Effects of Light on Reproduction of *Gibberella zeae* and Overwintering of Soil-Borne Conidia

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밀붉은곰팡이병균의 분생포자 및 자낭각 형성에 미치는 광선의 영향 및 토양에서의 분생포자월동

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적 요

밀, 보리 붉은곰팡이병균의 분생포자 및 자낭각형성에 미치는 광선의 영향을 수량적으로 연구하고, 분생포자의 제 1 차 전염원으로서의 가능성을 증명하기 위해서 토양에서의 월동상태를 조사하였다.

1. 균주 진주 1 은 연속광선조사구에서는 광암교호구 및 암처리구보다 분생포자 형성이 많았다. 균주 수원 3 은 암처리에서도 진주 1 의 광처리보다 분생포자 형성이 많았으며, 광암교호구에서는 배양일 8 일후 현저히 증가하였다.

2. 두 균주 모두 자낭각 형성에는 광선이 필요했고, 수원 3 은 연속광선처리에서도 미숙 자낭각만을, 진주 1 은 암처리를 제외한 다른 처리에서 많은 성숙자낭각 및 미숙자낭각을 형성했다. 분생포자 형성과 자낭각형성은 균주간에 차이가 있었고, 진주 1 은 분생포자 형성량은 적으나, 자낭각형성은 많았고, 수원 3 은 그와 반대였다.

3. 분생포자는 각각 10, 30, 50%의 토양습도에서 겨울동안 그 수가 현저하게 감소했으나, 이듬해 3 월부터 토양습도에 관계없이 그 수가 증가했다. 즉 밀, 보리 붉은곰팡이병균의 분생포자는 토양에서 월동할 수 있었으며, 1 차 전염원의 가능성이 있다고 본다.

Abstract

1. Continuous light induced more conidia than alternating light and darkness treatment in isolate Chinju 1. Isolate Suwon 3 produced much more conidia on synthetic medium than Chinju 1 in light. Conidial formation in Suwon 3 increased remarkably with alternate light and darkness in 8 days incubation.

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2. Light was essential for perithecial formation in Chinju 1. No matured perithecia were observed in Suwon 3 with any treatment. Abundant perithecia were produced in Chinju 1 but only perithecial initiation occurred in Suwon 3. Suwon 3 produced significantly more conidia than Chinju 1, while perithecial formation was reversed.

3. Conidial numbers in soil decreased significantly through the winter at 10, 30 and 50 per cent soil moisture, with the most striking decrease at 10 per cent levels, but the number recovered again beginning in March, regardless of the soil moisture. The above results provide us a useful clue to support the possbility the conidia may serve as a primary inoculum.

INTRODUCTION

In 1963, severe epidemics of wheat and barley scab caused by *Gibberella zeae* (Schw.) Petch. resulted in losses of an average of 40 to 60 per cent in southern Korea due to heavy rainfall during the flowering period of the crops[6,12]. Although extensive studies were made on the epidemiology and physiology of this important pathogen for a long time, still the effects of light on reproduction and importance of soil-borne conidia as a primary inoculum were discrepant yet.[1,7,8]

Nisikado [8] found that ultraviolet light and day light were more effective than incandescent light for conidial formation of *G. zeae*, while abundant conidia were produced on various media regardless of light [1]. Nisikado [3] also found that only those in the beakers near a window partly exposed to sunshine gave rise to perithecia and matured ascospores on sterilized rice straw. Inouye [5] reported that diffused light of summer with 3,500 to 4,000 lux was optimum for perithecial formation. In addition, continuous illumination with either 500 or 240 lux of fluorescent light at about 17°C in the growth chamber produced more perithecia than with lower light intensities.

It is well known that ascospores from overwintered perithecia are known to be an important primary inoculum [5,8]. In Korea, Lee et al.[7] found that perithecia on the infected straw of wheat, barley and rice stubble were the primary inoculum. However, soil-borne conidia of *Gibberella zeae* might be capable of causing seedling blight as well as scab of wheat and barley. This possibility was proved by Chung et al.[2] that soil infested artificially with conidia of *Gibberella zeae* resulted in varying degrees of pre-and post-emergence seedling blight of wheat, corn, barley and rice. Ishii [4] also suggested the possibility of conidia as a primary inoculum, while Inouye [5] detected negligible numbers of soil-borne conidia in late spring. The temperature of the soil is undoubtedly the most important single factor determining the extent of seedling blight of wheat and corn caused by *G. zeae*[3]. Stover [10,11] reported the effect of soil moisture and oxygen on survival of *Fusarium* spp. The possibility of soil-borne conidia as a primary inoculum is important for understanding the epidemiology of cereal scab.

