DYNAMICS OF SPORANGIAL PRODUCTION OF *SCLEROSPORA GRAMINICOLA* (SACC.) SCHRÖET IN RELATION TO TEMPERATURE AND HUMIDITY

BY
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CERTIFICATE

Mr. Prahalad Puranik has satisfactorily prosecuted the course of research and that the thesis entitled "**Dynamics of Sporangial Production of *Sclerospora graminicola* (Sacc.) Schröet in relation to temperature and humidity**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

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CERTIFICATE

This is to certify that the thesis entitled "**Dynamics of sporangial production of *Sclerospora graminicola* (Sacc.) Schröet in relation to temperature and humidity**" submitted in partial fulfillment of the requirements for the degree of "**Master of Science in Agriculture**" of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr. Prahalad Puranik**, under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Date: 27th August, 1999 **(PRAHALAD PURANIK)**

DECLARATION

I, **Prahalad Puranik** hereby declare that the thesis entitled "**Dynamics of sporangial production of *Sclerospora graminicola* (Sacc.) Schröet in relation to temperature and humidity**" submitted to **ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY** for the degree of "**Master of Science in Agriculture**" is a bonafide record of work done by me during the period of research at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India. This thesis has not formed in whole or in part, the basis for the award of any degree or diploma.

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ABSTRACT

Downy mildew caused by *Sclerospora graminicola* is a devastating disease of pearl millet n India. Some aspects of epidemiology of the disease are not clearly understood. Studies on the ffect of temperature and relative humidity on the sporulation process of *S. graminicola* was studied vith special reference to the production of sporangiophores and sporangia.

Sporangiophore and sporangial production increased with increase in temperature from 10 o 22°C and at 25 and 30°C their production was less. No sporangiophore and sporangia was roduced at 35°C. The number of sporangiophores and sporangia also increased with increase in ncubation time up to 8 h, but decreased at 24 h incubation period. An increase in length of porangiophore was observed with increase in temperature from 10 to 30°C but the sporangiophore iameter was not affected by temperature.

Sporangiophores did not emerge from stomata at or below 95% RH. At 96 and 97% RH, elayed (6-8 h) production of sporangiophores was observed. Only at 98 and 100% RH, production f sporangiophores began after 1.5 h of incubation. Sporangial production started after 2.5 h of ıcubation at 98 and 100% RH. Delayed (≥ 8 h) sporangial production was observed at 97% RH. porangiophore and sporangial size were not affected by RH.

Sporangiophores were viable for at least 30 minutes at RH ≥ 95%. The sporangiophores

collapsed and lost the ability to produce the sporangia when RH fell to 90% or below even for 10 minutes. The sporangiophores were very sensitive to RH and were unable to survive during the day after their production at night. This suggested that sporangia could be produced only on freshly emerged sporangiophores.

INTRODUCTION

CHAPTER I

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the important food grain crops of the world. It has been cultivated as a cereal or forage crop mostly in Africa and also in India for at least 3000 years. In India it covers an area of 10 million ha with a production of 7.91 million tonnes in 1991-92 (Directorate of Economics and Statistics, 1998). In Andhra Pradesh, it is grown in the districts of Srikakulum, Vizianagaram, Vishakapatnam, Ranga Reddy, Mahaboobnagar, Nalgonda, Prakasam, Chittor and Ananthpur, as a rainfed crop in kharif and as irrigated crop in summer. The crop is grown on the poorest soils and under harsh climatic conditions where no other crop can grow. Therefore, it is popularly called the food of the poorest of the poor. Although the crop is quite hardy it still suffers from various biotic stresses. One of the major biotic factors reducing yield is the downy mildew disease, caused by *Sclerospora graminicola*. The disease is of great economic importance in India and downy mildew epidemics caused substantial yield losses in F1 hybrids during 1970-76 (Safeeulla, 1977), 1983, 1984, 1987 and 1988 (Singh et al., 1993).

The disease was first reported in India (Butler, 1907). It has also been reported from more than 20 countries (Safeeulla, 1976). It is a major limiting factor for full exploitation of high yielding potential of hybrids in India. Grain yield losses of 10-60% have been reported in various countries in Asia and Africa (Nene and Singh, 1976).

The primary inoculum of the pathogen is seed or soil borne oospore, but the secondary

spread is by airborne sporangia. Asexual propagules are ephemeral and fragile. Thus production, liberation, deposition and germination are greatly influenced by environmental factors as are penetration, infection and disease development. Effects of environmental factors on asexual sporulation of *Sclerospora graminicola* was studied by Singh et al. (1987).

Epidemiological studies indicate that dew formation is the most important factor that govern the disease development. In addition, disease incidence positively correlates with relative humidity and maximum temperature (Shetty, 1987).

The difficulty of controlling humidity restricted the study of effect of humidity on pathogenic processes on living plants. Butler et al. (1995) constructed a set of controlled humidity chambers for single plant.

The role of sporangia in the epidemiology of pearl millet downy mildew was clearly established by Singh and Williams in 1980. But the role of sporangiophore in sporulation and effect of humidity on that was to be clearly established. Hence the present study was undertaken with the following objectives:

- Effect of temperature and relative humidity (RH) regimes on sporangiophore production.
- Longevity of sporangiophore productivity with reference to changes in RH and time interval.
- Effect of RH regimes on asexual sporulation process i.e., the time taken for differentiation, Sporangial initiation and quantum of sporangial production under constant and varying humidity regimes.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The downy mildew constitute a wide spread and highly destructive group of pathogens. They received their name because of the profuse white "down" produced on infected plant parts. The "down" growth consists of the sporangiophores or conidiophores and sporangia or conidia produced during the process of asexual reproduction (Williams, 1984). The downy mildew pathogens belonging to the family Peronosporaceae, are obligate parasites, although a few of them have been grown in tissue culture (Bhat et al., 1980).

Among the diseases affecting pearl millet (*Pennisetum glaucum* (L.) R. Br.), downy mildew caused by the fungus *Sclerospora graminicola* (Sacc.) Schröet, is the most destructive one and prevalent in the traditional pearl millet growing areas of Africa and the Indian Sub-continent. This is evident from several epidemics on hybrids in India (Dave, 1987; Rai, 1992). The pathogen and disease have been reported from Asia, Africa and Europe, but apparently not from South America or Australia (Safeeulla, 1976). *Sclerospora graminicola* infects several grasses including pearl millet, Italian millet (*Setaria italica* L.) and sorghum (*Sorghum bicolor* (L.) Moench). In the tropics the pathogen causes serious losses to pearl millet, which causes the 'green ear' symptoms (Shaw, 1975). The pathogen has been found on maize in Israel and USA (Shurtleff, 1980).

Sclerospora graminicola (Sacc.) Schröet, was originally named as *Protomyces graminicola* by Saccardo in 1876 who reported it on *Setaria verticillata*. Subsequently Schroeter in 1879

renamed it as *S. graminicola* in his work on the genus *Sclerospora* in Germany (Ullstrup, 1973). In 1884, Farlow described *S. graminicola* on *Setaria viridis* (L.) Beauv. In India, the earliest investigations on pearl millet downy mildew were carried out by Butler (1907) and Kulkarni (1913). The disease was not considered serious since damage was severe only where pearl millet was grown in low-lying, poorly-drained areas (Butler, 1918). Mitter and Tandon (1930) reported the seriousness of the disease in low-lying areas in the vicinity of Allahabad in North India. In India, the pathogen is present in all the states where pearl millet is cultivated. However, the disease has not been reported on pearl millet in the American countries (Singh et al., 1993).

