

## Some Environmental Factors Affecting Sporidial Germination of *Ustilago maydis*

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### *Ustilago maydis*의 소생자 발아에 영향을 주는 몇 가지 환경요인

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**ABSTRACT :** Sporidia of *Ustilago maydis* germinated over 98% on water agar 8 hr after inoculation. Germination rate on other four nutritional media including PDA was similar to that of water agar. Optimum temperature for sporidial germination was 30°C, followed by 25°C and 35°C, but sporidial germination decreased greatly at 20°C or lower temperature. Sporidia germinated more than 80% at pH 5-9 and best at pH 6-7. Light did not affect sporidial germination significantly. Sporidia germination reached 81% at the presence of free water (RH 100%) at 24hr after inoculation, but only 19-34% at RH 93% or lower. This result indicates that key environmental factors affecting sporidial germination are temperature and moisture, and that under Korean climatic conditions sporidia are able to germinate most vigorously in the rainy season of hot summer of July and August.

**Key words :** corn smut, *Ustilago maydis*, sporidia, germination.

Smut often causes considerable damage on cereal crops such as barley, wheat, and corn (1, 4, 8). Smut infection occurs through penetration of host tissues by germinated sporidia from teliospores of *Ustilago maydis* (2, 3, 9). However, not much information has been obtained on significance of environmental factors associated with sporidial germination. To induce successful colonization on host tissues by sporidia in an artificial inoculation test, it needs first to identify environmental conditions proper for sporidial germination of the pathogen.

This study was performed to examine factors affecting sporidial germination of *U. maydis* that is prerequisite for successful infections on host tissues. This study is a part of experiments for developing a fungicide screening technique against smut fungus. A preliminary report (6) and a part of the experiment (7) have been found elsewhere.

## MATERIALS AND METHODS

**Effect of nutrient sources on sporidial germination.** Five media; 2% water agar, Czapek-dox agar (Difco), carrot agar (carrot extract 20 g, agar 20 g, distilled water 1 l), corn meal agar (corn meal 17 g, agar 20 g, distilled water 1 l), and potato-dextrose agar (potato 200 g, dextrose 20 g, agar 20 g, distilled water 1 l) were compared for sporidial germination of *U. maydis*. Sporidial suspension preadjusted to 10<sup>6</sup> sporidia/ml was spread on each medium, and incubated at 28°C. Germination rate of sporidia was checked 2, 4, 6, and 8 hr after inoculation, based on a ratio of the number of sporidia germinated per the total number of sporidia observed under a microscope. Observation was made 5 times per Petri dish. The treatment was replicated 5 times.

**Examination of effects of temperature, pH, and light on sporidial germination.** To examine temperature effect, sporidia suspension of *U. maydis* at the concentration of 10<sup>6</sup>/ml was spread on 2% water agar

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in Petri dishes and incubated at 5 to 35°C at 5-degree intervals. Sporidial germination was examined 6 hr after inoculation by the same way described above. Observation was made five times per Petri dish, and the treatment was replicated 5 times.

To examine pH effect, 2% water agar was adjusted to pH 4.1, 5.1, 6.0, 7.0, 8.0, and 9.1 by using 1N HCl and 1N NaOH, and plated in Petri dishes. One ml of sporidial suspension ( $10^6$ /ml) was then spread on the water agar and kept at 28°C. Sporidial germination was checked similarly 7 hr after inoculation. The pH treatment was repeated 5 times.

In order to examine light effect Petri dishes containing 2% water agar were inoculated similarly with sporidial suspension of *U. maydis*. Light treatment was achieved by placing the petri dishes under a cool white fluorescent lamp at 15 cm high in an incubator at 28°C. For the dark treatment, the inoculated Petri dishes were wrapped with aluminum foil and kept in the same incubator. Germination rate of sporidia was examined similarly 6 hr after inoculation.

**Effect of RH on sporidial germination.** Corn seedlings at 3rd leaf stage were put into the glass bottles (18×20×7.5 cm) with lids containing 100 ml of water adjusted at RH 100%, 92.9%, 88.5%, 82.9%, 75.6%, and 66.8%. The desirable RH was controlled by adding different amounts of sulfuric acid following the method described (5). Prior to being put into the bottle, corn seedlings were sprayed with sporidial suspension of *U. maydis* and air-dried. The bottle was placed in an incubator at 25°C. Corn leaf samples were taken 3, 6, 12, and 24 hr after incubation, cut into 0.5×0.5 cm pieces and dyed in 0.3% fluorescent brightener for microscopic observations. Germination rate of sporidia was examined similarly under a microscope. The RH treatment was repeated five times.

## RESULTS

**Effect of nutrient sources on sporidial germination.** Sporidia germinated well on five media tested (Table 1). Sporidial germination of *U. maydis* was 0.7 to 3% at 2 hr after inoculation, depending on the media, but it reached over 95% at 8 hr after inoculation, regardless of the media used. Percentages of sporidial germination on water agar did not differ greatly with those on four different nutrient media tested.

