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Effect of water potential on mycelial growth, reproduction, and spore germination by *Cylindrocladium crotalariae*

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*Cylindrocladium crotalariae*의 菌絲자람, 胞子形成과 胞子發芽에 對한 Water potential의 效果

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Abstract

Mycelial growth and production of macroconidia by *Cylindrocladium crotalariae*. on either PDA or water agar containing soybean leaf pieces adjusted to different water potentials with KCl. were consistently maximal at −14bars. Mycelial growth by this fungus was nil at about −100 bars and below at 30C, −80 bars and below at 25C and −64 bars and below at 20C. Sporulation was prevented at −64 bars and below at 20°C and 25°C. Perithecial andmicrosclerotial formation was maximal at abot −1.4 to −3.0 bars (the basal medium without salt). Percentage of spore germination for this fungus was uniformly maximal at all water potentials between −1.4 bars (the highest tested) and −20 bars, was progressively less as the water potential lowered below −20 bars, and was prevented at −60 bars.

Introduction

Cyindrocladium (*Calonectria*) *crotalariae* (Loos) Bell & Sober causes root and stem rot of soybean in America (11), Japan (10), and Korea (12). The spore stages of the fungus are *Calonectria* ascospore in red perithecium and *Cylindrocladium* macroconidium. The mycelium and microsclerotium are produced in media and infected stem and root.

The diseases caused by soil-borne fungi are grouped in to those favoured by wet and those favoured by dry soil conditions (4). Those fungi which cause severe disease in wet soils have the highest water potential requirements for growth in vitro and, conversely, those fungi which cause severe diseases in dry soils have the lowest water potential requirements.

The effect of water potential on the behavior of numerous fungi has been studied (1, 2, 3, 6, 7). In general, there is an inverse and linear relationship between fungal growth rate and water potential below the optimal and down to the lower limit for growth of various fungi. Reports dealing with the water relations of *C. crotalariae* in artificial culture and in soil do not refer to mycelial growth, for-

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mation of conidia and microsclerotia and germination of spores.

This study is to examine quantitively the effects of water potentials on the mycelial growth, production of conidia and microsclerotia, and germination of spore. Information on the effetcs of water potential on mycelial growth, reproduction, and spore germination by this fungus is needed to provide a more complete picture of theier behavior.

Materials and Methods

Isolates: One single ascospore-derived isolate of *Calonectria crotalariae*, originally isolated from perithecium on infected stem and root and maintained on Potato Dextrose Agar (PDA) slants at 22~24C, were used experiments in vitro.

Media and osmotica: All experiments were carried out with homemade PDA or water agar containing sterile pieces of soybean leaves and each amended with KCI. Five pieces of soybean leaf tissue were used for each dish of the water agar medium. The required amount of salt for a given osmotic potential was dissolved in 1 liter of medium in an Erlenmeyer flask. The medium with disolved salt was then autoclaved at 121C for 20 min. The salt concentrations were varied to provide water potential down to −114 bars. The molten media after autoclaving were dispensed into petri dishes. PDA and water agar with soybean leaf pieces without the added salt had a water potential of about −3 bars and about −1.4 bars, respctively.

This fungus was grown first on PDA. Disks (3 mm in diameter) were cut with a cork borer from advancing margin of 14-day-old cultures and placed in the center of each petri dish of osmotcally adjusted or basal (no KCI) medium. Treatment was replicated three times. The dishes after seeding were sealed in new polyethylene bags and incubated at 20, 25 or 30C.

Measurement of mycelial growth, sporulation and perithecial and microslerotial formation: The mycelial growth was measured after 7days in culture. The amount of asexual sporulartion was estimated after 30 days in culture. To obtain the spores from a given dish, 20m*l* of distilled water containing Tween—20 was poured into each dish, and then the agar surface was rubbed gently with a came-hair brush. Spore numbers/m*l* in the resulting suspensions were counted with a haemacytometer.

