

水中におけるうどんこ病菌の形態形成(1)

オオムギうどんこ病菌 *Erysiphe graminis* の感染過程における水の影響

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Morphogenesis of the Powdery Mildew Fungus in Water (1) The Effect of Liquid on Infection Process of *Erysiphe graminis**

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Abstract

Barley coleoptiles that had been inoculated with conidia of *Erysiphe graminis* were immersed in 0.01 M CaCl₂ immediately after inoculation. Some of the immersed conidia showed normal differentiation giving rise to a haustorium, as similarly as those on coleoptiles that were not immersed. On the other hand, the percentage of elongated abnormal appressoria increased, and the penetration efficiency decreased slightly in water. However, *E. graminis* germlings that completed haustorium formation also elongated secondary hyphae, and eventually, sporulation occurred even in solution. The viability of the conidia and their associated structures was never affected at all while they were incubated in water without being dried. Thus, the water had no effect on the viability or on the differentiation process after formation of haustorium of *E. graminis*.

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Key words: *Erysiphe graminis*.

INTRODUCTION

Ectoparasitic powdery mildew fungi produce all vegetative and reproductive organs except haustoria on the surface of their host plants. In the literatures there are contradictory evidences concerning the effects of water on growth of powdery mildew fungi. For example, some researchers reported harmful effects of water on these fungi, such as bursting of conidia or damage of secondary hyphae^{1,4,7)}. In contrast, some researchers successfully inoculated grasses with powdery mildew conidial suspensions in water^{3,6)}. All of the latter researchers adopted water just for medium ingredient for spore suspension, and thus it is plausible that the water disappears soon by evaporating from the surface of leaves after inoculation before the water gives harmful effects, if any. However, no report is available concerning the effects of water on the differentiation process from spore germination to sporulation of powdery mildew fungi. In this paper, it is shown that there is no effect of CaCl₂ solution on viability of *Erysiphe graminis*, and that some colonies sporulated even in water.

MATERIALS AND METHODS

Fungi. *Erysiphe graminis* de Candolle f. sp. *hordei* Em. Marchal race I was maintained on barley (*Hordeum vulgare* L., cv. Kobinkatagi) grown at 20°C and 70% relative humidity under fluorescent lights (11.8 W m⁻² for 12 hr day⁻¹).

Specimen preparation and inoculation. Barley was grown from seed at 20°C under fluorescent lights (11.8 W m⁻² 14 hr day⁻¹). Coleoptiles were excised from seedlings 9 days after sowing, and single-cell thick epidermal layers of partially dissected coleoptiles were prepared as described

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Table 1. Frequency of abnormal appressoria and penetration efficiency of normal appressoria of *Erysiphe graminis* on barley coleoptiles incubated in air or liquid condition

Condition of incubation	Percentage of abnormal appressoria ^{a)}	Penetration efficiency of normal appressoria ^{b)}
Air	4.6±1.2	82.7±1.4 ^a
Liquid	22.9±3.5	68.4±4.9 ^b

a) (No. of abnormal appressoria/No. of germinated conidia)×100(%). Mean±S.E.

Structures of abnormal appressoria as shown in Plate I-2A and 2B.

b) (No. of haustoria/No. of attempted penetrations)×100(%). Mean±S.E.

Penetration efficiency was determined 48 hr after inoculation.

a, b : These are significantly different ($p=0.05$).

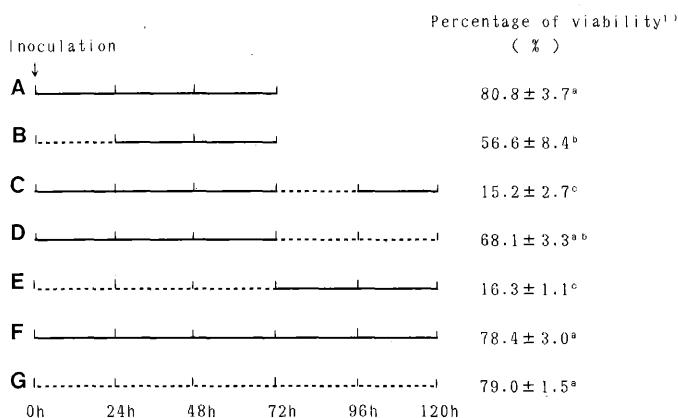


Fig. 1. Effects of immersion and timing of immersion in 0.01 M CaCl_2 on viability of *Erysiphe graminis* germlings. — : not immersed, : immersed. 1) (No. of turgid conidia, appressoria or secondary hyphae/Total no. of conidia, appressoria or secondary hyphae)×100(%). Mean±S.E. a-c : Analysis of variance was performed ; values followed by the same letter do not differ significantly ($p=0.05$).