**Temperature, pH, and light effects on sporidial germination.** Sporidial germination was best at 30°C,

**Table 1.** Temporal germination of sporidia of *Ustilago maydis* on various agar media at 28°C

Medium	Sporidial germination (%) <sup>a</sup> after			
	2 hr	4 hr	6 hr	8 hr
Water agar	3.5	29.6	76.6	98.5
Czapek-dox agar	3.0	28.5	77.6	95.0
Carrot agar	0.7	26.5	88.6	98.4
Corn meal agar	2.1	41.9	87.7	99.1
Potato-dextrose agar	3.0	25.4	80.7	99.3

<sup>a</sup> Values are means of 5 replications.

**Table 2.** Effects of temperature, pH, and light condition on sporidial germination of *Ustilago maydis* on artificial media

Environmental factor	Treatment	Sporidial germination	
		%	Std. <sup>d</sup>
Temperature <sup>a</sup> (°C)	5	5.8	2.6
	10	12.1	1.6
	15	22.6	8.6
	20	51.3	4.6
	25	75.9	20.7
	30	89.9	11.8
pH <sup>b</sup>	35	75.7	5.7
	4.1	72.8	5.6
	5.1	86.4	5.1
	6.0	88.1	3.5
	7.0	91.5	3.7
	8.0	81.9	3.4
Light/dark <sup>c</sup>	9.1	82.1	2.4
	light	75.7	5.8
	dark	85.2	9.8

<sup>a</sup> Sporidial germination was examined on PDA 6 hr after incubation. Values are means of 5 replications.

<sup>b</sup> Sporidial germination was examined on water agar 7 hr after incubation at 28°C. Values are means of 5 replications except treatments of pH 5.1 and 6.0, which have 4 replications, respectively.

<sup>c</sup> Sporidial germination was examined on PDA 6 hr after incubation at 28°C. Values are means of 5 replications.

<sup>d</sup> Standard deviation.

reaching 90% germination 6 hr after inoculation, followed by 25°C and 35°C (Table 2). Germination rate decreased sharply at 20°C and lower temperatures, resulting in only about 6% germination at 5°C.

Sporidia germination differed from 73% to 92% at the pH range of 4.1 to 9.1 (Table 2), and was best at pH 5.1 to 7.0, followed by pH 9.1 and 8.0, and least at pH 4.1. Sporidial germination did not differ significant-

**Table 3.** Effect of relative humidity on sporidial germination of *Ustilago maydis* on corn leaf surface at 28°C

Hours after inoculation	Sporidial germination (%) <sup>a</sup> at RH of					
	66.8	75.6	82.9	88.5	92.9	100
3	3.0±1.0	5.6±2.8	5.7±2.3	7.0±3.0	19.4±2.3	27.9±5.2
6	19.2±14.7	8.6±2.1	11.3±3.9	13.1±3.9	30.1±1.7	50.4±17.1
12	15.4±1.8	16.1±1.0	19.8±3.9	19.0±3.9	23.6±4.6	84.5±5.1
24	21.4±7.8	19.5±5.6	27.2±6.8	31.1±2.4	34.1±9.6	81.0±9.5

<sup>a</sup> Values are means of 5 replications with their standard deviations.

ly between light and dark treatments (Table 2). Sporidia germinated over 75% 6 hr after inoculation in both treatments.

**Effect of RH on sporidia germination.** Germination of sporidia increased as RH increased and as time after inoculation prolonged (Table 3). Sporidial germination was about 28% at RH 100% 3 hr after inoculation, but reached over 80% at 24 hr after inoculation. Sporidial germination decreased sharply at around RH 90% or lower.

## DISCUSSION

The two most important environmental factors affecting sporidial germination in this study were found to be temperature and RH. Of these two factors RH seemed to be more critical than temperature in a court of field infections, since sporidial germination occurred fairly well in a broad range of temperature such as 20 to 35°C with an optimum at 30°C. Meanwhile, germination rate of sporidia dropped sharply to below 34% as relative humidity decreased to 92.9% from RH 100%. Other factors such as source of nutrition, pH of the media, and presence of light appeared less important for germination of sporidia in field conditions, since sporidia germinated well in a broad range of this factors.

This study suggests that, in Korean condition, sporidia can germinate and infect corn plants any time in hot summer season of July and August under free moisture conditions. This study also indicates that sporidial germination can be induced artificially without difficulty for successful colonization on host tissues.

## 요약

*Ustilago maydis*의 소생자는 물한천배지에서 접종후 8시간 후에 98% 이상 발아하였다. PDA를 포함한 기타 영양배지에서의 발아율도 이와 비슷하였다. 소생자 발아에 가장 적당한 온도는 30°C였으며 25°C나

35°C의 순으로 양호하였으나 20°C나 그 이하의 저온에서는 발아율이 급격히 떨어졌다. 소생자는 pH 5~9의 범위에서 80% 이상 발아하였으나 최적 pH는 6~7이었다. 광조사는 소생자 발아에 큰 영향을 미치지 못하였다. 소생자는 자유수분(RH 100%) 하에서 24시간 후 81%가 발아하였으나 상대습도가 93%나 그 이하에서는 발아율이 19~34%였다. 이 시험결과를 소생자 발아에 가장 큰 영향을 미치는 환경요인은 온도와 습도였으며 우리나라 기후조건에서 소생자는 7~8월의 한여름철 장마기간 동안 왕성하게 발아할 수 있음을 뜻하고 있다.

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