Although pearl millet downy mildew has been recognized as an important cereal pathogen since the early part of this century, it received relatively little research attention until the early 1960s. But the true magnitude of the losses has now been fully appreciated. In East China, Porter (1926) estimated a 6% yield loss, while Mitter and Tandon (1930) reported a 45% yield loss due to *S. graminicola* in Allahabad, India. Mathur and Dalela (1971) revealed disease incidence ranging from 0-27% in a survey in Rajasthan, India during 1962 and 1964. With the release of high yielding hybrids, the disease occurred in epidemic proportions and devastated the hybrid crop, which was cultivated on more than one million hectares (Safeeulla, 1977). According to reports from Africa, there was 60% loss in Mozambique (DeCarvalho, 1949), 10% in Nigeria (King and Webster, 1970) and 0-50% in other western African countries (Saccas, 1954; Selvaraj, 1979; CILSS 1986; CILSS 1987).

Although comprehensive data for grain yield loss in pearl millet due to this pathogen is not available (Singh et al., 1993), the actual yield reducing potential of the disease has been fully

recognized. This was clearly demonstrated in HB3, a popular hybrid in India in the early seventies when pearl millet grain production was reduced from 8.2 million t in 1970-71 to 5.3 million t in 1971-72 by an epidemic of the disease (AICMIP, 1972). Subsequently grain yield losses continued to occur quite frequently due to downy mildew epidemics in India (Singh et al., 1987). The yield losses can be directly proportional to the disease severity (Williams and Singh, 1981). Downy mildew is considered a major biotic yield reducing factor in pearl millet not only in India, but also in many countries in Africa (Singh, 1995).

Following infection, *S. graminicola* readily induces systemic symptoms after colonizing the meristematic segments of young seedling. Local lesions are rarely produced (Kenneth, 1981). Systemic symptoms generally appear at any stage of development from the young seedling (usually from second seedling leaf) up to flowering and once these appear, all the subsequent leaves and panicles also develop symptoms, except in case of recovery resistance where plants outgrow the disease (Singh and King, 1988). In the first leaf to develop systemic symptoms, a general mild yellowing is observed, usually confined to the basal portion of the lamina. All subsequent leaves are fully diseased. In some instances the leaves may not show symptoms but the inflorescence may be totally or partially diseased. The area of leaves with mild yellowing support massive asexual sporulation from the abaxial surfaces, producing a thick white "down". In highly susceptible varieties, under ideal conditions for spore production, a mere sparse asexual sporulation can occur on the adaxial leaf surfaces. In pearl millet, the pathogen infection generally results in the production of grossly malformed inflorescence with various degrees of transformation of inflorescence tissue into leafy structures (Williams 1984).

Singh and Gopinath (1985) observed that highest incidence of downy mildew occurred on pearl millet plants in Growth stage I (i.e., ≤ 5mm long) and the incidence declined progressively when seedlings were inoculated after Growth stage III (>10 mm long, first leaf still folded). However, susceptibility was noticed up to seven leaf stage (Safeeulla, 1976). According to Subramanya et al. (1981), *S. graminicola* is apparently incapable of systemically infecting plants after it had reached a certain age. The main shoot was susceptible to downy mildew up to 26 days from emergence. However, the susceptibility of pearl millet crop throughout its growth phase is due to continued emergence of tillers.

Sclerospora graminicola produces both asexual (sporangia, zoospores) and sexual spores (oospores). Sporangia are hyaline, thin-walled, ellipsoid, and papillate, measuring 15 to 22 x 12 to 21 µm. Oospores are thick-walled resting spores produced in the infected leaves. A mature oospore is brownish yellow and spherical, and measures 32 µm (22 to 35 µm) in diameter (Singh et al., 1993; Singh, 1995).

Sporangia germinate indirectly by producing zoospores. The number of zoospores per sporangia may vary from 1 to 12 (Ramakrishnan, 1963; Shetty, 1987). Zoospores emerge through a pore produced by the opening of the operculum in the apical region of the sporangium. Zoospores swim for 30-60 minutes (min), encyst, and then germinate by forming a germtube. Sometimes zoospores may germinate within the sporangium, in which case, the germ tube grown through the apical pore giving the appearance of direct germination of sporangium (Shaw, 1981). However, direct germination, without the formation of zoospores also occurs (Maunch-Mani et al. 1989; Singh, 1995).

Effect of environmental factors on sporulation

The asexual spores are of great importance for the local spread of the downy mildew within and among crops in a season. Thus knowledge of the asexual reproductive process and factors affecting asexual spores will contribute to the understanding of the epidemiology of these diseases.

Sporangia of *S. graminicola* serve as the primary source of inoculum that spreads downy mildew from infector rows to test rows in disease screening trials. Disease pressure in screening trials is often variable and the availability of inoculum may determine the extent to which disease spread occurs. Sporangia are produced on the leaf surface and the extent of production is moderated to a large extent by environment. An understanding of the influence of weather factors on sporulation is necessary to better appreciate the inoculum pressure under which screening is carried out.

Environment can affect the development and spread of plant disease in different ways. It can affect the perpetuation or overwintering of the pathogen, the build up of both primary and secondary inoculum, the dissemination of inoculum, germination, and host penetration. The environment can also affect growth and development of the host prior to being infected in such a way as to affect its susceptibility. Finally, it can influence the actual development of disease after the plant has become infected (Chalam, 1996).

Sporulation in downy mildew occurs only under moist dark conditions following a period of light. Under continuous light or darkness and high humidity, there was little or no sporulation

(Weston, 1923, 1924; Yarwood, 1937). The members of the genus *Sclerospora* have a special position in that their spores are produced in a very few hours in the middle of the night and discharged immediately afterwards. After dawn, only scanty remnants of the previous night's crop are usually evident as the sporangiophores are killed by drying and disintegrate rapidly (Weston, 1923; Jones, 1971; Dogma, 1975; Payak, 1975).

According to Weston (1924), asexual sporulation in *S. graminicola* on the surface of plant was nocturnal and occurred in the presence of dew. He observed that if dew formed 02.00 h, mature sporangiophores and sporangia were present by 02.00 h, peak sporulation occurred at 03.00 h, and sporangial production continued until dew disappeared. For nearly thirty years, nocturnal sporulation in different species of *Sclerospora* was taken as an established fact.

Miyake (1911) induced sporulation by incubating infected leaves in moist chambers. Subsequently, Thirumalachar and Narasimhan (1952) repeated the experiment with *Sclerospora dichanthicola* Thirum and Naras. Thirumalachar et al. (1953) obtained the sporangial stage of *Sclerospora macrospora* (Sacc.). Thirum, Shaw, and Naras on infected leaf bits of ragi at any required time and concluded that there was no periodicity in its production.

Safeeulla and Thirumalachar (1955) observed no sporulation of *S. sorghi* on dry or submerged leaf areas, and sporulation on areas covered with heavy dew, under lab conditions. Dew chambers have been used for inducing sporulation in *S. sacchari* (Bonde and Melching, 1979) and *S. sorghi* (Schemitt and Freytag, 1974).

