Environmental Factors Regulating Ascospore Discharge by Mycosphaerella ligulicola

R. E. McCoy and A. W. Dimock

Former graduate student and Professor, Department of Plant Pathology, Cornell University, Ithaca, N.Y. 14850. Present address of senior author: Agricultural Research Center, University of Florida, Fort Lauderdale 33314.

Based on a portion of a Ph.D. thesis presented to the Graduate School, Cornell University, August 1971.
Accepted for publication 17 November 1972.

ABSTRACT

Ascospore discharge by *Mycosphaerella ligulicola* is regulated by moisture and, depending on isolate, by light, at a constant air temperature of 20 C. Explosive discharge of ascospores is effected by sprinkling water or accumulating heavy dew upon perithecium-bearing chrysanthemum tissue. Approximately 0.1 mm of moisture accumulation is necessary to initiate such discharge. Light prevented or stopped discharge, even in the presence of adequate moisture, in three of four isolates tested. These three isolates discharged ascospores only in darkness, whereas ascospore discharge by the fourth isolate occurred in either light or darkness. Ascospore trapping during an outbreak of Mycosphaerella blight of field-grown chrysanthemums revealed peak aerial concentrations just after an evening thundershower; much lower concentrations were present during nighttime dew periods.

Phytopathology 63:586-589

Additional key words: Ascochyta chrysanthemi, Chrysanthemum morifolium, chrysanthemum ray blight.

The role played by the perfect stage of *Mycosphaerella ligulicola* in the epidemiology of Mycosphaerella blight of chrysanthemum has been a matter of conjecture since its description by Baker et al. in 1949 (1). Although it was assumed that ascospore dissemination was by air currents, this fact had not been demonstrated. Also, the environmental parameters that might effect such ascospore discharge into the air were not known. This study was undertaken to obtain information on these aspects of ascospore dispersal and to determine if the perfect stage could actively participate in the disease cycle.

MATERIALS AND METHODS.–Ascospore discharge by four isolates of *M. ligulicola* was studied in a dew chamber in which wind velocity, temperature, and relative humidity (RH) were programmed. The dew chamber was an isolation cabinet made of clear plexiglass and measuring 90 X 40 X 40 cm (Fig. 1). Filtered air was supplied continuously at a rate equivalent to a complete air exchange every 2 min. The dew chamber was placed within a large walk-in controlled environment chamber. The advection of ambient growth chamber air into the dew chamber gave it the temperature and humidity characteristics of the large chamber, which was maintained at 20 ± 0.5 C and 70 ± 3 RH. The lighting conditions of the dew chamber were also those of the growth chamber in which it was placed; i.e., a combination of fluorescent and incandescent lamps with a 14-hr photoperiod, a radiant intensity of 1,700 $\mu w/cm^2$, and an illumination level of 2,300 ft-c as measured by a YSI-Kettering radiometer and a Weston ft-c meter, respectively.

Growth chamber air was blown into the dew chamber with a centrifugal fan. This ambient input blower was connected to the dew chamber through a Y-connection. A Walton spinning-disc type humidifier with a reservoir fed by a distilled water tap was connected to the other arm of the Y. The humidifier and the ambient input fan were adjusted to give equal inputs of air into the dew chamber so that air flow through the chamber was constant. These two inputs were controlled by a relay energized by a time clock so that either the humidifier or the ambient input fan was on at all times. When the relay was energized by the time clock the humidifier was switched on, blowing fog into the dew chamber and raising the RH to 100%. Deactivation of the relay switched the current to the ambient input blower, thereby introducing air of 70% RH. Condensation of moisture occurred on all surfaces within the dew chamber when the RH was 100%.

