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EFFECTS OF ENVIRONMENTAL FACTORS ON ASEXUAL SPORULATION OF *SCLEROSPORA GRAMINICOLA*

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Abstract : Effects of temperature, relative humidity (RH) and light on sporangial production of the pearl millet downy mildew pathogen were investigated. Though the process of sporulation was completed in about 6 h, high RH (95–100 per cent) was essential only during the last 3 h. Maximum sporulation occurred when infected leaves were incubated for 6–12 h at 30°C prior to exposure to high RH for sporulation. Sporulation occurred from 10–30°C with an optimum at 20°C. Sporulation occurred well in fluorescent light (2000 lux), in the dark and in near ultra-violet light (160 lux). The size of the sporangia and sporangiophores was affected by temperature, and the largest were produced at 25 and 15°C, respectively. The implications of these results are discussed.

Keywords : Effect of environmental factors, Sporulation, *Sclerospora*

Asexual spores (sporangia) of the downy mildew pathogen (*Sclerospora graminicola* (Sacc.) Schroet.) of pearl millet (*Pennisetum americanum* (L.) Leeke) play a major role in the epidemiology of this disease (Singh and Williams, 1980). These spores have been successfully used for the identification of resistance in plants in the laboratory (Safeeulla, 1976; Singh and Gopinath, 1985), and field using infector rows (Williams, *et al.*, 1981). For reliable field screening, a high production of sporangia is required and the factors that promote sporangial production increase the reliability of screening (Williams *et al.*, 1981). Several workers have studied the effects of environmental factors on the sporulation of *S. graminicola* (Nene and Singh, 1976), but the optimum levels of these factors and their durations have not been clearly defined. The participants in the Bellagio Conference on Graminaceous Downy Mildews indicated the need to determine the physiological and environmental factors controlling the production and germination of spores (Durbin *et al.*, 1979). In this paper, we report the effects of temperature, light and RH on the sporulation of *S. graminicola* on pearl millet.

MATERIALS AND METHODS

Plants of a susceptible pearl millet cv. 7042, which were grown in pots in the greenhouse, were used.

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Production and collection of sporangia

Systemically infected plants obtained by seedling inoculation method (Singh and Gopinath, 1985) and grown in normal day light during February to April were used. Plants were given fluorescent light (2000 lux) in addition to day light (supplemental light), for desired durations during night. Immediately following the light treatment, diseased leaves were detached and their old growth removed by washing with camel hair brush. The leaves were cut into small segments (4–6 cm) and placed abaxial side up in Petri plate humidity chambers (95–100 per cent RH) and incubated in darkness at 20°C. After 6 h, sporangia were harvested from the leaf segments with a measured quantity of cold (2–4°C), distilled water.

Determination of concentration

Sporangial concentration was determined using a haemacytometer. Six haemacytometer counts were taken in each treatment and an average was calculated. Leaf area involved in sporangial production was measured by an automatic areameter (Model AAM 7, Hayashi Denkoh Co. Ltd, Tokyo, Japan) and the number of sporangia produced per unit area of leaf was determined.

Effects of pre-sporulation temperature and day length on sporulation

A set of five pots containing 24-day old, systemically-infected plants were given supplemental light treatment for six durations, 2, 4, 6, 8, 10 and 12 h in separate incubators. Each light treatment was given at 20°, 25°, 30°, 35°, 40° and 45°C. Light treatments were initiated such that they all ended at 0800 h. The leaves from each treatment were separately incubated for sporulation. Each treatment was replicated in three humidity chambers. Every hour for 6 h, one leaf segment from each treatment was removed, examined for sporulation and discarded.

Effect of temperature during sporulation

Infected plants were incubated at 20°C and 70 per cent RH in fluorescent light at 1700 h. At 0830 h the following day, leaves were cut into small segments and incubated for sporulation in humidity chambers. Two humidity chambers were incubated for sporulation at each of 7 temperatures—5°, 10°, 15°, 20°, 25°, 30° and 35°C. The number of sporangia produced per cm^2 of leaf area was determined. The experiment was done thrice.