Safeeulla & Thirumalachar (1956), determined that three factors were required for sporangial production on pearl millet: i) a period of 15-20 h since the previous event of sporangial production, ii) the presence of moisture on the surface of leaves; and iii) a temperature of about 25°C. At 15°C and below, sporangial formation was sparse and delayed. Melhus et al. (1927) and Suryanarayna (1965) considered temperatures as low as 10°C favourable for sporulation. According to Suryanarayana (1965) sporulation did not occur at temperatures greater than 28°C. Under favourable conditions, *S. graminicola* produces 35 x 10^3 sporangia per square centimeter (cm^{-2}) leaf area of infected pearl millet plants per sporangial crop, and as many as 11 crops on successive nights are formed (Safeeulla, 1976). Sporangia are discharged forcibly up to 2.5 mm (Melhus et al., 1927) and contact with liquid water is required for sporangial germination. Each sporangium produces 3-8 zoospores (Suryanarayana, 1965).

Thakur and Kanwar (1977) have reported that downy mildew phase of the disease initiated from sporangial infection was maximum in young seedling. Further they have also reported that sporangia play a significant role in secondary spread of downy mildew. Their study showed that only secondary sporangial infection caused 13.2% plant infection. Wind direction had an effect on the extent of secondary spread.

Singh and Williams in 1980 reported that the sporangia play a major role in the epidemiology of pearl millet downy mildew. They reported that sporangia remained infective for up to 340 m downwind source during the rainy season and 80 m during dry period from a sporangial source.

Thakur (1986) through detailed studies on various aspects of sporangial infection observed that inoculation of sporangia produced downy mildew seedlings within six days under high humidity (≥ 90%) and at a temperature range of 18-20°C.

Hirata and Takenouti (1932) reported sporangial germination through the release of zoospores when placed in water at 12.5 to 29°C. Germination was not affected by light. According to Nene and Singh (1976), the pathogen from Italian millet had minimum, optimum and maximum sporangial germination at temperatures of 5-7, 18 and 30-33°C, respectively. The optimum temperature range for germination of the pathogen was 14-18°C (Melhus et al., 1927). In contrast, the sporangia of *S. graminicola* from pearl millet germinated at 18-29°C, the optimum being 24-25°C (Safeeulla et al., 1963). Zoospores germinate by producing germ tubes. Zoospores of the pathogen from pearl millet readily germinate and swim at 16-22°C (Suryanarayana, 1965). These germ tubes exhibit a chemotactic response by growing towards pearl millet roots (Safeeulla, 1976).

Dew formation was the most probable variable that could be useful in predicting the downy mildew incidence of pearl millet. In addition, relative humidity and temperature were also found to be positively correlated with disease incidence (Muthusamy, 1979). Distribution of rainfall was important in the expression of downy mildew (Nene and Singh, 1976), rather than total rainfall (Suryanaryana, 1965).

Effect of different environmental factors such as temperature, light, relative humidity, and dew formation on germination of sporangia/conidia, and infectivity of different downy mildew pathogens were studied in several other crops such as sorghum (Safeeulla et al., 1974; Schmitt and

Freytag, 1974; Schuh et al., 1987), Maize (Exconde et al., 1967; Change & Wu, 1969; Bonde and Melching, 1979; Bustaman and Kamiga Fukuro, 1981; Bonde et al., 1992), Chinese cabbage (Shao et al., 1999), Cucurbits (Tsai and Hsu, 1989), alfalfa (Patel, 1926; Melhus and Patel, 1929; Fried and Stuteville, 1977), Soyabean (Kirik et al., 1985), Peas (Singh et al., 1988) and onion (Hilderbrand and Sutton, 1984a,b).

Weston (1923) noticed whip-like elongations of conidiophores in place of conidia in *Sclerospora philippinensis* Weston due to the effect of excess moisture. Under excessive moisture conditions, elongation and proliferation of sporangiophores of *S. graminicola* were observed by Safeeulla and Thirumalachar (1956). They also studied the effect of temperature on the asexual phase. Their observations indicated that at 15°C and below, the sporangial formation was delayed considerably extending up to 24 to 36 h. The sporangial formation was delayed considerably extending up to 24 to 36 h. The sporangiophores formed at 10°C were stout and dwarf with complete suppression of branches. There were only 1-3 sporangia borne directly on the main axis with well developed sterigmata. At 15°C, the branches were shortened, and all the sporangia appeared to be clustered at the top.

Williams (1984) reported that the process of asexual reproduction of downy mildews is dependent on temperature, relative humidity and the supply of photosynthate in the infected host organ. The need for a good supply of photosynthate, indicated by the requirement for exposure of diseased plant to several hours of sunshine between successive crops of asexual spores, together with the requirements for relatively low temperatures and high relative humidities, results in natural sporulation generally occurring in the early hours of the morning (02.00-04.00 h).

S. graminicola can be induced to sporulate during the day time provided the incubation period (7-8 h) is initiated at the appropriate time with leaves that have received at least 3-4 exposure to daylight after production of the previous crop of asexual spores (Schmitt and Freytag, 1974; Dange and Williams, 1980; Williams et al., 1981).

The temperature ranges over which sporulation occurs will probably vary somewhat with species and location, and will probably reflect the nocturnal temperatures during the early part of the cropping season where the disease occurs. Most of the downy mildews infecting graminaceous hosts appear to sporulate well in the 20-24°C range (Williams, 1984).

The sporangiophores emerge through the stomata within 2-4 h after the initiation of incubation. The most critical period for high humidity is probably from the time of the emergence of sporangiophores from the stomata to the time of spore maturation. Experiments with pearl millet downy mildew at ICRISAT, Hyderabad indicates the occurrence of profuse nocturnal sporulation following a 30-mm mist-irrigation shortly after sunset (Williams and Singh, 1981), which leads to dew formation as the temperature falls during the night. Excessive free moisture is detrimental to the sporulation process, and sporulation is considerably reduced or precluded if heavy rains occur following the period when the sporangiophores emerge from the stomata (Williams, 1984).

The role of sporangia of pearl millet downy mildew has been controversial for many years (Safeeulla, 1975; Nene and Singh, 1976). Kenneth (1966) reported no evidence of secondary spread in Israel, and Safeeulla (1976) reported that asexual spores have little role in disease spread in

Mysore (Southern India).Girard (1975) in Senegal, however, obtained evidence that sporangial inoculum does play a role in secondary infection. Singh and Williams (1980) showed that sporangia produced on infected plants play a major role in the secondary spread of the disease. The asexual phase of the pathogen, leading to the production of sporangia is very efficient in that the sporangial cycle repeats every 24 h under favourable conditions, the number of sporangia produced per unit infected surface area is large, spore dispersal and dissemination is rapid, and spores can travel up to 340 m downwind during the rainy season with the disease spreading up to 80 m from the source of inoculum.

Frederiksen and Rosenow (1967) reported that the conidia of *S. sorghi* are air-borne and can be trapped several feet away from diseased plants. Under ideal conditions these spores were carried several hundred meters away from the site of production. Conidia were liberated on clear nights around midnight and remained abundant in the air at 01.00-03.00 h and none was found in the air after 06.00 h.

The effects of temperature, relative humidity and light on sporangial production of *Sclerospora graminicola* was studied by Singh et al. (1987). They reported that though the process of sporulation was completed in about 6 h, high RH (95-100%) was essential only during the last 3 h. Maximum sporulation occurred when infected leaves were incubated for 6-12 h at 30°C and ambient humidity before exposure to high RH for sporulation. Sporulation occurred at 10-30°C with an optimum at 20°C. The size of sporangia and sporangiophores were affected by temperature and the largest were produced at 25 and 15°C respectively.