Perithecia and microsclerotia were counted under a dissecting microscope 20 days after the cultures were started. The counts were made within 50-mm^2 areas that were marked at random on the soybean leaves or on the PDA surface with an 8-mm-diameter cork borer. Only those perithecia which appeared mature were counted.

Measurement of conidial germination: The influence of osmotic potential on conidial germination was determined on water agar adjusted osmotically with KCl. Macroconidia were obtained from 15-day-old infected soybean stem on water agar. The macroconidia were suspended in distilled water (0 bar) or distilled water containing KCl of the same molalities (osmotic potentials) as the respective osmoticlly adjusted water agar subsequently used to germinate them. Approximately 10m*l* of water or KCl solution containing the test spores was poured on the surface of 10m*l* of solidified water agar in a petri-dish. The spores were allowed to settle on the agar for a few minutes and then was determined after 6, 9, 12, 24hours, respectively. Each experiment was conducted three times.

Results

Influence of water potential on mycelial growth —Mcelial growth was maximal at about −14 to −20 bars on PDA or water agar with soybean leaf pieces adjusted water potential with KCl (Fig. 1). The minimum water potential value that allowed radial growth on either PDA or water agar was −64 bars at 20C, −80bars at 25C and −100 bars at 20C, respectively. The fungus grew gradually larger when wates potential decreased from −1.4 bar to −14 bars and then grew gradually smaller when water potential decreased from −14 to −64 bars at 20C.

Influence of water potential on formation of ma-

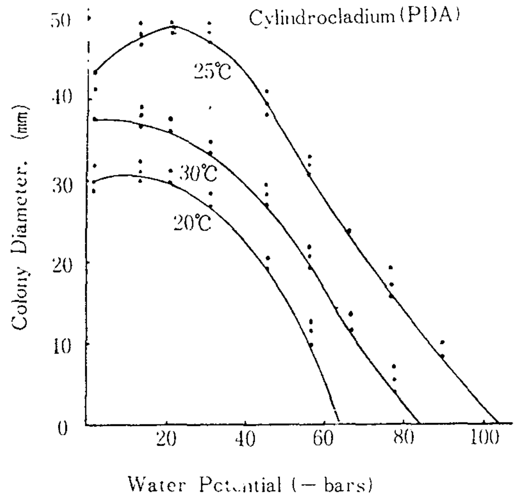

Fig. 1. Relationship between water potential and mycelial growth by *Cylindrocladium crotalariae*.

croconidia—Formation of macroconidia by *C. crotalariae* was maximal at about −14 bars on either PDA or water agar adjusted water potential with KCl (Fig. 2). This fungus produced macroconidia on water agar with soybean leaf pieces down to −64 bars. The number of macroconidia increased as water potential decreased from −1.4 to −14 bars but number of macroconidia decreased as water potential decreased from −14 bars to −64 bars. The number of macroconidia produced by *C. crotalariae* on the different water potentials could be predicted by oubic function $Y=b_0+b_1X+b_2X^2+b_3X^3$, where X=water potential and Y=predicted number

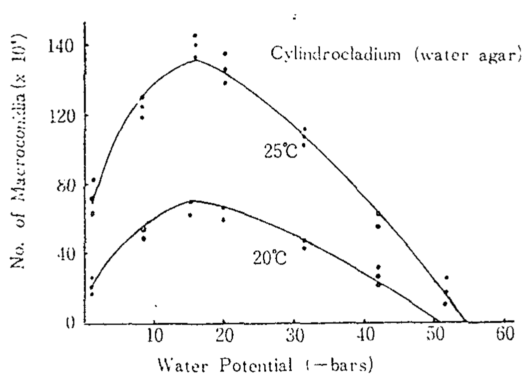

Fig. 2. Relationship between water potential and production of macroconidia by *C. crotalariae*.

of maconidia. Correlarion coefficient between the theoretical and actual numbers of macroconidia at different water potentials was 0.95 for production of macroconidium.