Effect of lighting on sporulation

Three infected leaves, one from each of three different plants that had been incubated at 70 per cent RH and 20°C (in an incubator to prevent sporulation) from 1700 to 0800 h, were detached and their old downy mildew growth was removed. Three leaf segments (one from each of the three leaves) were kept on moist blotting paper in an uncovered Petri plate. One such plate was exposed to NUV light (160 lux), another to fluorescent light (2000 lux) and the third was kept in darkness. The light sources were 50 cm above the Petri plates. High humidity (95–100 per cent) necessary for sporulation was provided in each incubator. Number of sporangia produced per cm^2 of leaf area was determined. The experiment was done twice.

Effect of RH during sporulation

Uniformly sporulating leaves from 20-day old infected plants were cut into small segments. The leaves were kept in Petri plates with different RH levels created as follows: (a) old downy growth was removed with dry cotton (dry-), and the leaf segments were kept in Petri plates lined with dry blotters (dry chamber) (T1); (b) old downy growth was removed with wet cotton (wet-), the leaf segments were blotter dried and kept in the dry chambers (T2); (c) as in (a) but the leaf segments were sprayed with water before placing them in the dry chamber (T3); (d) as in (a) except that the leaf segment were kept in Petri plates lined with moist blotters (moist chamber) (T4); (e) as in (b) but blotter dried segments were kept in moist chambers (T5); and (f) as in (b) but sprayed with water and kept in moist chambers (T6). Each treatment was repeated in three chambers which were incubated at 20°C for 6 h. A digital psychrometer was used to measure RH at the beginning and end of the incubation period. The dry and wet sensors were inserted into the chamber through two separate holes cut into the wall, and left in each chamber for 15–20 s for each measurement. The chambers were sealed with parafilm. The experiment was repeated twice.

Dew period requirement

Twentyfive-day old infected plants, with several intact leaves, were cut at the base and their cut ends were placed in water in flasks. The flasks were incubated at 20°C in fluorescent light from 1700–0800 h. Leaves of similar age were detached, old downy growth removed, and their cut ends were placed in water. The leaves were maintained in a position vertical to the water surface in a grooved plastic container. The container was incubated at 20°C and 70 per cent RH in an incubator (pre-sporulation incubation). After 1 h, washed, blotter-dried leaves were incubated for sporulation in a humidity chamber at 20°C. Every hour for 9 h, four 5-cm long leaf segments were removed from the incubator and incubated for sporulation. Thus each successive sample that was incubated for sporulation, received increasing pre-sporulation incubation period and decreasing sporulation incubation period, with the ninth sample receiving no sporulation incubation.

Effect of temperature on the size of sporangiophores and sporangia

Sporangia were produced at five temperatures—10°, 15°, 20°, 25° and 30°C. They were harvested with cold water and 100 measurements (312.5×) made of sporangiophore length (base to first branching), sporangiophore width (broadest point), sporangial length (operculum to base) and maximum sporangial width.

RESULTS

Effects of pre-sporulation temperature and light duration

Onset of sporulation : The effects of the treatments applied prior to sporulation and their interactions were observed only during the early process of sporulation. Leaves incubated at 20° and 25°C with 10 h supplemental light and also from 30°C with only 2 h supplemental light (during pre-sporulation) began to produce sporangiophores in 1 h following their incubation for sporulation at 20°C. The number of sporangiophores

increased with increase in time of humid incubation. However, differences in the effects of pre-sporulation treatments were not evident after 4–5 h of sporulation incubation when fully developed, branched sporangiophores had formed in all treatments. Leaves exposed to pre-sporulation temperatures exceeding 30°C showed erratic development of sporangiophores which were comparatively short, fewer in number and unevenly distributed on the leaf surfaces. The emergence of sporangiophores appeared to be a continual process and young sporangiophores were seen emerging even after 6 h at 95 per cent RH. Tip-bulging of sporangiophores, irrespective of temperature and light duration, was an interesting feature. The swellings occurred prior to branching which originated from the swellings.

Spore production and germination : Leaves from 30°C pre-sporulation incubation temperature, produced the maximum number of sporangia in all cases except the 4 h supplemental light treatment (Fig. 1). Any deviation from 30°C during pre-sporulation, markedly decreased sporulation in the majority of the light exposure treatments, with little or no sporulation on leaves from the 45°C pre-sporulation incubation. No clear effects of supplemental light were observed.