Singh and Gopinath (1990) reported that the germination of sporangia occurred at 10-45°C. There was a gradual increase in germination with the increase in incubation time from 30 min to 2 h at temperatures from 10-35°C. Germination was most rapid at 40 and 45°C, but total germination at these temperatures did not increase more than 60% even after 3 h. Optimum temperature for germination was 29.6°C and little germination occurred after 2 h 40 min. Germ tubes grew at 15-35°C and temperature has no influence on the germ tube length.

Singh and Gopinath (1990), further reported that the infectivity of sporangia (zoospores) as judged by systemic infection of young seedlings was greatly influenced by preinoculation temperature of the sporangial suspension and post inoculation temperature. Sporangia stored at 10°C and 15°C for up to 12 h prior to inoculation produced >70% downy mildew when used as inoculum. Sporangial infectivity generally declined more rapidly at the higher incubation temperatures, with no infectivity after 2 h incubation at the two highest temperatures, 40 and 45°C. Inoculated seedlings incubated at 30°C developed downy mildew symptoms as early as 3 days after inoculation. At 4 to 5 days after inoculation, seedling at 20, 25 and 35°C also developed downy mildew symptoms. Seedling at 15°C remained symptomless for 8 days, but when they were moved at 20°C, >75% downy mildew develops within five days. Downy mildew did not develop at 40 and 45°C up to 13 days after inoculation.

Ramesh and Safeeulla (1982) reported that the sporangia can germinate in the soil and liberate zoospores. The zoospores can move against gravity and remain viable and infective for 5 h in the soil. Survival of zoospores in the soil indicated that, they may serve as a potential secondary source of inoculum through soil under field conditions.

Humidity is a key weather variable which strongly influence the majority of foliar pathogen. Except powdery mildew, spores of many fungal pathogens require liquid water to germinate, although a number of species have been reported to germinate when the air is not saturated (Yarwood, 1978). Clayton (1942) was able to distinguish pathogens *in vitro* which germinated on a dry surface at relative humidities of 99% and above (e.g., *Venturia inequalis*) from those which required liquid water (e.g., *Sclerotinia fructicola*).

Harrison (1992) stated that humidity within the leaf boundary layer is usually different from the surrounding air and is dependent on wind speed. Accurate control of humidity is difficult, especially in situation where plants are transpiring. Saturated salt solutions provide a convenient method to control relative humidity *in vitro*, but when living plant material is introduced into such a system the equilibrium humidity changes.

Harrison & Lowe (1989) described systems to control RH for detached leaves and whole plants using water baths to control the dew point and chamber temperatures. In their plant chamber, they were able to obtain RH within ±2% of the desired value in studies on sporulation of *Phytophthora infestans*.

Because of the difficulty in controlling humidity.relatively few studies on the effect of humidity on pathogen processes on living plants have been reported. Most reports describe the effects of different levels of constant humidity on processes such as sporulation and infection.In nature, diurnal changes in humidity can be large and little is known of the response of pathogens to

a dynamic situation.

Butler et al. (1995) constructed a set of controlled humidity chambers for single plant. They reported that accurate control of RH between 40 and 99% was achieved by mixing dry air with a saturated air stream using solenoid valves controlled by a programmable data logger. The system responded to a step change of RH in about 6-10 min. and diurnal changes of RH were reproduced.similar to those observed in nature. The stability of control made it possible to differentiate between the requirement of liquid water and very high RH (98%) for conidial germination and subsequent infection of groundnut by *Phaeoisariopsis personata* causing late leaf spot disease. Controlled environment studies of conidial production by *Phaeosariopsis personata* on groundnut are described by Butler et al. (1995). Under constant RH, conidia were produced above a threshold(94.5% RH) and there was a linear increase between 94.5 and 100% RH. Conidial production was less with continuous leaf wetness (resembling heavy dew) than with continuous 98-99% RH, but it was similar with intermittent leaf wetness and intermittent (98-99%) RH (8h at 70% RH each day). With alternate high (≥ 97% RH) and low humidity daily conidial production depended both on the duration of high RH and low RH value.

Viranyi (1973) apparently concluded that the pathogen *Peronospora destructor* may sporulate and infect leaves during single period of high humidity (RH ≥95% for 12-14 h) or during two periods of high humidity on successive nights (RH ≥6 h and ≥ 8 h respectively).

Conidia were produced in continuous light when the photon flux density was 2 $\mu mol\ m^{-2}\ g^{-1}$. but production was completely inhibited with 60 $\mu mol\ m^{-2}\ g^{-1}$. With constant RH.more conidia were

produced with a 12 h photoperiod than in continuous darkness.With continuous darkness, more conidia were produced during the night (18.00-06.00 h) than during the day, but this biological rhythm was over come with a light-night, dark-day regime. With constant RH of 98-99% there was a linear increase in conidial production with temperature between 10 and 28°C, and virtually no conidia were produced at 33°C. The daily production of conidia increased with time for 2 to 6 days, depending on the treatment.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The studies on the "Dynamics of sporangial production in relation to temperature and humidity in the pearl millet downy mildew" were carried out at International Crops Research Institute for the Semi-Arid Tropics, Asia Center,Andhra Pradesh, India. The materials and methods used in the present investigation are broadly described under the following heads:

1. General
2. Time course study on sporangiophore development and sporangia production.
3. The effect of temperature and relative humidity (RH) regimes on sporangiopore production.
4. Viability of sporangiophores and production of sporangia under intermittent low and high humidity regimes.
5. Effect of RH regimes on the asexual sporulation process.
6. Statistical analysis.

3.1. General

This section includes the materials and method common to all other sections.

3.1.1. Plant material

Downy mildew susceptible genotype 7042S plants was used in this study. 7042S is a selection of IP 2696 from the Republic of Chad, which normally develops >80% downy mildew incidence under moderate disease pressure (Singh and Gopinath, 1990).

3.1.2. Production of systemically infected plants

Seeds of 7042S were planted in thirty pots (20 seeds in each pot) on every alternate day in the greenhouse. Plastic pots (sizes: 10 cm & 17.8 cm) were filled with a mixture of Alfisol and Sand (2:1, v/v). At the time of sowing few diammonium phosphate granules were added to the soil for healthy growth of plants. Seedlings at two-leaf stage were inoculated with sporangial inoculum and immediately after the expression of first symptoms (chlorosis) and before the occurrence of sporulation, seedlings were shifted to an environmental chamber maintained at low (60%) RH.

3.1.3. Preparation of inoculum

Sporangial inoculum was obtained from systemically infected leaves (Plate 1) of 7042S inoculated with an ICRISAT isolate of the pathogen. Leaves were thoroughly washed with moist cotton to remove old downy growth of the pathogen and were kept in humidity chambers made by lining both the lids of plastic trays with moist filter paper.The humidity chambers were incubated in dark at 20°C for 6 h for sporulation. The incubator was programmed in such a manner, that after this incubation period the temperature automatically drops to 0°C to prevent sporangial

Plate 1. Systemic infection of downy mildew on pearl millet leaf

germination. Sporangia were collected by washing the leaves with cold (5-6°C) distilled water. The concentration of sporangia was estimated using a Haemocytometer and adjusted to a desired concentration.

