Ecological Aspects of Ascospore Discharge in Gibberella zeae

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ABSTRACT

Ascospore discharge from perithecia of *Gibberella zeae*, incubated under controlled environment conditions, is initiated during an atmospheric moisture saturation deficit. If mature ascospores are present, ascospore discharge is regulated by perithecial dehydration. Ascospore maturity appears to be light dependent. Ascospore discharge is inhibited if a saturation deficit is preceded only by a period of darkness.

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Sexual reproduction in *Gibberella zeae* (Schw.) Petch and its role in the disease cycle of stem rot of carnations, stalk rot of corn, or headblight of small grains is poorly understood. Initial data acquired (P. E. Nelson, *unpublished*) in a greenhouse containing carnation plants showing a severe outbreak of stub dieback indicated a periodicity of spore discharge apparently associated with several environmental factors. Ascospore counts during peak discharge periods were as high as 480 ascospores per 0.027 m^3/hour (ft^3/hour). Spore counts taken in a minimum-tillage corn field were twice as high. Ascospores and conidia of isolates from both corn and carnation readily infect carnation to produce the stub dieback phase of Fusarium stem rot (P. E. Nelson, *unpublished*). The perfect stage of this fungus is apparently capable of ejecting its ascospores, which allows them to be carried in wind currents. This study was undertaken to determine factors that affect ascospore discharge.

MATERIALS AND METHODS.—Ascospore discharge in *G. zeae* was studied in environmental growth chambers using a dew chamber similar in design to the one constructed by Mc Coy (2). Temperature within the walk-in growth chamber (Model M-1, manufactured by Environmental Growth Chambers, Chagrin Falls, Ohio) was maintained at either 23± 1 or 16 ± 1 C with a relative humidity (RH) of 70 ± 3%. Illumination was supplied by Sylvania 40-W VHO warm-white fluorescent and 25-W incandescent lamps with an intensity of 22,600 ± 1,000 lx at culture level in the 23 C chamber and 15,000 ± 1,000 lx in the 16 C chamber as measured by a cosine-corrected Model 756 Weston illumination meter. The light was filtered through two Plexiglas barriers, each 4.5-mm thick, one separating the light cap from the chamber proper, and the other being the walls of the dew chamber. Photoperiod in both chambers was 14 hours. Temperatures in the dew chamber ranged as high as 3 C above ambient growth chamber temperature between humidification cycles during the illumination periods, and returned to normal shortly after the initiation of a humidification period.

Dew chamber RH was either approximately 70%, the growth chamber RH, or approximately 100% and was controlled by either a centrifugal fan or a Walton spinning-disk type humidifier. The centrifugal fan circulated ambient growth chamber air through the dew chamber, while the humidifier provided the moisture to raise the RH to 100% and allow dew formation. Air inputs were controlled through a relay, regulated by a time clock so that either the fan or humidifier was operating at all times. A small Muffin fan within the dew chamber was operated at a slow speed to increase the air turbulence over the cultures of *G. zeae*, which enhanced spore collection.

Temperature data were monitored continuously with two gold-ball thermocouples connected to a 24-point Honeywell Electronic 16 recorder which recorded ambient air temperature inside and outside the dew chamber. The presence and duration of dew was measured by electrical resistance sensors similar in design

to those used by Davis and Hughes (1). The sensors were calibrated against free moisture to insure proper function. Dew formation began approximately 30 minutes after the initiation of humidification and relative humidity reached approximately 100% at approximately the same time.

A rotating-drum type spore trap was attached to the air outlet of the dew chamber to continuously monitor ascospore discharge. The spore trap utilized the Scotch-tape collecting method of Mc Coy and Dimock (3). Tapes were removed from the rotating drum every 24 hours. The number collected each hour was determined by counting the ascospores in a 450-μm wide transect of the tape every 7 mm, the length of tape which had passed the orifice each hour. Half-hour readings were taken during peak discharge periods. The tape and ascospores were stained for 15 minutes in a 0.1% aqueous solution of fast-green FCF and placed on a cover slip adhesive side down. Pressure was applied to the tape through absorbent tissue removing excess stain and bonding the tape to the cover slip. Permount was the mounting medium.