The sporangia produced on leaves that experienced pre-sporulation temperatures of up to 30°C gave >90 per cent germination and normal zoospore release; those from higher temperatures had decreased germination and inactive, irregularly shaped zoospores.

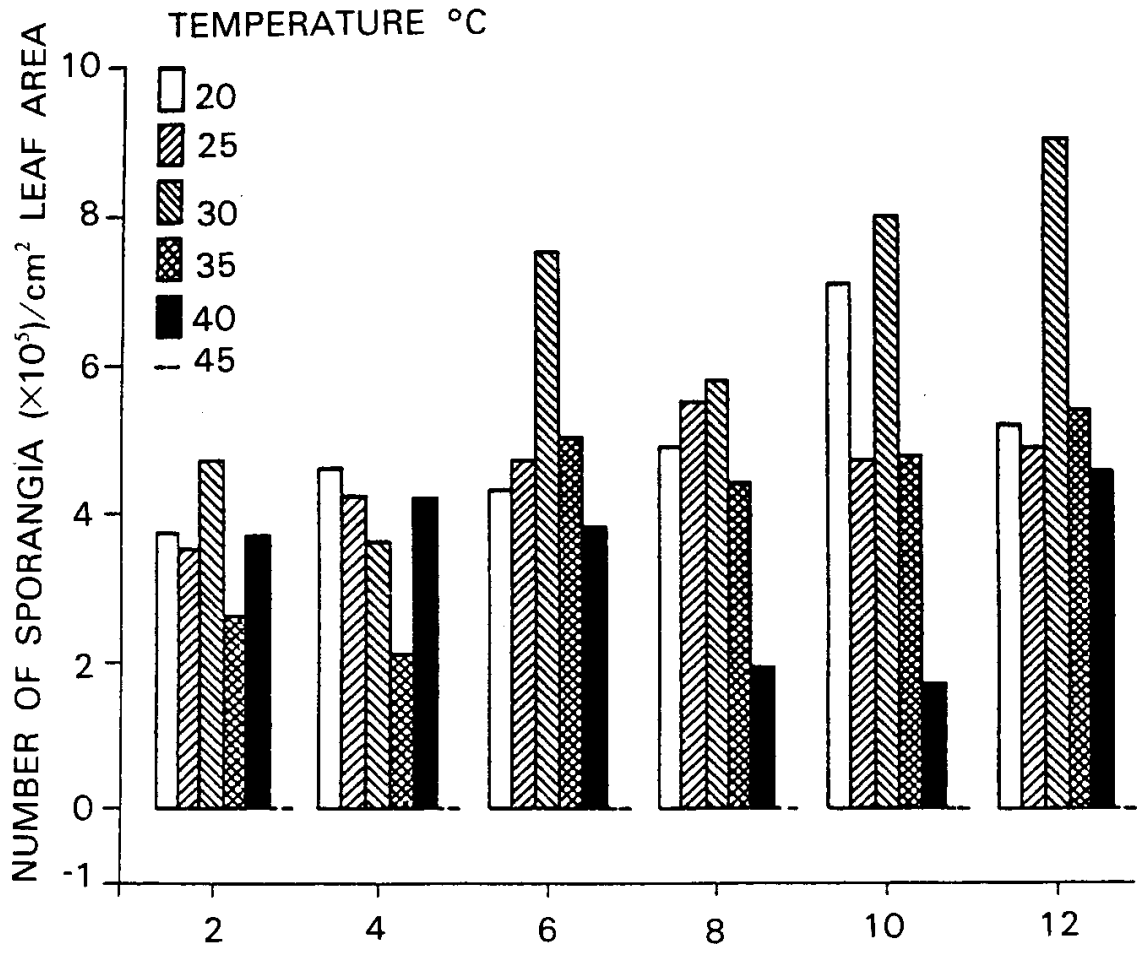

Fig. 1 : Effect of pre-sporulation temperature and light duration on subsequent sporulation at 20°C and 95 per cent RH.