3.1.4. Inoculation

Seedlings at two-leaf stage were shifted from greenhouse to downy mildew inoculation chamber where temperature was maintained at 20°C and relative humidity of >95%. Freshly prepared inoculum (as described above) was delivered to seedlings by a mass inoculation technique using a sprayer (Singh, 1993). After inoculation, the pots were covered with plastic covers. After 24 h the pots were shifted back to greenhouse.

3.1.5. Maintenance of systemically infected plants

Immediately after the expression of first symptoms (chlorosis), 4-5 well established infected plants were selected. Before the occurrence of sporulation, the plants were shifted to a plant growth cabinet (E15 Conviron,Winnipeg, Canada) (Plate 2). The cabinet can be programmed to desired temperature, humidity and lighting. The light intensity can also be maintained by adjusting the height of the bulbs. The cabinet was programmed to a temperature of 25°C and humidity to 60%. The lights (450 $\mu molm^{-2} s^{-1}$) were turned off from 09.00 to 21.00 h and turned on from 21.00 to 09.00 h. Almost all the experiments were given the same preincubation treatment expect for few initial experiments where lights were off from 18.00 to 06.00 h and vice versa.

Plate 2. Plant growth cabinet (E.C. Conviron, Winnipeg, Canada).

3.1.6. Microscopes

Olympus CHB-2 light microscope was used to count the number of sporangia. Olympus Vanox Fluorescence microscope and Olympus AH 70 Fluorescence microscope were used to study the sporangiophore development and sporangia production, to count the number of stomata, sporangiophore initials, sporangiophores and the dimensions of sporangiophores and sporangia.

3.1.7. Glassware

"Borosil", "Corning" & "Pyrex" brand glassware were used after cleaning and sterilization.

3.1.8. Stains

Fungiflour (solution A, Polysciences Inc. Warmington, PA) was the only stain used for all the experiments. It is a fluorescent stain for observing fungal structures on leaf samples under fluorescence microscope.

3.1.9. Sampling

All leaf samples were collected from the same leaf at regular intervals as per requirements of the experiment. Pieces of leaf lamina (2-5 cm) were sampled from the 3rd or 4th youngest leaf of the plant using forceps and scissors. Leaf tip (2-3 cm) and the midribs were discarded while

collecting the samples. Utmost care was taken in collecting the samples so as not to disturb the sporulating structures elsewhere in the plant.

3.1.10. Methodology of observation

Many samples were collected at each sampling time and observation of samples was a time consuming process.Hence the samples were stored in a refrigerator immediately after collection to restrict further fungal growth as the fungus fails to sporulate at $\leq 5^{\circ}C$ (Chalam, 1996). Later, each sample was taken out and cut into 3-4 mm pieces. Each piece was placed on a slide flooded with Fungifluor, mounted on the slide with the help of stain and topped with a coverslip. Excess stains was blotted out and wiped from the slide using an absorbent paper. Fluorescence microscope was used to count the number of stomata containing sporangiophore initials, number of sporangiophores, their dimensions, and asexual sporulation process at 80x and 160x magnifications.

Light microscope was used to count the sporangia number. Leaf sample was washed thoroughly in known quantity of distilled water with the help of a brush. After washing, the quantity of water was measured again. A drop of spore suspension was mounted on a Haemocytometer and five observations of sporangia count was taken in 5 different fields. The average of sporangia number in five fields times the quantum of spore suspension after washing multiplied by 10^4 becomes the number of sporangia present in the particular leaf sample.

3.2. Time course study on sporangiophore development and sporangia

production

The production of sporangiophores and sporangia is a continuous process. Not all the sporangiophore primordia give rise to fully developed sporangiophores at a single point of time.

Six experiments were conducted to study the time required for development of sporangiophores and sporangia from the stomata. The infected plants were given preincubation conditions of 60% RH, 25°C and 18.00 to 06.00 h light in growth cabinet (E15) to prevent sporulation.

Three infected but nonsporulating leaves from three different plants were collected and their old downy growth (if any), was removed. Three leaf segments (one from each of the three leaves) were kept on moist blotting paper in an uncovered petriplate (Singh et al. 1987). They were incubated at 100% RH, 20°C for 6 h and observations were taken at every 15 min of incubation. At each sampling five microscopic fields were observed. Observations were taken at 160x magnification except for dimensions of sporangiophores and sporangia (observed at 80x magnification). Sporangia were counted with the help of Haemocytometer.

3.3. Effect of temperature and relative humidity (RH) regimes on sporangiophore production

To examine the effect of temperature on sporangiophore production, dew cambers (Butler et al. 1994) were used (Plate 3). Each dew chamber consisted of an outer cabinet and inner chamber

(containing the plants) with heated water bath. Substantial leaf wetness, resembling heavy dew, was achieved by maintaining the outer cabinet at about 10°C less than the inner chamber.High RH (98-99%) with little or no leaf wetness was achieved by maintaining the outer cabinet at 1-2°C less than the inner chamber. The humidity in the inner chamber was measured with an aspirated wet and dry bulb psychrometer, with thermistor sensors calibrated individually to within ±0.05°C. The temperature of the inner chambers were controlled by means of a data logger (CR 10,Campbell. Logan, UT, USA).

Eight dew chambers were used for the experiment. They were set at eight different temperatures (10°, 14°, 18°, 20°, 22°, 25°, 30° and 35°C). Three infected plants of the same age (25 days) and size grown in E15 (growth chambers) were shifted into each dew chamber. Each plant was considered as a replicate. Pieces of the samples for examination were taken at every half an hour interval from second or third leaf excluding top or basal portion of the leaf. The leaf samples were examined directly under the microscope using Fungiflour stain. The number of stomata containing sporangiophore initials and the number and size of sporangiophore in relation to time at different temperatures were recorded. A total of eight experiments with three replications each were conducted.

To study the effect of humidity on sporangiophore production,single plant chambers made of glass jars (Butler et al. 1995) were used (Plate 4). These chambers can be controlled individually to cater to experiments with six treatments and they can be programmed to give diurnal changes in humidity. The chambers are housed in a basement room (below ground level) where the diurnal temperature changes by about 0.7K between day and night. Each chamber was supplied with moist

Plate 3. An internal view of dew chamber containing downy mildew infected pearl millet plants.

and dry air,mixed in an appropriate ratio to achieve the desired humidity. Relative humidity in the plant chambers was calculated from the chamber temperature and the absolute humidity of air leaving the chamber.

The chamber temperature was measured using five copper/constant theremocouples in series and air humidity was measured either with a condensation dew-point meter (General Eastern; Wooburn, MA, USA) or with a thin-film capacitative Hunicap sensor (Vaisala Sensor Systems, Helsinki, Finland).

At the time of experiment, five single plant chambers were set to five humidity levels (95%, 96%, 97%,98% and 100%). Uniformly infected plants of 25 days old and of same size were shifted from E15 to single plant chamber room. Each plant was placed in one chamber and at every half an hour of incubation time the glass chambers were lifted and samples were taken out. The samples were mounted on a slide with a drop of stain and observed under fluorescence microscope. A total of fifteen experiments were done at different lengths of incubation time (6, 8 and 24 h) to study the effect of humidity and time interaction on sporangiophore growth.