Influence of water potential on formation of perithecia and microsclerotia— The production of perithecia and microsclerotia by *C. crotalariae* was maximal on the basal water agter plus soybeen leaf pieces at water potential of −1.4 bars (Fig. 3, 4). The fungus formed progressively fewer perithecia and microsclerotia as water potential decreased from −1.4(basal media) to −50bars or slightly lower. This fungus produced fewer microsclerotia at 20C than at 25C. The relationship between water potential and number of perithecia and mi-

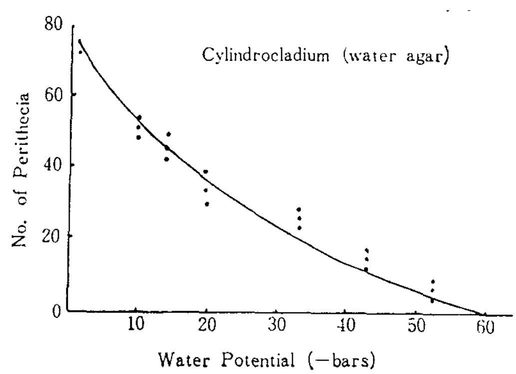

Fig. 3. Relationship between waterpotential and production of perithecium by *C. crotalariae*.

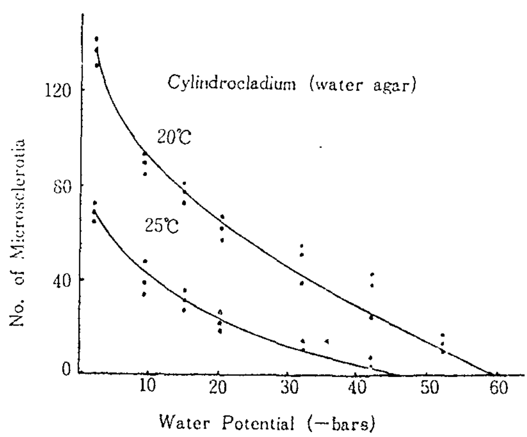

Fig. 4. Relationship between water potential and production of microsclerotium by *C. crotalariae*.

crosclerotia fitted the equation $Y=b_0+b_1X+b_2X^2$, where X=water potential and Y=predicted number of perithecia or microsclerotia. Production of perithecia or microsclerotia by *C. crotalariae* decreased quadratically as the water potential decreased from −1.4 to −60bars. The correiation coefficient between the predicted and actual number of perithecium and microsclerotium at different water potentials were 0.99 for both perithecia and microsclerotia.

Influence of water potential on germination of macroconidia－Macroconidia germinated maximally at all water potentials between −1.4 and −20bars, progressively slower with each increment drop in water potential below about −20bars(Fig. 5). Germination of macroconidia on water agar adjusted different water potentials was best expressed and could be predicted by the piece wise coubic function $Y=(X-a)^* \cdot (b_0-100+b_1X+b_2X^2+b_3X^3)+100$, $(X-a)^*=\begin{cases}1 : \geq Xa \\ 0 : < Xa,\end{cases}$ where X=water potential and Y=predicted percentage of macroconidium germination. Germination of macroconidium kept 100% as the water potential values decreased from −1.4 through approximately −20 bars, and then decreased cubically as the water potential values decreased from −20 to −80 bars. Correlation coefficient between the theoretical and actual percentage of macroconidium germination at different water potetials are above 0.96.