The present study investigates the effect of light on conidial and perithecial production and the survival of soil-borne conidia of *Gibberella zeae* as a primany inoculum for seedling blight and scab of cereal crops.

MATERIALS AND METHODS

All of the experiments were made at about 25±1°C in an incubator. Microscopic observation with haemocytometer at 100×magnification was carried out to investigate the formation of conidia at different light treatments.

Two isolates of *Gibberella zeae* (Schw.) Petch., were obtained from naturally infected wheat at Chinju and Suwon, collected in 1966 by researchers of the Plant Pathology Section, Office of Rural Development,Suwon, Korea. The isolates were studied and designated as Chinju 1 and Suwon 3 because preliminary tests indicated that amounts of conidial formation and other cultural characters were distinct among several isolates. The inoculum was produced on a modified glucose-asparagine medium which contained the following ingredients per liter: Urea 1.908g, Mannitol 10.11g, $MgSO_4$ 1g, K_3PO_4 1.2g, agar 20g.

The effect of light on conidial production was studied on the modified glucose-asparagine medium in Pyrex

Table 1. Effects of light on conidial formation on a synthetic medium and perithecial production on rice stems by two isolates of *Gibberella zeae*.

Treatment	Isolate	10^3 conidia/ml after indicated no. of days/[a]			No. of perithecia after 45 days incubation	
		4	8	12	matured[b]	initiation[c]
Contiuous light	Chinju 1	6.1	3.4	2.0	235	85
(1,200lux)	Suwon 3	14.0	32.0	29.4	0	105
Alternating light & darkness	Chinju 1	3.8	2.2	3.1	152	103
(12 hr. period)	Suwon 3	14.2	60.0	63.6	0	0
Darkness	Chinju 1	0.9	0.5	0.4	0	0
	Suwon 3	13.1	18.6	16.0	0	0

a: Based on two 1-cm^2 discs from each of three replicates in each of two experiments. Differences between isolates were statistically significant ($p=0.99$).

b: Averages of matured perithecia based on five 10 cm-long rice stems from each of three replicates.

c: Isolate Chinju 1 initiated perithecia after 20 days while isolate Suwon 3 after 30 days.

petri plates seeded with conidial suspension(5×10^4 conidia/plate). Test tubes containing 5 stems of rice cut in 10 cm length with one ml of tap water were autoclaved at 120°C for 30 minutes. The rice stems thus treated were inoculated with 0.2ml of conidial suspensions(5×10^4 conidia/ml) in order to test the effects of light on perithecial formation.

Of the plates or test tubes thus treated, some were exposed continuously to 1,200 lux of cool white fluorescent light, some to alternating light and darkness for 12 hours while the others received no light. The number of conidia were counted in 4-day old cultures and at two succeeding 4-day intervals. Perithecial production was enumerated after 45 days incubation.

Survival of soil-borne conidia was examined on modified PCNB agar by the dilution plate method [9]. Uniform inocula of 5×10^4 conidia were applied to vial of 15-cc volume in which 10 grams of sterilized soil was placed. The soil moisture was maintained by adding water to contain initial soil moisture levels of 10, 30, and 50 per cent, respectively. The vials thus treated were wrapped with vinyl and buried outdoors at 10cm depth on November, 30, 1969. Two 1 gram samples were taken from each vial five times, at monthly intervals. Each sample was diluted to 1/10,000 prior to inoculation on modified PCNB medium in each of 3 replicates in each case.

RESULTS

1. Effect of light on conidial and perithecial formation.

In both isolates, more conidia were produced in light than in darkness. Among the treatments, the alternating light and darkness was most favorable at both 8 and 12 day incubations in Suwon 3. In Chinju 1, the number of conidia was decreased as the incubation period was increased except in alternating light. Suwon 3 showed considerably more sporulation even in darkness regardless of treatments. Light was more effective for sporulation in Chinju 1 than in Suwon 3. Conidial production in the two isolates differed significantly at 1 per cent level.