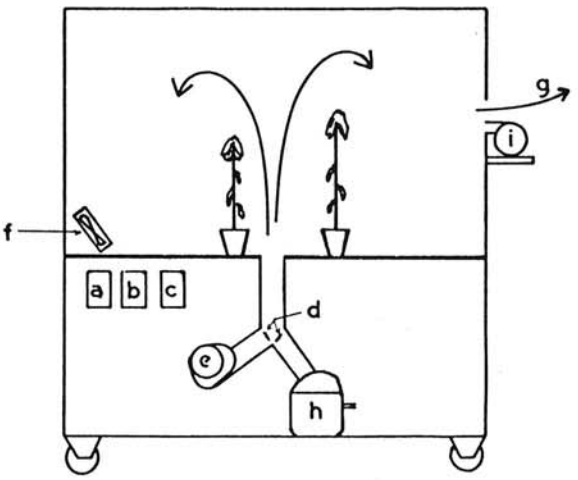

Fig. 1. Diagrammatic representation of dew chamber: a) timer; b) relay; c) variable autotransformer for fan control; d) one-way valves to prevent reverse flow of air; e) ambient input fan; f) mixing fan; g) air outlet; h) humidifier; i) spore trap.

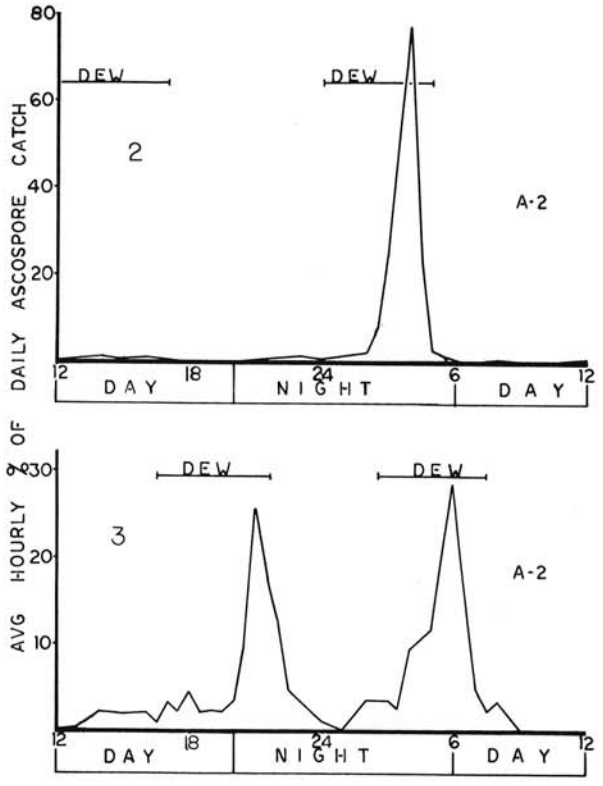

Fig. 2-3. 2) Ascospore discharge by isolate A-2 subjected to two dew periods daily, one in light and one in darkness. **3)** Ascospore discharge by isolate A-2 subjected to two dew periods daily, one beginning in light and ending in darkness, the other beginning in darkness and ending in light.

A Muffin fan controlled by a variable autotransformer and placed within the dew chamber was used to vary the turbulent mixing velocity of the air within the chamber independently of the air flow rate through the chamber. The rates of condensation and evaporation of dew could be varied by changing the speed of this fan. The rate of condensation varied inversely with wind velocity, whereas the rate of evaporation varied directly. The duration of dew periods and relative amounts of dew present were measured by electrical resistance sensors previously described (4), and were recorded by a YSI Model 81 strip chart recorder. The sensors were calibrated by absorbing the dew from a prescribed area of the chamber floor on a tared filter paper. The resulting change in weight gave the amount of dew present in mg/cm^2. The maximum rate of condensation was 4.5 mg/cm^2 hr, obtained when the Muffin fan was switched off. This is a moderate to high rate of condensation and well within the natural limit set by Monteith (10).

