Factors Affecting Conidial Germination of *Entomophthora delphacis* Hori (Entomophthorales: Entomophthoraceae)

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The effects of relative humidity, temperature, and lighting conditions on germination of conidia of *Entomophthora delphacis* were investigated. A large number of conidia germinated in water drops, but few at 100% RH without water drops, and none germinated below 93% RH. The germination occurred at temperatures between 15 and 35°C in water drops, and the optimum temperature for germination was 25°C. The germination rate was not affected significantly by light.

INTRODUCTION

*Entomophthora delphacis* Hori has been known as a pathogen of planthoppers and leafhoppers in Japan. The effects of temperature and pH on the hyphal growth of this fungus were reported by Shimazu (1976). To utilize this fungus as a control agent of insect pests, it is important to know the factors affecting not only hyphal growth but also conidial germination, since it is believed that infections by entomophthoraceous fungi arise from penetration of the body wall of insect hosts by germinating conidia. Environmental factors are important to the germination of conidia, and germination and infection will not occur unless favourable conditions exist. It is generally thought that temperature, humidity, and light are especially effective factors for conidial germination of fungi. The effects of temperature and humidity on conidial germination of entomophthoraceous fungi were investigated on *Entomophthora apiculata*, *Entomophthora coronata*, and *Entomophthora virulenta* by Yendol (1968), and on *Entomophthora gammae* by Newman and Carner (1975). They suggested a need for both moderate temperature and high humidity. In the present paper, the effects of various temperatures, humidities, and illumination on the germination of *E. delphacis* conidia were investigated.

MATERIALS AND METHODS

A stock culture of *E. delphacis* which was isolated from the brown planthopper, *Nilaparvata lugens*, was subcultured on Sabouraud's dextrose agar containing 1% of yeast extract at 25°C. The fungus was cultured on the same medium in petridishes of 9-cm diameter at 25°C for 3 to 5 days. When the cultures sporulated and discharged conidia abundantly, the cultures were inverted over glass slides with or without water drops for 5 to 10 minutes to collect discharged conidia. These glass slides were incubated at various humidities, temperatures, and light intensities as described later.
Germination of *Entomophthora delphacis*

Table 1. **Effect of Relative Humidity on Conidial Germination of *Entomophthora delphacis***

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Percent germination(^b) after:</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>In water drops(^a)</td>
<td>3.4</td>
</tr>
<tr>
<td>100%</td>
<td>0.0</td>
</tr>
<tr>
<td>93%</td>
<td>0.0</td>
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<tr>
<td>75%</td>
<td>0.0</td>
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</table>

\(^a\) Conidia were collected in water drops on slides.

\(^b\) Average of three replicates.

After various durations of incubation, the glass slides were collected, covered with cover glasses and observed under a microscope to count the germinating conidia. Three hundred conidia were checked at random for germination, and the examination was replicated three to five times for each condition.

*Germination at various humidities.* The conidial germination was examined at four grades of humidity. For three of these, dry glass slides were kept in desiccators at various humidities, obtained using saturated solutions of NaCl for 75% RH and KNO\(_3\) for 93% RH, and distilled water for 100% RH. For the fourth grade of humidity, the discharged conidia were collected in water drops on the glass slides, and kept in a desiccator containing distilled water. Each test was conducted at 25°C and in darkness.

*Germination at various temperatures.* Glass slides with water drops were used to determine the germination at various temperatures. The slides were kept in petri dishes at temperatures of 15, 20, 25, 30, and 35°C. Each dish contained a piece of moistened filter paper to keep the relative humidity at 100%. Each test was conducted in darkness.

*Germination in light and darkness.* Glass slides with water drops were kept in petri dishes containing moistened filter paper at 25°C. Half of the slides were constantly illuminated with fluorescent lamps, and the other half were kept in total darkness.

**RESULTS AND DISCUSSION**

The appearance of the conidia varied according to the presence or absence of water drops on the slides: those discharged onto dry slides usually contained one large oil globule and had conspicuous basal papilla, while those discharged into the water drops contained numerous small oil globules and basal papilla were inconspicuous. Such a phenomenon was also observed in the case of *Entomophthora aphidis* (Shimazu; unpublished). Secondary conidia were formed as the result of conidial germination in all experiments. The germination rate of primary conidia decreased with the increase of the secondary conidia, because they were the same shape as the primary conidia.

