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SHORT COMMUNICATION

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Effects of humidity and temperature on ascospore discharge of *Graphostroma platystoma* in the laboratory and in the field

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Abstract The effects of air humidity and temperature on the ascospore discharge of *Graphostroma platystoma* were experimentally investigated. The ascospores were not discharged from the stromata in air at 100% relative humidity (RH). However, they were discharged from the wetted stromata at 3°, 10°, and 24°C under 100% RH or nearly so. The amount of the discharged ascospore was large at 24°C, medium at 10°C, and small at 3°C. The ascospores in the rainwater that washed down the stromata were counted after rainfall in the field. The discharge was observed from September to the following May.

Key words Ascospore · Discharge · *Graphostroma platystoma*

Graphostroma platystoma (Schw.) Pirozynski forms flat and widely effused perithecial stromata in the periderm of bed-logs for shiitake [*Lentinula edodes* (Berk.) Pegler] cultivation in May to July and removes the outer bark (Abe 1986; Barr 1985; Pirozynski 1974; Ohira et al. 1979; Tsunoda et al. 1996). The fungus grows into the bed-logs, occupies the substrate, and finally causes reduction of shiitake harvest (Tsunoda et al. 1996). Therefore, the fungus is one of the troublesome fungi in shiitake cultivation (Furukawa and Nobuchi 1986; Przybylowicz and Donoghue 1990).

The ascospores of the fungus appear to be the main agent in infection (Tsunoda et al. 1999). The invasion season of the fungus to the bed-logs was estimated in early spring, corresponding to the inoculation time of shiitake into the bed-logs, and in winter, from the results of repeated inoculation experiments of the fungus into the first-year bed-logs (Tsunoda et al. 1999). Latent infection of the fungus in live trees was also estimated (Tsunoda et al. 1999). However, the weather conditions inducing discharge of the ascospores have not been investigated yet.

The purposes of the present investigation were to determine the effects of relative air humidity and air temperature on ascospore discharge of *G. platystoma* in vitro and also to observe the seasonal fluctuation of the amount of ascospores discharged in the field.

To estimate the effect of relative humidity (RH), the following experiment was done. Mature stromata of *G. platystoma* on the bed-logs (*Quercus acutissima* Carruth.) with bark tissue were cut into pieces, 7–15 mm wide and 10–40 mm long (Fig. 1). Seven or eight pieces of the stroma were fixed upward on 80-mm-square netting (steel mesh, 3 mm) by a copper wire (diameter, 0.2 mm) (Fig. 1) and placed downward on 90-mm Petri dishes. Two Petri dishes with stromata were placed in a closed container at 20°C for 4 days. The RH in the container was kept at 100% during the incubation. The presence or absence of discharged ascospores was verified every day by microscope.

To investigate the effect of temperature on the discharge of the ascospores, dripping equipment consisting of 2-l plastic bottles, glass cocks, rubber stoppers, and vertical glass cylinders (diameter, 22 mm; length, 120 mm) were placed in incubators kept at 3° and 10°C and in a room at 24°C (Fig. 2). Cylindrical baskets (diameter, 20 mm; depth, 30 mm) were made of 1-mm-mesh stainless steel netting (Fig. 2). The mature stromata were cut into rectangular polygons (0.5–1.5 cm^2), and the tissue of the bark was removed by a cutter knife. The pieces of the stroma were photographed and the surface area of the each stroma was measured by a planimeter on enlarged copies. The number of ostioles of 8 pieces of stromata was also counted on enlarged copies to calculate the number of perithecia per unit area of the stroma. Each basket was filled with 24–26 pieces of the stroma (total surface area, 19–21 cm^2) and hung in the middle part of the glass cylinder by the copper wire (Fig. 2). About 200 ml water was dripped on the basket every hour. As a result, the stromata were kept wet during the experiment. Water that passed through the basket was collected every hour using a glass funnel and an Erlenmeyer flask (300 ml) (Fig. 2). The experiment was carried out for 10 h.