A rotating drum spore trap was placed at the air outlet so that air passing from the chamber was continuously sampled. Scotch brand Magic Transparent Tape was used as the spore-trapping substrate (8). Perithecium-bearing chrysanthemum tissue was placed within the dew chamber and hourly ascospore counts were recorded. Spore counts were made by counting spores present in a 420 μ-wide transect of the tape every 7 mm of tape length, 7 mm representing the distance traveled by the tape past the intake slit of the trap in 1 hr. In heavy discharge periods, when greater accuracy was desired, counts were taken at 30-min intervals. Trapping efficiency for *M. ligulicola* ascospores was calculated to be 95% according to the method described by May (7). Calculated efficiency of collection of particles 4 μ in diam. was 50%.

RESULTS AND DISCUSSION.—*Moisture-light interaction.*—Ascospore discharge was studied in several tests in which the timing of dew periods was varied in a light regime where lights were switched on at 0600 hr and switched off at 2000 hr each day. In the first test, two dew periods were programmed each day by switching on the humidifier from 0 - 0400 hr and 1200 - 1600 hr. Surfaces within the dew chamber remained wet for approximately 1 hr after the humidifier was switched off and evaporation had commenced. This resulted in two daily dew periods of 5 hr each, one in darkness and one in light.

When perithecium-bearing chrysanthemum tissue inoculated with *M. ligulicola* isolate A-2 was placed in the dew chamber, a spore discharge pattern like that depicted in Fig. 2 was obtained. Almost all ascospores were caught during the "nighttime" dew periods. Less than 2% of the spore catch occurred in the "daytime" dew periods. Both sampling periods were identical in duration and intensity. A rapid drop in spore count occurred at 0400 hr when the ambient input fan was switched on, thereby producing a saturation deficit within the dew chamber and commencing an evaporative regime. This test was carried out over eight daily periods with an average sample per day of 1,200 ascospores.

A second test, also using isolate A-2, was conducted over a 7-day period in which the two daily dew periods were interrupted by switching the lights on or off. This was accomplished by timing the dew periods to run from 1630 - 2130 hr and from 0230 - 0730 hr. The first dew period ran 3.5 hr in light followed by 1.5 hr in darkness, whereas the second period ran 3.5 hr in darkness then 1.5 hr in light. Again, an inhibition of discharge under lighted conditions was observed (Fig. 3). Discharge remained low in the first dew period until the lights were switched off. Discharge then began, rising to a peak at the time drying commenced. The second dew period began in darkness and the rate of discharge rose rapidly after 2 hr. A rapid drop in discharge occurred when the lights were switched on at 0600 and before the evaporative regime began at 0700.

A third test using isolate LI-2 was repeated in five independent daily periods in which the humidifier was operated continuously to produce a 24-hr dew period each day. The majority of the ascospores were trapped during the dark period, with a relatively broad peak of discharge (Fig. 4). Again, there was an apparent restriction of discharge by light. A similar

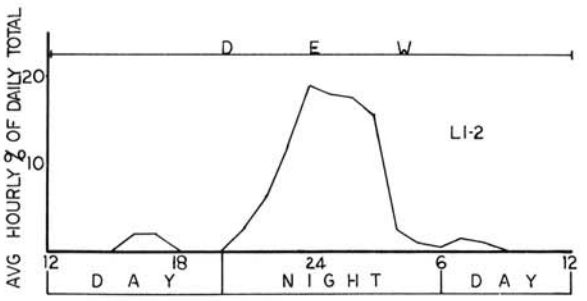

Fig. 4. Ascospore discharge by isolate LI-2 subjected to constant moisture.

test with isolate A-1 gave essentially the same result; discharge was predominantly during darkness.

Another isolate, mA_2, was tested with dew periods from 0 - 0400 hr and 1200 - 1600 hr. Contrary to expectation, isolate mA_2 discharged ascospores readily during dew periods in light and in darkness (Fig. 5). This test was replicated over nine independent daily periods with an average sample of more than 1,700 ascospores per day.