Effect of sporulation temperature : Sporulation occurred at 10–30°C, with a maximum at 20°C (Fig. 2).

Effect of light : Light or its absence appeared to have no effect on sporulation. The number of sporangia produced were 6.5×10^5, 6.4×10^5, and 6.0×10^5 sporangia per cm^2 leaf area in fluorescent light, NUV light and darkness, respectively. The sporangia from these three treatments germinated normally.

During the early sporulation period, sporangiophores produced in darkness appeared to be shorter than those produced in either of the two light treatments. These differences disappeared with an increase in the incubation time.

Effect of RH : Sporulation occurred in all the treatments. There was good sporulation in treatments where RH was maintained above 94 per cent (Table 1). Poor sporulation was recorded in treatments with RH below 90 per cent.

TABLE 1 : Effect of relative humidity on the production of sporangia by *Sclerospora graminicola*

Treatment	RH (per cent)		sporangia* ($10^3/cm^2$)
	Initial	Final	
T1	76	89	1.7 (7.44 ± 0.155)
T2	80	89	2.1 (7.62 ± 0.551)
T3	82	89	15.2 (9.56 ± 0.189)
T4	96	94	139.3 (11.84 ± 0.084)
T5	97	96	142.0 (11.86 ± 0.123)
T6	98	97	154.5 (11.95 ± 0.050)

*Log transformed values and their standard errors are given in parentheses

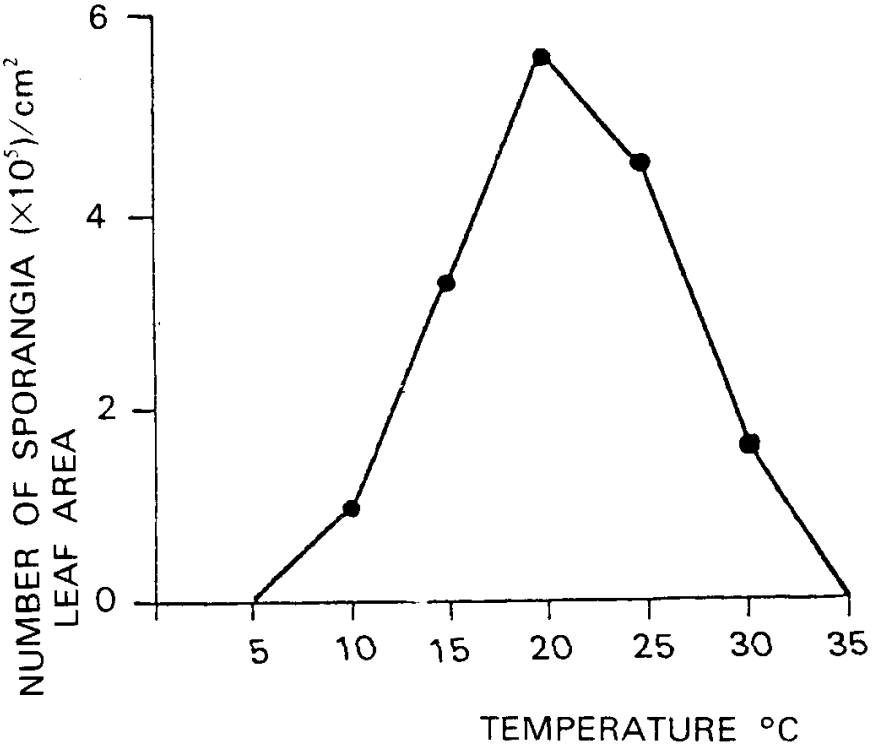

Fig. 2 : Effect of sporulation temperature on the production of sporangia

Dew period requirement : A minimum of 3 h incubation under 95-100 per cent RH was required for the completion of sporulation after the leaves were maintained at 20°C and 70 per cent RH. In the absence of a high RH, there was no sporulation for 9 h (on leaves kept in the incubator under < 70 per cent RH), except on the leaf areas that were close to the water surface. Sporangiophores emerged and sporangia began to develop during the first 2 h of incubation at a high RH. The sporangia matured (capable of germinating) in an hour. After sporulation, sporangiophores collapsed leaving a bed of sporangia on the leaf surface. Since the process of sporulation is continuous, the collapse of earlier formed sporangiophores and the emergence of new sporangiophores were seen simultaneously.