previously⁸⁾. Coleoptiles were inoculated with 40-50 freshly harvested conidia of *E. graminis* with a brush, and immersed immediately in 0.01 M CaCl_2 and incubated at 20°C (Plate I-1B). This experimental condition will be referred to as the "liquid condition" below. For a control, inoculated coleoptiles were incubated by floatation on 0.01 M CaCl_2 under the same environmental condition (Plate I-1A). This condition will be referred to as the "air condition" below. All the experiments were repeated 3 times, and 300-700 and 200 conidia were observed in each replicate for the data shown in Table 1 and Fig. 1, respectively. Data were expressed by mean±S.E. and analyzed by the *t*-test in Table 1 or least significant difference (LSD) in Fig. 1.

RESULTS AND DISCUSSION

In the air condition, an AGT (an appressorium germ tube) usually increased in thickness to form a club shape with a lobe at its apex (Plate I-3). Since this shape is typical for the appressorium of the present fungus, this was designated as an appressorium in this paper. In the liquid condition, some AGTs formed an appressorium (Plate I-2A, left), but others produced an undifferentiated long tube maintaining almost the same thickness from their base to their apex regardless of age (Plate I-2A right). Thus we distinguished these germ tubes from appressoria, and designated them as germ hyphae, following terminology of Gay *et al.*²⁾. Moreover, in the liquid condition, AGTs exhibited another abnormal growth as illustrated in Plate I-2B. These were named pseudoappressoria, because they did not initially contact the host surface but increased in thickness as similarly as appressoria followed by producing a slightly abnormal lobe-like structure at its apex. However, they never attempted penetration into host cells.

Germ hyphae and pseudoappressoria were collectively expressed as abnormal appressoria below. In the air condition, no pseudoappressorium was observed.

In both air (Plate I-3) and liquid conditions (Plate I-4), haustoria were formed from appressoria, and subsequently secondary hyphae emerged. On the other hand, the percentage of abnormal appressoria increased in solution, and the penetration trial never occurred from them. As shown in Table 1, penetration efficiency (No. of haustoria/No. of appressoria inducing a papilla or forming a haustorium $\times 100\%$) was slightly lower in the liquid condition than in the air condition. In contrast, the percentage of abnormal appressoria in the liquid condition was several times higher than in the air condition. The reason why penetration efficiency of *E. graminis* in the liquid condition was lower than that of in the air condition is not clear. However, if *E. graminis* secretes a substance like a suppressor in the infection process as reported by Komura *et al.*⁵⁾, it is plausible that the substance is readily washed out during differentiation by immersing coleoptiles after inoculation. Further studies are needed for this phenomenon.

Immersed *E. graminis* which had succeeded in forming haustoria in barley coleoptile cells elongated secondary hyphae as frequently as that in coleoptiles which had not been immersed. In addition, sporulation occurred eventually at 120 hr after inoculation when the inoculated coleoptiles were continuously incubated in the liquid condition (Plate I-6) as similarly as in the air condition (Plate I-5). The amount of sporulation per germling was not determined because of the abundance of mycelia on the coleoptile surface.

Similar differentiation patterns of *E. graminis* were observed on coleoptiles immersed in either water or 0.01 M CaCl_2 . However, the penetration efficiency obtained was much less in water than in 0.01 M CaCl_2 .

As mentioned above, *E. graminis* was able to complete its differentiation even in water. Moreover there was no effect of water on the viability of this fungus. This result supports the paper of Lumbroso *et al.*⁶⁾ who reported no effect of water on the infection of spores immersed in water for 170 min. However, the effect of water on conidia has been disputed. Sakurai and Hirata⁷⁾ reported that conidia of *E. graminis* in water burst when the specimens in water were covered with a cover glass, followed by absorbing the water from the edge of a cover glass with a filter paper. Furthermore, they reported that drying of the specimens after the emergence of secondary hyphae was very harmful to the fungus. In our experiment, no bursting conidium was observed in the liquid condition when the specimens were not covered with a cover glass.