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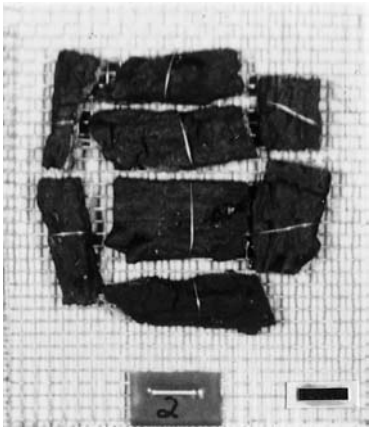

Fig. 1. Pieces of stroma with bark tissue fixed on 8-cm-square netting. *Bar* 1 cm

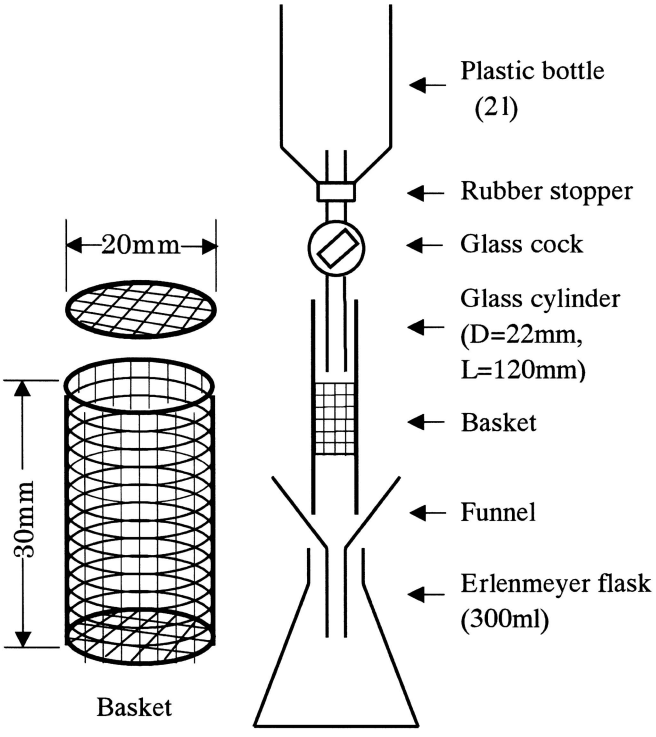

Fig. 2. Dripping equipment. *D*, diameter; *L*, length

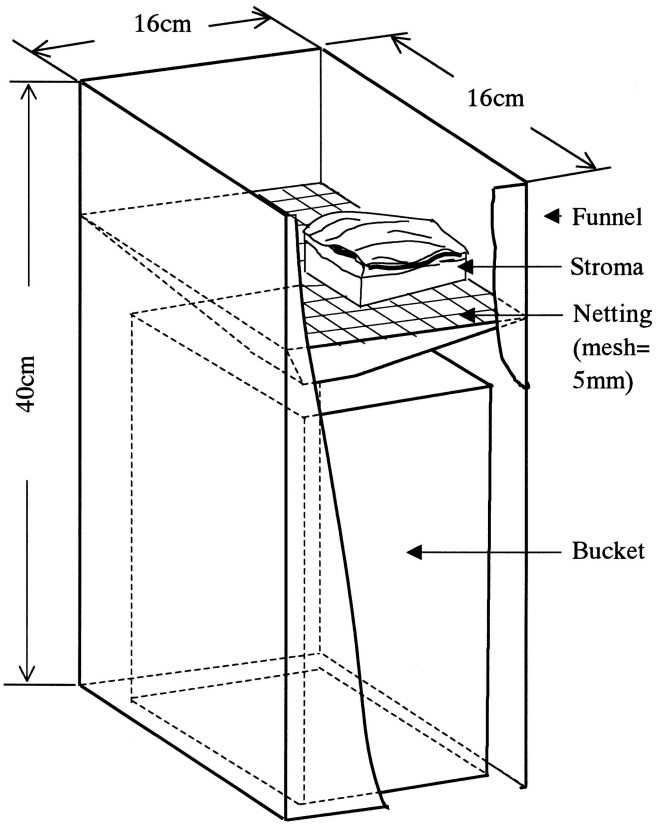

Fig. 3. Water trap

The concentration of the ascospores was counted using a Thoma hematocytometer, and the numbers of ascospores per unit area of the stroma were compared.