**Effect of humidity**

Conidial germination did not occur at the humidities of 93% or lower, and very little occurred even at 100% RH (Table 1). However, the conidia discharged into water drops on the slides showed considerable germination. This started within 1 hour after discharge, and 33.7% of the conidia were germinating 3 hours after discharge.
These results show that the conidia of this fungus are almost unable to germinate without water. The humidity requirements for germination of conidia of the other entomophthoraceous fungi were 100% RH for *E. apiculata* and *E. virulenta* (Yendol, 1968), 98 to 100% RH for *E. gammae* (Newman and Carner, 1975), 95 to 100% RH for *E. coronata* (Yendol, 1968), and 70% RH or more for *Entomophthora sphaerosperma* (Sawyer, 1931). The humidity requirements of entomophthoraceous fungi are generally believed to be rather stringent. The conidia of this fungus seem to require more humidity than the other entomophthoraceous fungi, but as long as water is present the period before germination is shorter in this species than in the other entomophthoraceous fungi. This character seems to be inconvenient when this fungus is used as a control agent of insect pests. Because the method of spraying with a conidia suspension cannot be used, owing to the quick germination of conidia in water. Such a water requirement for germination of *Entomophthora* conidia has not been reported but, according to MacLeod (1963) unless free water was present, no infection was obtained when conidia of *E. sphaerosperma* were applied to larvae of *Plutella maculipennis*. This fact suggests that water is required for the germination of *E. sphaerosperma* conidia, contrary to the observation by Sawyer (1931).

*E. delphacis* causes epizootics of the brown planthopper, *N. lugens*, and the green
Germination of *Entomophthora delphacis*  

![Graph](image)

Fig. 2. Effect of light on conidial germination of *Entomophthora delphacis*. Germination rates in light are indicated by open circles, and in darkness by solid circles. The mean rates are indicated by broken line (in light) and solid line (in darkness).

rice leafhopper, *Nephotettix cincticeps*, in Kyushu in Japan. They occur in paddy fields, which have very moist conditions adequate for the conidia of this fungus to germinate.

**Effect of temperature**

Conidia germinated at all temperatures tested (Fig. 1). In the range of 15 to 30 °C, the velocity of germination, which is shown as the gradient of the germination curve, increased and the period before the peak grew shorter in proportion to the increasing temperature. At 15°C, the period before the beginning of germination was long, and the germination velocity and rate were lower. At 20 to 30°C, germination began within 1 hour and the peak germination rate was attained 1.5 to 2 hours after discharge. The peak germination rate was highest at 25°C, followed next at 20 and 30°C. At 35°C, the period before the beginning of germination was within 1 hour, as at 20 to 30°C, but the germination velocity and rate were very low. These results on the temperature range of conidial germination agreed with those for the other entomophthoraceous fungi (*Yendol*, 1968; *Newman* and *Carner*, 1975), and suggest that the temperature requirement of this fungus is not as severe as the humidity requirement. *Newman* and *Carner* (1975) also considered that temperature requirements for sporulation and germination of *E. gammae* were not as critical as humidity. The results also coincided with those on hyphal growth of this fungus (*Shimazu*, 1976), and it may be inferred that the optimum temperature for activity of this fungus is 25°C. An epizootic was caused in green rice leafhopper by this fungus early in September 1976 in Chikugo, Japan. The mean temperature of that period was 24.1°C, which is reasonable for the occurrence of an epizootic.

**Effect of light**

The effect of light on germination was not significant (Fig. 2). *Sawyer* (1931) investigated the development of *E. sphaerosperma* and stated that in darkness the conidia
of *E. sphaerosperma* germinate more favorably than in light, and capillary conidiphores (forming secondary conidia) were fewer than the usual germ tubes. Aoki and Yanase (1975) reported that the conidia of *Entomophthora thaxteriana* formed secondary conidia in light, but they formed germ tubes as a result of germination in darkness. However, all conidia of *E. delphacis* in this investigation formed secondary conidia as the result of germination and the form of the germ tubes was not affected by illumination.

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**REFERENCES**