3.4. Viability of sporangiophores and production of sporangia under intermittent low and high humidity regimes.

Sporangiophores are normally produced within 1.5 h after incubation at 98-100% RH and sporangia are subsequently produced after 1 h. Experiments were conducted to determine the

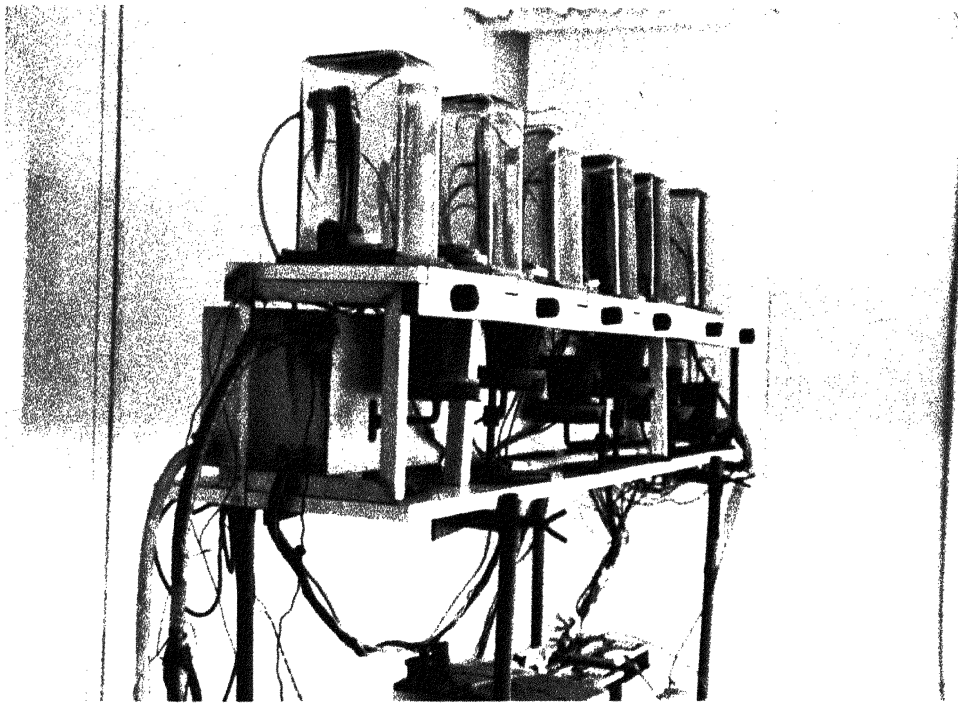

Plate 4. Set of six single plant chambers each containing a downy mildew infected pearl millet plant.

viability of sporangiophore after exposure to different periods of variable sub-normal RH. Two kinds of experiments were conducted.

In the first type of experiment, the effect of variable number of exposures of different duration (10,30, and 60 min) under a fixed low RH level (60%) was examined. Infected plants were exposed to 100% RH in the dew chambers for 1.5 h to allow the production of sporangiophores, and then these sporangiophores were subjected to the low RH level for 1 h duration. This was followed by exposure again to 100% RH for 1.5 h to allow production of new sporangiophores. Such cycle of exposure to low humidity was repeated for 1,2,3, and 4 times. A treatment without any exposure to low RH was also included as control.Two other durations (30 and 10 min) of low RH exposures were also examined in addition to the 1-h low RH exposure following the experimental protocol mentioned above. The low RH levels were maintained in the E 15 environmental chamber with light. Three plants were used per treatment, a single plant being a replicate. The timings followed for the experiment with 1 h low RH exposure is given below:

Dew chamber	Low RH (60%) period (time)	High RH (100%) period (time)	Samplings
1.	-	08.00-09.30	taken
	09.30-10.30	10.30-12.00	taken
	12.00-13.00	13.00-14.30	taken
	14.30-15.30	15.30-17.00	taken
	17.00-18.00	18.00-21.30	taken
2.	-	08.00-09.30	"
	09.30-10.30	10.30-12.00	"
	12.00-13.00	13.00-14.30	"
	14.30-15.30	15.30-21.30	"
3.	-	08.00-09.30	"
	09.30-10.30	10.30-12.00	"
	12.00-13.00	13.00-21.30	"
4.	-	08.00-09.30	"
	09.30-10.30	10.30-21.30	"
5.	-	08.00-21.30	"

In the second type of experiment, the effect of variable number of exposures of different time period (10, 30, and 60 min) under variable low RH level (70, 80,90 and 95%) was examined. Five single plant chambers were used for this study. At the beginning, all the chambers were maintained at 98% RH, and infected plants were incubated in the chambers for 1.5 h to allow the production of sporangiophores.As per the requirement of the treatment, the RH levels in four single plant chambers were lowered to 70, 80, 90, and 95% for 1 h. RH of the fifth chamber was not changed and thus continued at 98% RH. This subjected the sporangiophores to variable RH levels for 1 h. Immediately thereafter, RH levels of all chambers were increased to 98% RH for 1.5 h followed by exposure at the same respective low RH level for 1h. Therefore, only two cycles of

exposure to low RH was used in this experiment. Plants in the fifth chamber served as control. A similar experimental protocol was followed to determine the effect of two other durations (30 and 10 min) of low RH exposures. A single plant was used per chamber. The experiments with 30 and 60 min duration were repeated three times each, whereas the experiment with 10 min duration was repeated four times. Each repetition served as a replicate. The timings followed for the experiment with 1 h low RH exposure is given below:

Dew chamber	Low RH		High RH		
	Set value (%)	period	Set value (%)	period	samplings
1.	-	-	98	08.00-09.30	taken
	70	09.30-10.30	98	10.30-12.00	taken
	70	12.00-13.00	98	13.00-19.00	taken
2.	-	-	98	08.00-09.30	taken
	80	09.30-10.30	98	10.30-12.00	taken
	80	12.00-13.00	98	13-00.19.00	taken
3.	-	-	98	08.00-09.30	taken
	90	09.30-10.30	98	10.30-12.00	taken
	90	12.00-13.00	98	13-00-19.00	taken
4.	-	-	98	08.00-09.30	taken
	95	09.30-10.30	98	10.30-12.00	taken
	95	12.00-13.00	98	13-00-19.00	taken
5.	-	-	98	08.00-19.00	taken
	-	-	98		
	-	-	98		
	-	-	98		

Plants were incubated for 6 h at high RH (100% RH for the first set of experiments and 98% RH for the second set of experiments) after the final exposure to low RH in a given treatment to determine the capacity of sporangiophores to produce sporangia. Leaf samples were collected before exposing the plants to dew chambers (first set of experiments) and single plant chambers (second set of experiments) for the first time before starting the experiment to determine the condition of sporulation apparatus of the fungus in the plant at the time of initiating each low RH period (invariably immediately after exposure to high RH to induce sporangiophore production) to

inspect for the number of sporangiophores and the presence or absence of sporangia, and at 3 and 6 h after the final low RH period the number of sporangiophores and sporangia were counted.

3.5. Effect of RH regimes on the asexual sporulation process.

Uniformly infected plants (25 days old) of same size were brought from E15 and arranged in single plant chambers. During the incubation time at every half an hour interval the glass jar of the chambers were lifted and samples were collected separately in petriplates. They were placed on slides with a drop of stain and observed for sporangial differentiation, initiation and quantum of sporangial production at different humidity conditions.