To observe ascospore discharge in nature, the following experiments were conducted. A water trap consisting of a funnel and a bucket was made of galvanized sheet steel and a square netting (16 × 16 cm; mesh, 5 mm) was placed in the funnel (Fig. 3). Four water traps were placed in the artificial laying yard for shiitake cultivation at the Kyushu branch of the Forestry and Forest Product Research Institute in Kumamoto City. To pass raindrops freely, the laying yard was shaded by hanging plastic nets. The mature stromata (width, 3–5 cm; length, 5–12 cm) with sapwood of bed-logs were cut from the bed-logs. The periphery of stromata was traced on polyethylene films and their areas were measured by a planimeter. Three to six stromata were placed in each funnel of two traps, while no stroma was placed in each funnel of two traps for a control. The total surface area of the stromata in each of two traps was 231 and 234 cm^2 in the first experiment and in the second was 98 and 112 cm^2. Because the stromata on the water traps deteriorated sooner than those on the bed-logs in the field, the ascospore discharge was measured for about 6 months. The first experiment was carried out from November 1984 to May 1985 and the second from September 1986 to February 1987. After the end of every rainfall, the volume of water in the buckets was measured and the concentration of the ascospores was counted using the hematocytometer.

Ascospores of *G. platystoma* were not discharged in air at 100% RH from stromata with bark tissue even after 4 days. Thus, the high RH alone is not sufficient to initiate ascospore discharge. The same phenomenon was observed for *Hypoxylon fragiforme* (Pers. ex Fr.) Kickx and *Lopadostoma turgidum* (Pers.) Trav. (Walkey and Harvey 1968) and *H. pruinatum* (Klotzsch) Cke (Gruenhagen 1945). Therefore, ascospores of *G. platystoma* may be discharged from the stroma when it is wetted by rain or dew (Ingold 1964).

The number of ostioles per unit area of stroma was 3999 ± 565, which means that the number of perithecia per unit area of stroma was almost the same so that the number of

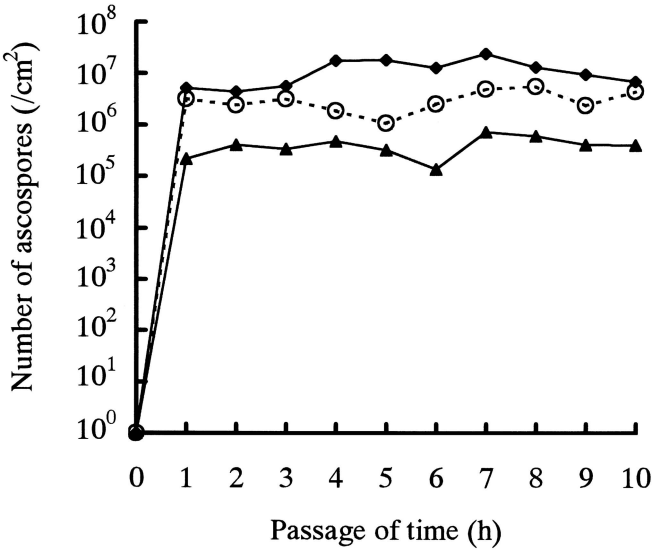

Fig. 4. Effect of temperature on discharge of ascospores of *Graphostroma platystoma* in dripping experiment: —◆—, 24°C; --⊙--, 10°C; —▲—, 3°C. *Vertical axis* is number of ascospores per unit area of the stroma

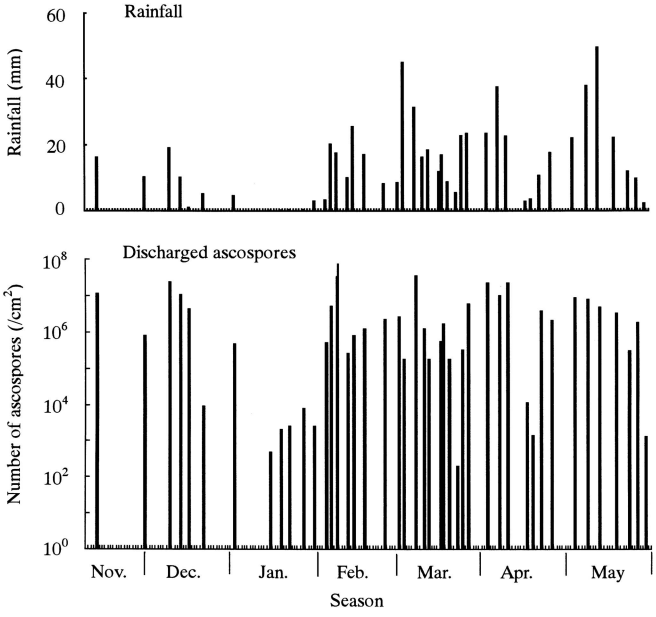

Fig. 5. Number of discharged ascospores of *G. platystoma* and rainfall from November 1984 to May 1985

ascospores discharged from unit area of stroma could be compared.