Cultures were prepared and inoculated following standard procedures outlined by Tschanz (4). *Fusarium roseum* 'Graminearum' (isolate R-670 isolated from corn) from the collection of the Fusarium Research

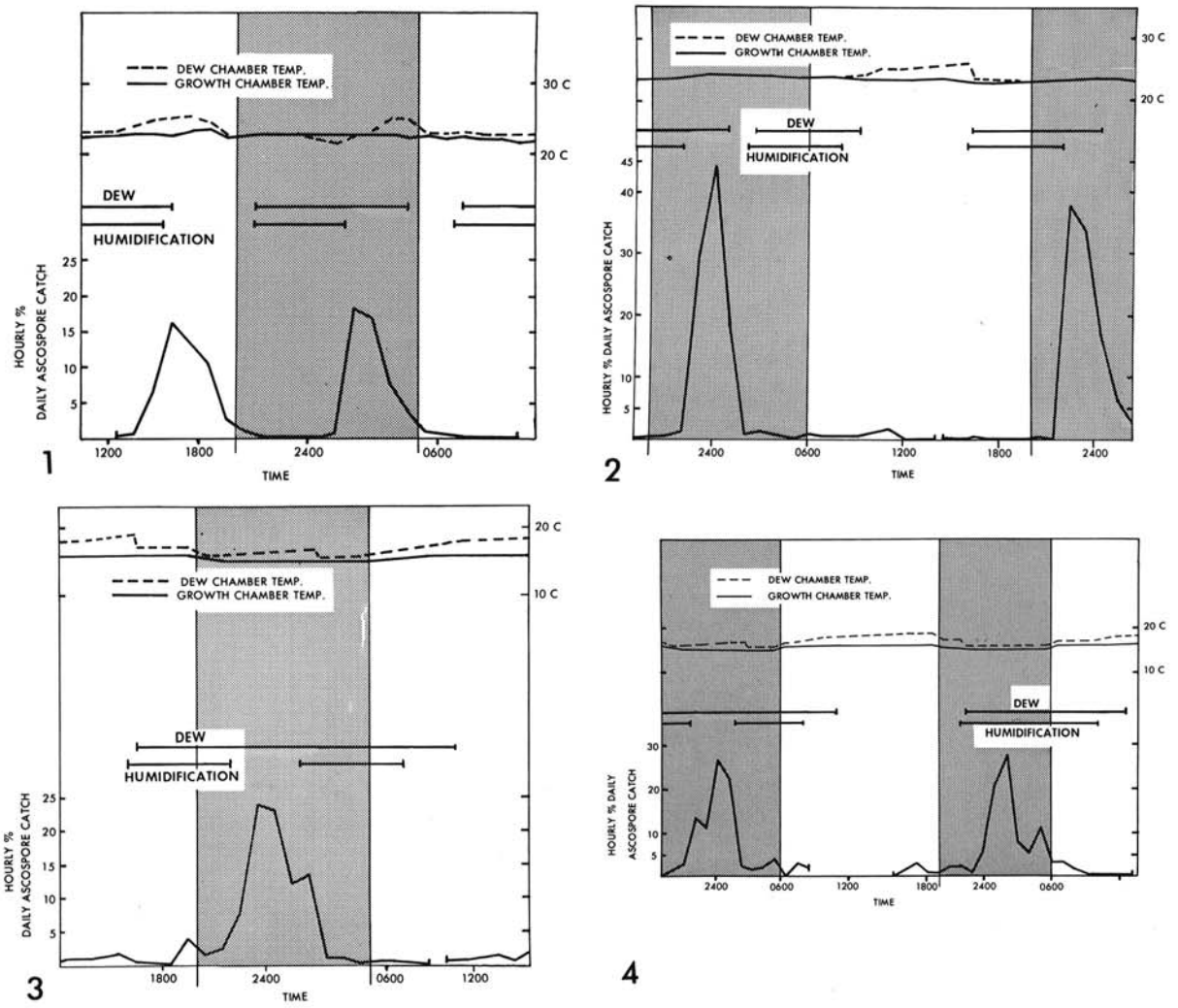

Fig. 1 - 4. In all figures duration of humidification and dew periods are indicated by horizontal solid lines. Shading indicates dark periods. **1)** Ascospore discharge from perithecia of *Gibberella zeae* expressed as a percentage of total daily catch from cultures subjected at 23 C to two daily dew and humidification periods, one in darkness and the other in light. **2)** Ascospore discharge from perithecia of *G. zeae* expressed as a percentage of total daily catch from cultures subjected at 23 C to two daily dew and humidification periods, one initiated in the light and ended in the dark, and the other initiated in the dark and ended in the light. **3)** Ascospore discharge from perithecia of *G. zeae* expressed as a percentage of total daily catch from cultures subjected at 16 C to two daily dew and humidification periods, one initiated in the light and ended in the dark, and the other initiated in the dark and ended in the light. **4)** Ascospore discharge from perithecia of *G. zeae* expressed as a percentage of total daily catch from cultures at 16 C subjected to two daily dew and humidification periods which was changed to one daily dew and humidification period that was initiated in the dark and ended in the light.