3.6. Statistical analysis

The data were statistically analyzed by applying square root transformation. The analyses were carried out using sigma stat program. The graphs were prepared using Freelance packaging.

RESULTS

CHAPTER IV

RESULTS

The present study reports the effect of temperature and relative humidity on the process and extent of sporulation of *S. graminicola* on pearl millet.

It is essential to understand the normal development events of sporangiophore and sporangia under ideal conditions before studying environmental effect on the sporulation process. Sporangiophore began to grow within 15 min after exposure to 100% RH and started branching 90 min after incubation (Table 1). Sporangial initials formed 135 min after incubation, and sporangia were fully developed and detachable 165 min after incubation.

Table 1. Developmental stages of sporangiophore and sporangia by *S. graminicola* in relation to time

Incubation time (min)	Observations
0	Sporangiophore initials deeply seated within the stomata. Some fluid was seen on the edges of stomata containing sporangiophore initial which were yet to come to the surface of stomata (Plate 5).
15	5-7 Sporangiophore initials covered each stomata.
30	1-3 Sporangiophores started their aerial growth (Plate 6).
60	Sporangiophores were elongating (Plate 7).
90	Sporangiophores started branching (Plate 8).
105	Sporangiophores were branched. Sporangiophore length was 180-215 µm and diameter 19-26 µm. The length was observed from base to first branching and diameter to its broadest point (50 observations from each sample) (Plate 9).
120	Sterigmata formed on Sporangiophore branches.
135	Sporangiophores with sporangia initials were seen (Plate 10).
150	Sporangia developed but attached to the sporangiophores (Plate 11).
165	Sporulation was 0.96-1.98 x 10^5 spores cm^{-2} leaf area. Sporangia size was 13-20 x 15-22 µm (50 observations from each sample) (Plate 12).
360	2.5-4.26 x 10^5 spores cm^{-2} leaf area.

4.1. Effect of temperature and incubation time

4.1.1. Effect of temperature and incubation time on the number of productive sporangiophores $stomata^{-1}$

The relationship between stomata containing sporangiophore primordia and fully developed sporangiophores present in an infected area helps in better understanding of sporulation aspects of the downy mildew pathogen and in turn epidemiology of the disease.

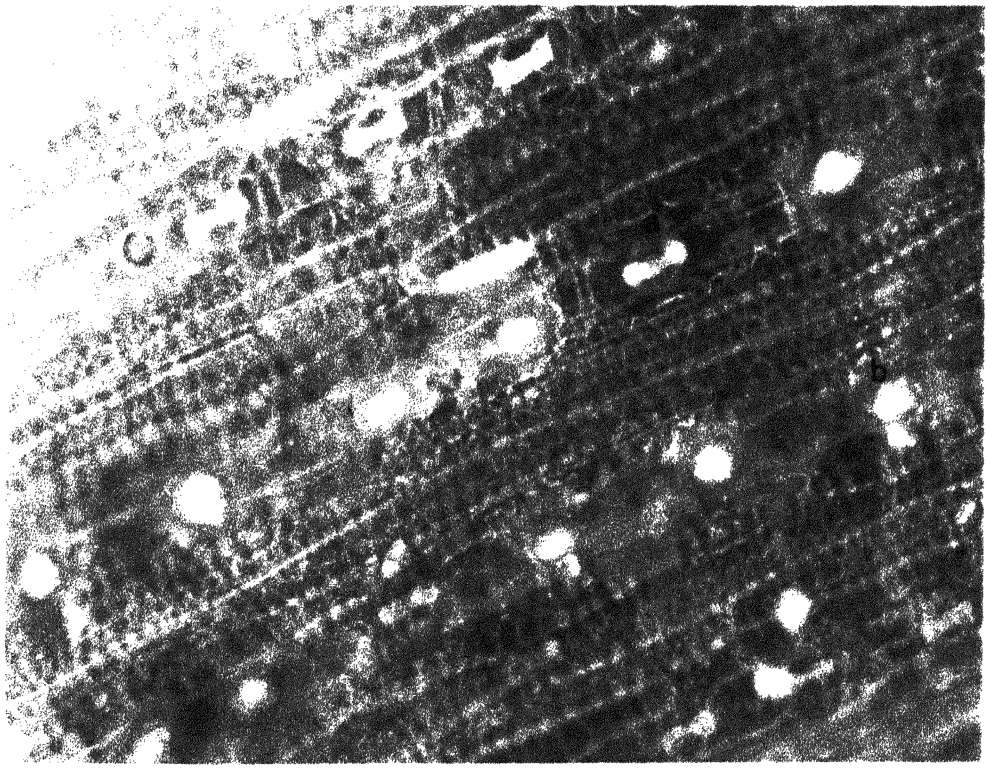

Plate 5. Micrograph of a pearl millet leaf infected with S. graminicola

a. Stomata without sporangiophore primordia.
b. Stomata with 5-7 sporangiophore primordia.

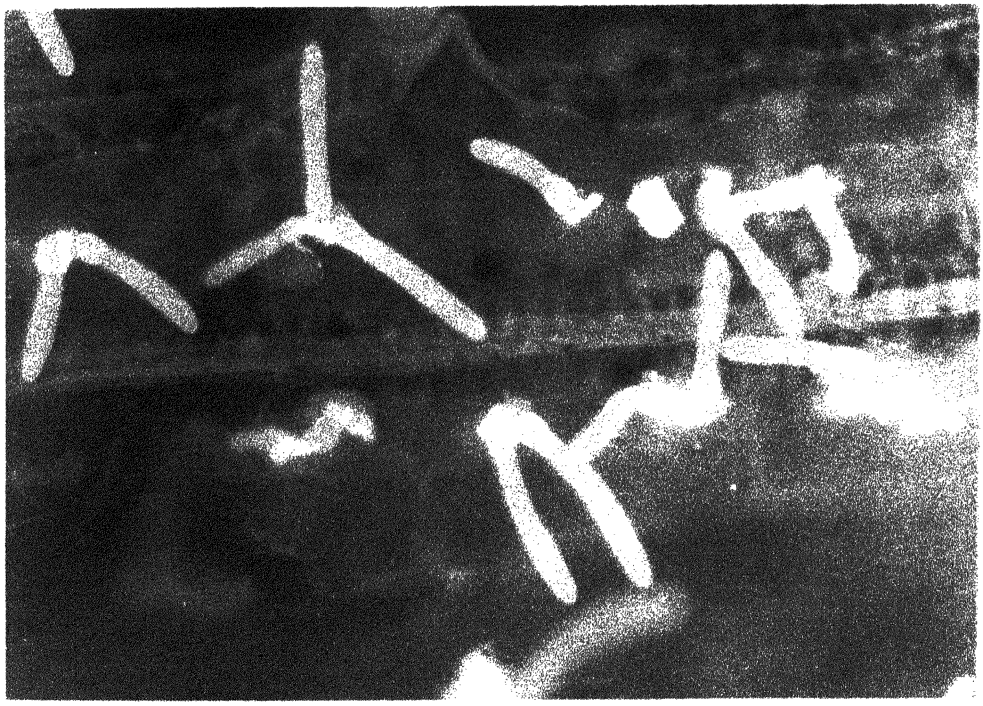

Plate 6. Micrograph of pearl millet leaf sample showing 1-3 sporangiophores from each stomata.