McCoy et al. (9) found that isolates LI-2, A-1, and A-2 require light for the development of reproductive structures. On the other hand, isolate mA_2 produced pycnidia and perithecia in either an alternating light and dark regime, or in continuous darkness. Examination of other isolates indicated that they fell into one of two categories, sensitive or insensitive to light stimulation for development of reproductive structures. Sensitivity to light appears to be carried through to ascospore discharge as well; those isolates sensitive to light stimulation of reproductive development were inhibited by light (i.e., sensitive to light) for ascospore discharge. Isolate mA_2, which developed reproductive structures in either light or darkness, also exhibited no inhibition of ascospore discharge by light.

Brook (2) reported that ascospore discharge by *Venturia inaequalis* was stimulated by light and inhibited in darkness. He related this fact to the necessity for *V. inaequalis* ascospores to be carried from the orchard floor up into the apple foliage in order to initiate infection. Since vertical transport of spores by turbulent air is greatest in afternoon and minimal at night, the ecological advantage is for afternoon discharge by *V. inaequalis*. However, *M. ligulicola* ascospores are discharged within the crop canopy and have the greatest potential for producing infection when carried in a stable atmosphere, a condition which is most likely to occur at night.

Moisture requirement for discharge.—In Fig. 2 it is seen that ascospore discharge began approximately 2 hr after the onset of dew condensation. The rate of condensation was 4.5 mg/cm^2 per hr. When this same tissue was wet by sprinkling or by immersion in distilled water before placement in the dew chamber, ascospore discharge began within 30 min. This indicates that a requisite amount of moisture must be present for initiation of discharge. Based on dew condensation rate, this amount is approximately 0.1 mm of moisture accumulation. This agrees closely with the results of others (3, 5). Carter (3) found that a minimum of 0.007 inches (0.18 mm) of rainfall was necessary to trigger measurable ascospore discharge by *Mycosphaerella pinoides.* Hirst & Stedman (5) reported that the equivalent of 0.2 mm of rain stimulated heavy ascospore discharge by *V. inaequalis* with far more ascospores liberated in periods of rainfall than in periods of dew. This readily is explained by the fact that even a light rain can deliver 1 - 2 mm of moisture per hr, whereas an extremely heavy dew may deliver at most 0.07 mm moisture per hr (10).

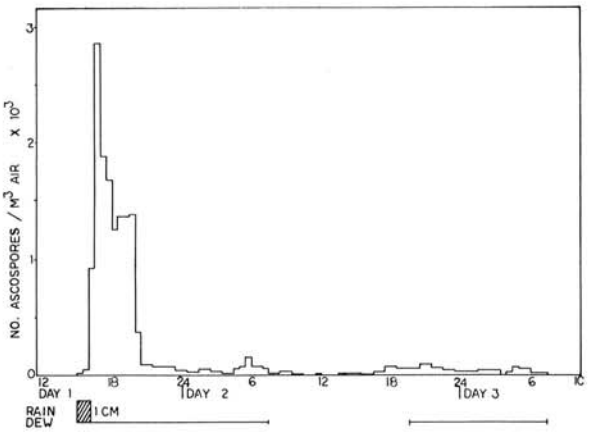

Fig. 6. Ascospore catch over a 3-day period from a naturally occurring field outbreak of Ascochyta blight.

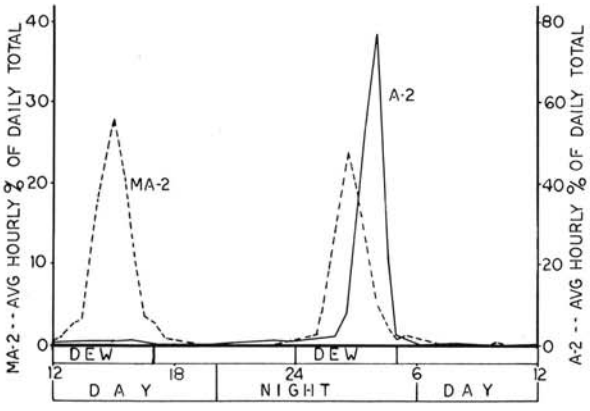

Fig. 5. Ascospore discharge by isolates mA_2 (dotted line) and A-2 (solid line) subjected to two dew periods daily, one in light and one in darkness.