When the stromata were wetted by water that was dripped on them, ascospore discharge occurred in 1 h at 3°, 10°, and 24°C and continued for 10 h. The number of discharged ascospores from a unit area of the stroma was large at 24°C, medium at 10°C, and small at 3°C (Fig. 4).

Discharge of ascospores is probably arrested below 5°C, and a rise in temperature tends to increase the rate of spore liberation between 5° and 35°C (Ingold 1964). Although the amount of the discharged ascospores of *G. platystoma* was very small at 3°C, it was confirmed that ascospores of the fungus were discharged below 5°C. It was estimated that the amount of the discharged ascospores increased with temperature in the range of 3°–24°C.

The discharged ascospores in the rainwater that was collected in the buckets of the water traps were counted. The ascospores were discharged in every rainfall from November 1984 to the following May and from September 1986 to the following February (Figs. 5, 6). Some spores having the same shape and size as the ascospores of *G. platystoma* were found in rainwater in the control traps. The concentration of ascospores in the rainwater collected from the stromata-placed traps was more than 6.8×10^5/ml and that of the control was less than 3.8×10^3/ml. It is clear that the amount of spores in the control was apparently less than that of the stromata-placed traps.

The daily mean air temperature of the rainy days in winter in Kumamoto City was above 3°C, referring to the data of the Kumamoto Meteorological Observatory in Kumamoto City. This observation suggests that the ascosopores would be discharged even in winter. Because the ascospores of Pyrenomycetes were discharged through narrow ostioles and the amount of discharged ascospores

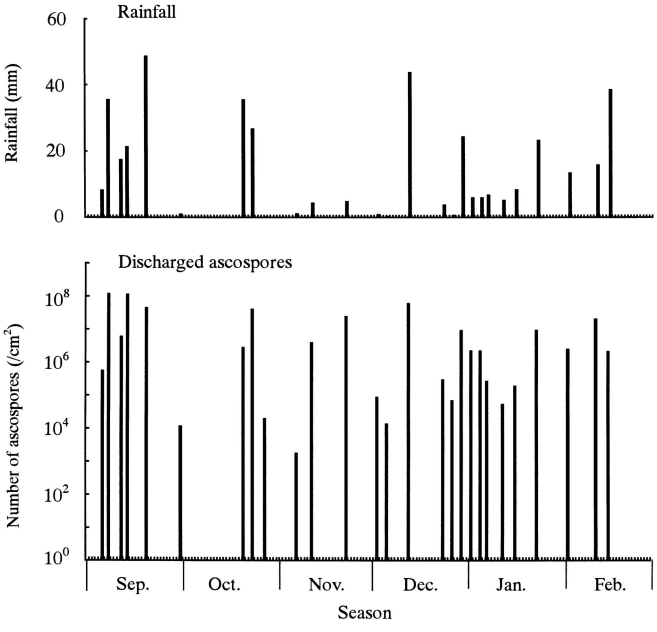

Fig. 6. Number of discharged ascospores of *G. platystoma* and rainfall from September 1986 to February 1987

was limited at each discharge, the ascospores were discharged over a long period (Ingold 1964). It has been confirmed that the ascospores of *G. platystoma* are discharged every month from September to the following May by the collection of rainwater that had washed down the stomata. *G. platystoma* likely discharges ascospores throughout the year, much like *Eutypa armeniacae* Hansf. & Carter (Pearson 1980) and *H. mediterraneum* (De Not.) Mill. (Vannini et al. 1996).

Preparatory work for Shiitake cultivation such as felling trees, logging, and inoculation with shiitake spawn is done from autumn to spring. This routine provides many opportunities for *G. platystoma* to invade the logs and the bed-logs (Tsunoda et al. 1999). *G. platystoma* also has opportunity throughout the year to invade standing trees.

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