Center, The Pennsylvania State University, was used throughout these studies. Cultures were grown in 5-cm box-type plastic petri dishes (Falcon Plastics #1006) and incubated in an environmental growth chamber at 24± 1 C under 3,200 ± 200 lx illumination provided by four Sylvania 40-W black-light blue fluorescent lamps, four 40-W VHO warm-white fluorescent lamps and four 25-W incandescent lamps, with a 12-hour photoperiod. An incubation of 5 days was used to insure uniform perithecial initiation and development, after which six petri dishes were transferred to the dew chamber. The agar plus leaf disk substrate was carefully lifted out and placed on top of the inverted petri dish bottom to prevent condensation from flooding the culture. Cultures were replaced every 10 days. The four experiments were each conducted over at least four daily periods.

RESULTS.—The photoperiod in all studies was from 0600 hours to 2000 hours. Two relative humidity regimes were used during these studies. In the first regime, humidification periods were from 0800 to 1600 hours and 2100 to 0200 hours. The first period was entirely within the light, whereas the second was entirely in the dark. This regime was only programmed for the 23 C chamber and resulted in two daily dew periods, one of 8 hours duration during the day, and the other for 9 hours at night. This humidification regime resulted in the ascospore discharge pattern depicted in Fig. 1. All data are actual measurements of a 24-hour period during the 4 days of the experiment, and show the trend for this discharge period. The dew and humidification periods are represented graphically by horizontal solid lines. Shading indicates dark periods. Two discharge periods were recorded.

In the second regime, humidification periods were from 1600 to 2200 hours and 0200 to 0800 hours, or the opposite of the previous regime. The first humidification period started 4 hours before dark and lasted 2 hours after the lights were turned off, resulting in an 8.5-hour dew period. The second period started 4 hours before light and lasted 2 hours into the illumination period, resulting in a 7-hour dew period. Both dew periods started approximately 0.5 hour after the onset of humidification. This humidification regime was conducted at 23 and 16 C. Ascospore discharge under this regime is illustrated in Fig. 2 and Fig. 3 for the 23 C and 16 C conditions, respectively.

There is an indication that ascospore discharge is a rhythmic phenomenon that continues for a short period after the original stimulus has been removed or changed. When the humidification schedule in the second regime (Fig. 2, 3) was changed to a single period of high humidity extending from 2200 to 1000 hours at 16 C (Fig. 4), there was no influence upon ascospore discharge. The ascospore discharge pattern was similar to that of the previous regime; however, the influence of the previous regime on ascospore discharge decreased rapidly.

DISCUSSION.—Ascospore discharge, except as noted in Fig. 4, was initiated during an atmospheric moisture saturation deficit that caused the perithecia and the substrate to dry out. The rate of desiccation may determine the number of ascospores released under an ideal situation; i.e., an unlimited supply of mature ascospores. However, this may not be true when asci and ascospores are found at various stages of development within the same perithecium. In *G. zeae*, the supply of mature ascospores is limited by the rate at which they mature (P. E. Nelson, *unpublished*). It can, therefore, be assumed that the number of ascospores discharged is regulated not only by the rate at which the perithecia dry, but also by the availability of mature ascospores, which in turn is affected by the conditions that regulate ascospore maturation. This can be seen by comparing the spore discharge patterns in Fig. 1 and Fig. 2. In Fig. 1, atmospheric moisture saturation deficits apparently were preceded by a period in which conditions were favorable for maturation of ascospores. In Fig. 2, these conditions were absent prior to the second moisture deficit. Analysis of the humidification regimes reveals that in the first regime (Fig. 1), the initiation of spore discharge was preceded by a period of light of 4 or more hours duration following a moisture deficit. In the second regime (Fig. 2, 3), moisture conditions favoring discharge were preceded by 2 hours of light in the one instance, and 12 hours in the other. Significant ascospore discharge was only recorded after the 12-hour period of light. Timing of discharge was unaffected by temperature. The above phenomenon supports the hypothesis that light is required for ascospore maturation (4).

Ascospore discharge in these studies seemed to occur in a rhythmic pattern that continued even though the stimulus was changed (Fig. 4). However, this may be an artifact induced by the precisely structured environmental conditions (light, temperature, RH) within the growth chamber.

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