Discharge under field conditions.—In October 1970 during a naturally occurring field outbreak of Mycosphaerella blight, a spore trap was placed in the field for a period of 3 days. The spore trap, which

was non-directional (8), was placed at the center of a focus of disease approximately 1 m in diam.; the orifice was directed downwards to minimize sampling error due to changes in wind direction. The spore counts (Fig. 6) indicate a broad peak in ascospore catch, up to 2,800 ascospores/m^3 of sampled air, beginning shortly after an evening thundershower on day 1. Counts ranging up to 200 ascospores per cubic meter of sampled air were found in the dew periods of days 2 and 3. Very few ascospores were caught during the dry daytime periods.

The moisture requirements for ascospore discharge by *M. ligulicola* are similar to those of most other Ascomycetes which explosively discharge their spores (6). The rapidity of discharge after sprinkling tissue with water as compared to delayed and lighter discharge after slow wetting by dew is consistent with the threshold discharge requirement of 0.1 mm moisture. The heavy ascospore catch in the field shortly after a rain shower also exhibits the effect of moisture availability on ascospore discharge.

The inhibition of ascospore discharge by light is a very real phenomenon in those isolates sensitive to light for perithecium development but the distribution in nature of these isolates is not known. The majority of isolates seen by us are sensitive to light; however, J. P. Blakeman (*personal communication*) has indicated that most British isolates would fall into the light-insensitive group. Before any broad generalizations are made regarding the diurnal timing of dispersal of *M. ligulicola* ascospores, it would appear necessary to determine the categories of isolates present in any particular situation.

Perithecia of *M. ligulicola* mature on senescent chrysanthemum tissue at the flowering stage of the crop (9). Consequently, the airborne phase of dispersal may become active just as the greatest volume of susceptible host tissue is being produced. It is our conclusion that the flower blight phase of this disease is in large part initiated by ascospore infections.

LITERATURE CITED

1. BAKER, K. F., A. W. DIMOCK, & L. H. DAVIS. 1949. Life history and control of the Ascochyta ray-blight of chrysanthemum. Phytopathology 39:789-805.
2. BROOK, P. J. 1969. Effects of light, temperature, and moisture on release of ascospores by Venturia inaequalis (Cke.) Wint. N. Z. J. Agr. Res. 12:214-227.
3. CARTER, M. V. 1963. Mycosphaerella pinoides II. The phenology of ascospore release. Aust. J. Biol. Sci. 16:800-817.
4. DAVIS, D. R., & J. E. HUGHES. 1970. A new approach to recording the wetting parameter by the use of electrical resistance sensors. Plant Dis. Reptr. 54:474-479.
5. HIRST, J. M., & O. J. STEDMAN. 1962. The epidemiology of apple scab [Venturia inaequalis (Cke.) Wint.] II. Observations on the liberation of ascospores. Ann. Appl. Biol. 50:525-550.
6. INGOLD, C. T. 1965. Spore liberation. Clarendon Press, Oxford. 210 p.
7. MAY, K. R. 1967. Physical aspects of sampling airborne microbes. p. 60-80. *In* P. H. Gregory & J. L. Monteith [ed.]. Airborne Microbes, 17th Symposium of Soc. for Gen. Microbiol. Cambridge Univ. Press.
8. MC COY, R. E., & A. W. DIMOCK. 1971. A Scotch tape method for the trapping and examination of airborne spores. Plant Dis. Reptr. 55:832-834.
9. MC COY, R. E., R. K. HORST, & A. W. DIMOCK. 1972. Environmental factors regulating sexual and asexual reproduction by Mycosphaerella ligulicola. Phytopathology 62:1188-1195.
10. MONTEITH, J. L. 1963. Dew: facts and fallacies. Brit. Ecol. Soc., Symp. No. 3. p. 37-56.