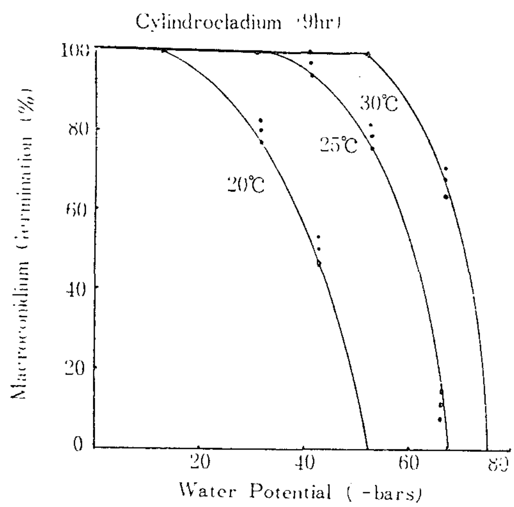

Fig. 5. Relationship between water potential and germination of spores of *C. crotalariae*.

Disussion

The resuls of this study showed that *C. crotaariae* could grow and sporulate under relativcy dry conditions. Radial growth and production of macroconidum were even increased when water potential was reduced from −14 to −20 bars (Fig. 1). A similar increase in radial growth rate also was observed by Ioannou (8), Manandhlar (9), and Sung (13), The sporulation was maximal when PDA or water agar with soybean leaf pieces adjusted −14 bars osmotic potential with KCl was used. Mycelial growth and prouction of macroconidia by this fungus was prevented at about −100 bars and −50 bars, respectively. Production of macrocondium was water potentials higher than those limiting to their mycelial growth. It was not uncommon for fungi to require a more narrow range of environmental conditions for sporulation than for mycelial growth (5). This fungus could form perithecia and microsclerotia under relatively moist conditions. The fungus formed progressively fewer perithecia and microsclerotia as water potential decreased from −1.4 to −50 bars or slightly lower. In contrast, the perithecia formation by *Fusarium roseum* 'Graminearum' (13) and the production of microsclerotia by *Verticillium dahliae* (8) were increased by reduction of water potential from −2 to −20 bars. We suppose that *F. roseum* 'Graminearum' makes perithecia during periods on the drying plant surface in nature but *C. crotalariae* makes perithecia and microsclerotia during periods of free moisture on plant surface in below ground part in nature.

Germination of macroconidia was maximal and uniformly high over the entire range of water potentials between near 0 and −20 bars irrespective of incubated time or temperature. It was different from mycelial growth which was progressively better as the osmotic potential lowered over this range (2, 5, 14). The stimulation in mycelial growth by osmotic potentials down to −20 bars has been interpreted (3) as due to ion uptake by the hyphal cells, and with these ions and the generation of cell osmotic potentials more ideal for cell functions and maintenance of tungor.

C. crotalariae causes black root rot of soybean and peanut and functions mainly below ground in humid climates where plant surfaces are commony wet such as water potential about 0 bar. The fungus is then well suited to form a perithecia and microsclerotia maximally under the environment in which they must operate.

This fungus grows best at a less low water potential similar to water potential of soybean host, makes macroconidia during periods on the drying plant surface in nature, germinate and must grow in plant tissues which are rarely wetter than −8 to −10 bars, and form perithecia and microsclerotia maximally on below ground stem equal to high water potential like free moisture.

摘　要

콩의 黑色뿌리썩음病을 일으키는 *Cylindrocladium crotalariae*은 감자한천배지와 콩의 組織을 함유한 한천 배지를 서로 다르게 Water potential을 調節하여 이 病原菌을 接種한 結果 菌絲伸長과 大型分生胞子形成은 −14bars에서 좋았고 30°C에서는 −100bars 25°C에서는 −80bars 20°C에서는 −64bars에서 菌絲伸長이 중지되었으며 胞子形成도 −64bars에서 중지되었다. 子囊殼과 微少菌核形成은 소금을 첨가하지 않은 −1.4나 −3.0bars에서 잘 되었고 이 菌에 對한 胞子發芽率은 −1.4bars에서 −20bars의 모든 Water potential에서 똑 같이 좋았고 −20bars에서 Water potential이 낮아지므로 發芽率이 떨어지다가 −60bars에서 억제되었다.

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