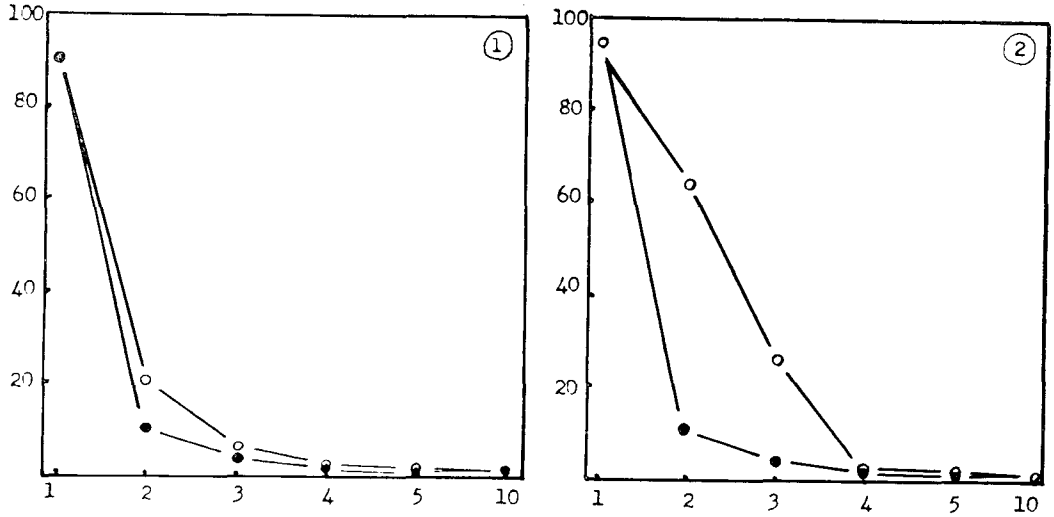


Fig. 2. Germination of washed(—●—) and non-washed conidia(—○—) of *Septoria glycines* 24 hours (1) and 48 hours after seeded (2) with six different concentration of conidia on wet agar plates



. 3. Cermination of washed(—●—) and non-washed conidia(—○—) of *Septoria glycines* 24 hours(1) and 48 hours after seeded(2) with six different concentration of conidia on dry agar plates

nitrogen, phosphorous, sulfur and few natural mins for conidial germination(1, 15, 16), no infor- on is available for nutritional requirement of lial germination in *Septoria glycines*. Present es showed that exogenous supply of carbon sou- was needed for conidial germination of *S. gly-* . Conidial germination was significantly decre- by increase in number of conidia, suggested ing self-inhibitor. The conidial matrix seemed lay a role for protecting the conidia from ger- tion inhibitor. Septoria brown spot resistance in ile leaves was associated with the inhibition of lial germination in the leaf diffusates which ed to contain a fungistatic substance.

ucose as a carbon source for conidial germination be substituted by oligosaccharides which have alactopyranosyl-a-D-glucopyranose linkage. It natural that the conidia could germinate on the ied water agar as appeared in this study, since gar is also composed of mostly d-l-galactose lactan esterized with sulfur(10). It was evident carbon compounds conditions specific changes in onidia which were conducive to germination, t was not clarified whether the nature of the ination-promoting properties of carbon com- ds was due to its rate of permeating the conidia similation in carbon synthesis. Therefore, more critical data for the effectiveness in supporting conidial germination and their relationship to specific metabolic pathways are needed.

It was suggested that *S. glycines* produced a substance inhibting conidial germination in the present study. As the concentration of conidia were increased, conidial germination was proportionally decreased and this trend was more obvious on dry agar blocks. Chung and Wilcoxon(4) also found marked decrease in conidial germination of *Phoma medicagnis* on dry agar blocks as the number of conidia were increased, in contrast to those did not affected on wet agar blocks. They suggested that it was due to presence of water enough for diluting the inhibitor in wet agar blocks. Starvation can be one possible explanation of reduced germination in dense populations of conidia, but can ruled out if nutrients do not overcome the inhibition. The author observed that conidial germination was not increased by adding glucose solution to the media in the present study. This convinced that the crowding effect of *S. glycines* conidia was responsible for an inhibitory substance, and probably the self inhibition was independent from nutrients in view of the fact that addition of glucose did not nullify the inhibitory effect.

It was not clear why the germination of the wa-

shed conidia was decreased more than these of non-washed conidia in the present study. It could be only supposed that the matrix carrying conidia might play a role of protecting the conidia from germination inhibitors in view of the suggestions that matrix may serve as a nutrient to support growth (2) and make conidia resistant to germination inhibitors (4). Informations concerning the mode of action of germination inhibitors are still limited and therefore, more works should be done to learn how the matrix affect the conidial germination of *S. glycines*.

摘 要

대두갈색무늬병균의 포자발아(分生胞子發芽)에 미치는 외부영양공급(外部營養供給) 및 self-inhibitor의 영향(影響)을 조사하여 다음과 같은 결과(結果)를얻었다.

1. 갈색무늬병균의 포자발아율은 감자한천배지 및 대두잎조각을 첨가한 증류수에서는 양호하였으나 살균된 증류수에서는 극히 불량하였다.

2. 갈색무늬병균의 포자발아에는 탄소원의 외부공급이 절대 필요한 것으로 보였으며 인산, 가리 등은 큰 영향이 없는 것으로 보였다.

3. 탄소원으로서는 가용성 전분이 가장 효과적이었고 다음이 포도당, 유당 등이었으며 포도당의 경우 5×10^{-2}mol. 농도에서 가장 높은 발아율을 보였다.

4. 포자발아율은 포자밀도가 높을수록 현저히 감소하여 포자농도 10,000conidia/mm² 이상에서는 거의 발아하지 않는 것으로 보아 self-inhibitor가 존재하는 것으로 보였다.

5. 기질(基質)을 세척한 포자의 발아율은 세척하지 않은 포자의 발아율에 비해 낮았으며 이러한 현상은 포자농도가 2,000conidia/mm² 이상으로 증가할 때 더욱 현저하였고 건조된 한천배양기위에는 발아율 감소가 현저하였다.

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