Matured perithecia were produced abundantly on rice straw by Chinju 1, with perithecia initiation when rice straw was exposed to continuous light or to alternating light and darkness. Suwon 3 produced no matured perithecia at any treatments, but perithecia initiation occurred in continuous light. In darkness neither perithecia nor perithecia initiation occurred in either isolate. Perithecia initiation began after 30 days. Light was essential for perithecial formation in both isolates.

2. Survival of soil-borne conidia through the winter.

According to the data for soil temperature from the Suwon Meteorological Station (Table 2.), fluctuation of soil temperature at a depth of 5 cm was similar to

Table 2. Average temperatures of air and soil at Suwon in 1969—1970.

(Suwon Meteorological Station)

Point of measurement	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Air	3.4	−2.1	−6.7	−1.7	0.5	10.7
Soil Surface	4.9	−0.7	−3.2	−0.2	2.1	15.5
5 cm depth	6.3	−0.3	−3.2	0.9	1.2	11.5
10cm depth	6.7	0.9	−2.2	0.8	1.1	11.0

Average of daily observation at 10 a. m.

that at the soil surface. However, temperature fluctuated relatively less at a depth of 10cm. At this depth, the monthly average temperature was lowest (−2.2°C) in January; averages for December, February and March were about 1°C. The percentage of recovered colonies from surviving conidia of *G. zeae* decreased rapidly in December, and increased gradually beginning in March regardless of soil moisture. (Table 3).

Table 3. Percentage of recovered colonies on modified PCNB agar by survived conidia of *Gibberella zeae* at different soil moistures through the winter.

Soil moisture(%)	Percentage of recovered colonies[a] Nov.	Dec.	Jan.	Feb.	Mar.	April
10	100	74	6	10	35	60
30	100	56	40	13	35	80
50	100	120	44	40	39	70

a.: Based on two 1-gram samples (2×10^4 gram soil per plate) from each of three vials with 3 replicates.
The vials containing inoculated soil were buried under at ground 10cm depth on 30th of November.

Fluctuation of conidial numbers was most apparent at 10 per cent soil moisture while that at 50 per cent was relatively stable throughout the experiment. Survival of soil-borne conidia was highly influenced by temperature fluctuation of soil through the winter. However, the conidia showed remarkable ability to multiply on return to favorable temperature even with 10 per cent moisture. In March, the multiplication was similar regardless of soil moistures.

The results indicate that the conidia had considerable ability of multiplication even with exposure to soil temperatures below freezing.

DISCUSSION

Earlier workers [5,7,8] reported that ascospores of *G. zeae* discharged from perithecia on rice stubble are an important primary inoculum. However, not all of the conidia in soil would developed into perithecia because light is essential for perithecial formation and maturation. Moreover, Suwon 3 did not produce matured perithecia in light studies. Consequently, conidia would be ascertained also as a primary inoculum if the conidia could overwinter in the field.

The conidia of *Gibberella zeae* showed remarkable potentiality of survival at any soil moisture levels through the winter in this study. Temperature is reported to profoundly affect the survival of *Fusarium*[3]. The survival is also determined by the amounts of oxygen available to surface oxidized soil layer[10,11]. Temperature of the soil is undoubtedly the most important single factor affecting the survival of soil-borne conidia through the winter. However, low temperatures thus we have examined could be possible conditions for survival of conidia *G. zeae* in soil.

It has been previously reported that various crop seedlings, such as rye, soybean, rice, buckwheat, corn, and cotton in greenhouse were susceptible when planted in the pots artificially infested with conidia of *G. zeae.*[2]. Soil-borne conidia in natural conditions may infect wheat and maize seedling[3]. Such detailed information is very useful for crop rotation. Dickson [3] already suggested not to sow wheat after corn. It is apparent that the conidia of *G. zeae* can serve as an important primary inoculum to following crops when the conidial population is high in the soil. In present study, the amounts of conidial production of Suwon 3 were abundant while Chinju 1 produced few conidia, but perithecial production in the two isolates was vice versa. Furthermore, Suwon 3 produced no matured ascospores, but abundant conidia. This fact again provides us a useful clue to support the possibility that the conidia can serve as a primary inoculum.

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