Effect of temperature on the size of sporangia and sporangiophores : The sizes of sporangia and sporangiophores varied with temperature (Table 2). Sporangia tend to grow large at higher temperatures while reverse is the case with sporangiophores.

TABLE 2 : Effect of temperature on the size of sporangia and sporangiophores in *Sclerospora graminicola*

	Temperature (°C)	Length* (μm)	S.E. ±	Width* (μm)	S.E. ±
Sporangia	10	19.45	0.31	13.63	0.24
	15	19.73	0.32	13.84	0.27
	20	18.10	0.33	12.79	0.25
	25	20.35	0.29	15.57	0.26
	30	21.78	0.43	15.62	0.34
Sporangiophores	10	216.80	4.40	16.33	0.39
	15	208.50	3.19	18.10	0.35
	20	202.00	4.07	13.29	0.32
	25	191.00	3.83	17.85	0.41
	30	179.80	5.22	14.94	0.42

*Mean of 100 observations.

DISCUSSION

The effects of environmental factors on asexual sporulation of downy mildew pathogens have been reported by several researchers (Nene and Singh, 1976; Safeeulla and Thirumalachar, 1956; and Shetty and Ahmad, 1981). Weston (1924) reported asexual sporulation in *S. graminicola* to be nocturnal. Our results, however, and those of Safeeulla and Thirumalachar (1956) show that light does not inhibit sporulation. The critical factors for sporulation are temperature and humidity. Temperatures ranging from 15-35°C prior to a sporulation period (corresponding to day temperature) and 10-30°C during the sporulation period (corresponding to night temperature) favoured sporulation, though the optimum temperatures for the pre-sporulation and sporulation periods were 30° and 20°C, respectively. There is no published report on the effect of pre-sporulation temperature on asexual sporulation of this pathogen, but during the dew period the pathogen could sporulate at tempertures up to 30°C as against 28°C reported earlier (Suryanarayana, 1965).

The day and night temperatures during the rainy season at the ICRISAT Center generally fall within the 10–35°C range as reported above. Thus the temperature is suitable every night for sporulation provided that the RH is above 90 per cent. This level of RH is generally present in farmers' fields during the rainy season at night in all cases excepting non-rainy days when RH becomes the major factor limiting sporulation.

These results also show that a high RH is not required for the entire process of sporulation as has been reported in some other downy mildews (Michelmore, 1981). Internal development, the first of two developmental phases, involves the initiation and development of the sporangiophore primordia in the sub-stomatal cavities and is probably little affected by the external environment. Although the effect of high temperature (>20°C) on the internal development is not known, this initial phase was completed at 20°C and 70 per cent humidity in this study. However, the second phase, the development of sporulation (external development) did not occur under these conditions. The absence of sporulation can solely be attributed to low RH because the temperature was congenial for sporulation.

These findings have significance in screening for resistance to downy mildew in the field. During the non-rainy days in most rainy seasons at the ICRISAT Center, a high RH is built-up by the perfo-spray irrigation system (Williams *et al.*, 1981) which would provide favourable conditions for sporangial production. In an atypical season, however, such as in 1979 when a continuous wind blew for almost the entire season, the irrigation water evaporated within 2–3 h after spraying and very little sporulation occurred. Under these conditions, the internal development of sporulation during the night would probably remain unaffected, and perfo-spray irrigation carried out at midnight would be helpful in creating a high RH for the production of sporangia, for the reliable screening of downy mildew resistance.

The large variations in sporangia size with varying temperature clearly shows that the size of sporangia cannot be used as a criterion to separate pathogen variants as has been done earlier (Shetty and Ahmad, 1981), unless sporangia are produced at an optimum temperature. Further studies are needed to determine whether the size of sporangia is influenced by other factors such as nutrition and the host genotype.

The wide range of temperatures at which this pathogen can sporulate and infect plants (Singh *et al.*, unpublished), the ability of systemically infected plants to supply inoculum until maturity and the availability of juvenile tissue throughout crop growth are the contributing factors for the wide spread occurrence and destructive potential of this disease.

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