The timings and effects of immersion in 0.01 M CaCl_2 on viability of *E. graminis* were examined. The viability was evaluated by the incidence of shrunk conidia, appressoria, all secondary hyphae at 72 hr (Fig. 1. A, B) or 120 hr (Fig. 1. C-G) after inoculation. As shown in Fig. 1, there were no differences in viability between immersed and non-immersed *E. graminis* as far as the respective conditions were unchanged during incubation (Fig. 1. A, F, G). In contrast, once the solution was removed after immersion from the surface of coleoptiles, the viability decreased greatly (Fig. 1. B, C, E). As most of the secondary hyphae begin to emerge about at 24 hr after inoculation, it seems that the reduction of viability is more prominent when the solution was removed during the step of secondary hypha elongation (Fig. 1. C, E) than when it was done during the stages from conidium germination to haustorium formation (Fig. 1. B). Moreover, the viability was not significantly reduced even when coleoptiles were immersed at 72 hr after inoculation (during the step of secondary hypha elongation) if the solution was not been removed after immersion (Fig. 1. D). This suggests that drying after immersion might be responsible for the damage of *E. graminis*, as Sakurai and Hirata reported⁷⁾.

Based on these results it was concluded that immersion of *E. graminis* might have no effects on viability of the present fungus, and that it had an effect to enhance formation of abnormal appressoria. Further studies of the latter phenomenon are needed.

Applying the phenomenon that the immersed *E. graminis* with an appressorium penetrates as similarly as the non-immersed one without any serious damage, it will be possible to examine several questions which have remained unsolved because of difficulty in being tested in the aerial infection system of *E. graminis*: for instance, the effects of fungicides which require the accurate value of ED_{50} ,

the isolation of fungal products such as a suppressor or an elicitor at each differentiation step of the present fungus, or the induced resistance or susceptibility by other fungi which can infect only in the liquid condition. The detailed time course of morphogenesis of conidia and the pathogenicity of conidia formed in water will be discussed elsewhere.

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和 文 摘 要

山岡直人：水中におけるうどんこ病菌の形態形成 (1) オオムギうどんこ病菌 *Erysiphe graminis* の感染過程における水の影響

オオムギうどんこ病菌を接種したオオムギ子葉鞘細胞を、接種後ただちに 0.01 M CaCl₂ 水溶液中に浸せきし、培養した。水溶液中では空気中と同様に分生子の発芽、付着器形成、吸器形成が認められたが、その割合は空気中と比較して低く、また異常な徒長発芽管の割合が増加した。しかしながら、いったん正常な吸器形成を行った分生子の中には、その後の二次菌糸の伸長に続き、最終的には分生子形成まで至る場合も認められた。さらに水溶液中で連続的に培養を行い、途中の過程において乾燥という状態を経なければ、本菌は死ぬようなことはなく、従来よりいわれていた本菌の生存に対する水の影響はほとんどないと結論できる。

Explanation of plate

Plate I

- 1A. A conidium just after inoculation onto a coleoptile surface in the air condition.
- 1B. A conidium on a coleoptile surface after immersion in a CaCl₂ solution immediately after inoculation.
- 2A. Conidia with either an appressorium (left) or a germ hypha (right) on an immersed coleoptile surface in the liquid condition, 24 hr after inoculation.
- 2B. A conidium with a pseudoappressorium that was formed in the liquid condition.
- 3, 4. Conidia with appressoria that produced haustoria and elongating secondary hyphae by 36 hr after inoculation in the air (3) and liquid (4) conditions.
- 5, 6. Sporulation on coleoptile surfaces in the air (5) and liquid (6) conditions, observed 120 hr after inoculation.

a : appressorium, c : conidium, esh : elongating secondary hypha, ha : haustorium, gh : germ hypha, pa : pseudoappressorium. Bar (in 1A) = 10 μ m.

Plate I