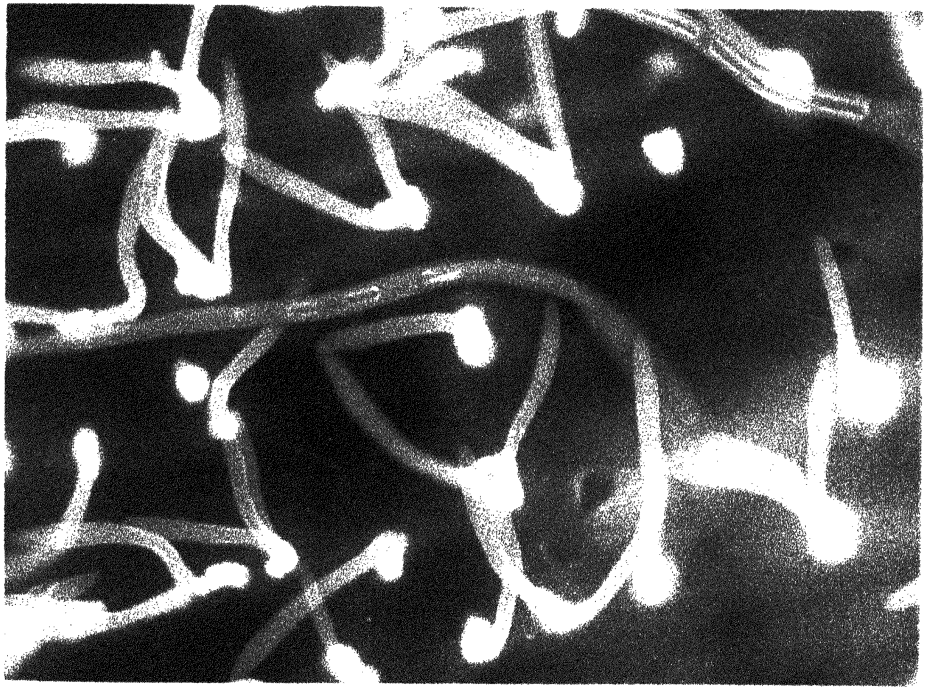

Plate 7. Micrograph of pearl millet leaf sample showing elongating sporangiopho

Plate 8. Micrograph of pearl millet leaf sample showing initiation of sporangiophore branching.

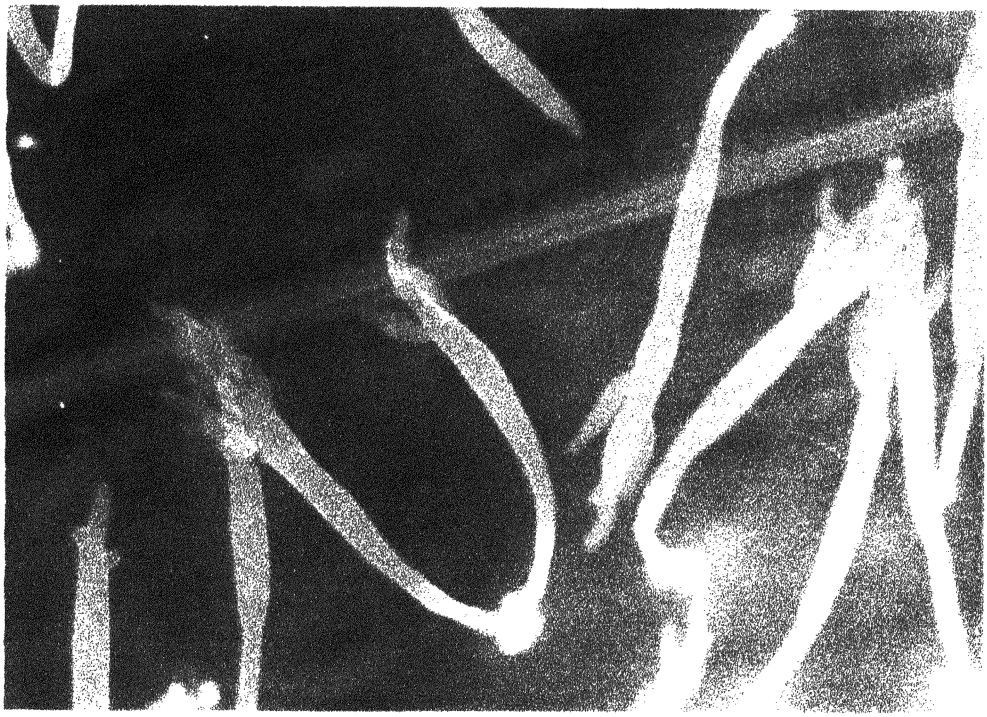

Plate 9. Micrograph of pearl millet leaf sample showing branched sporangiophores.

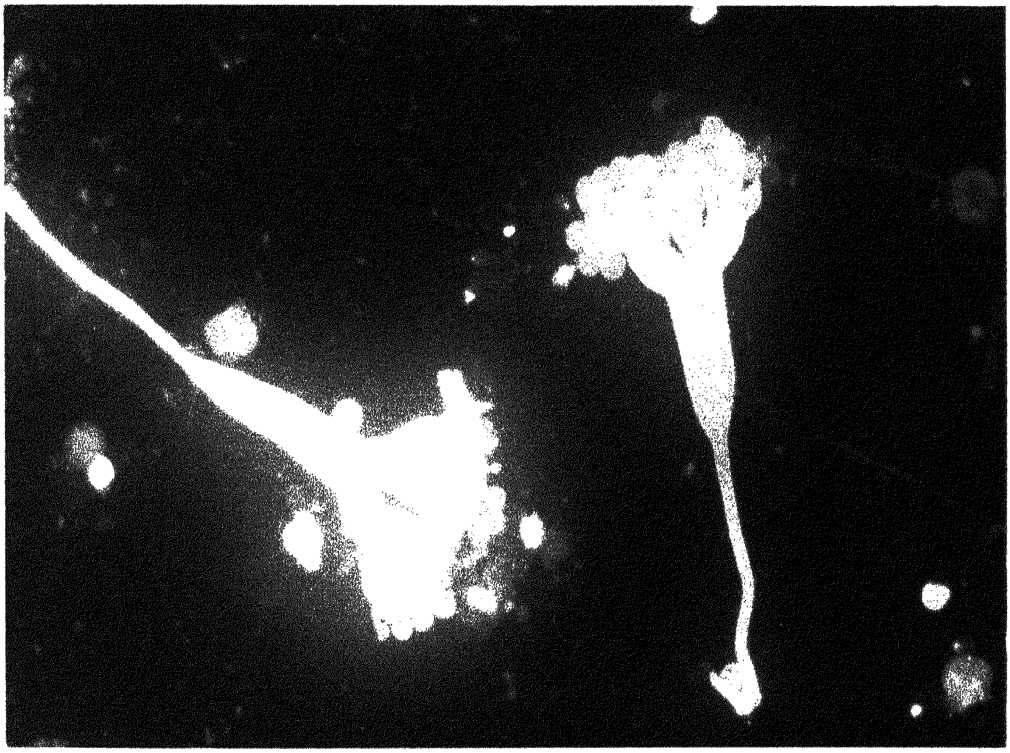

Plate 10. Micrograph of pearl millet leaf sample showing empty sterigmata, sporangia initials and developed sporangia on sporangiophore.

Plate 11. Micrograph of pearl millet leaf sample showing matured sporangia on sporangiophores.

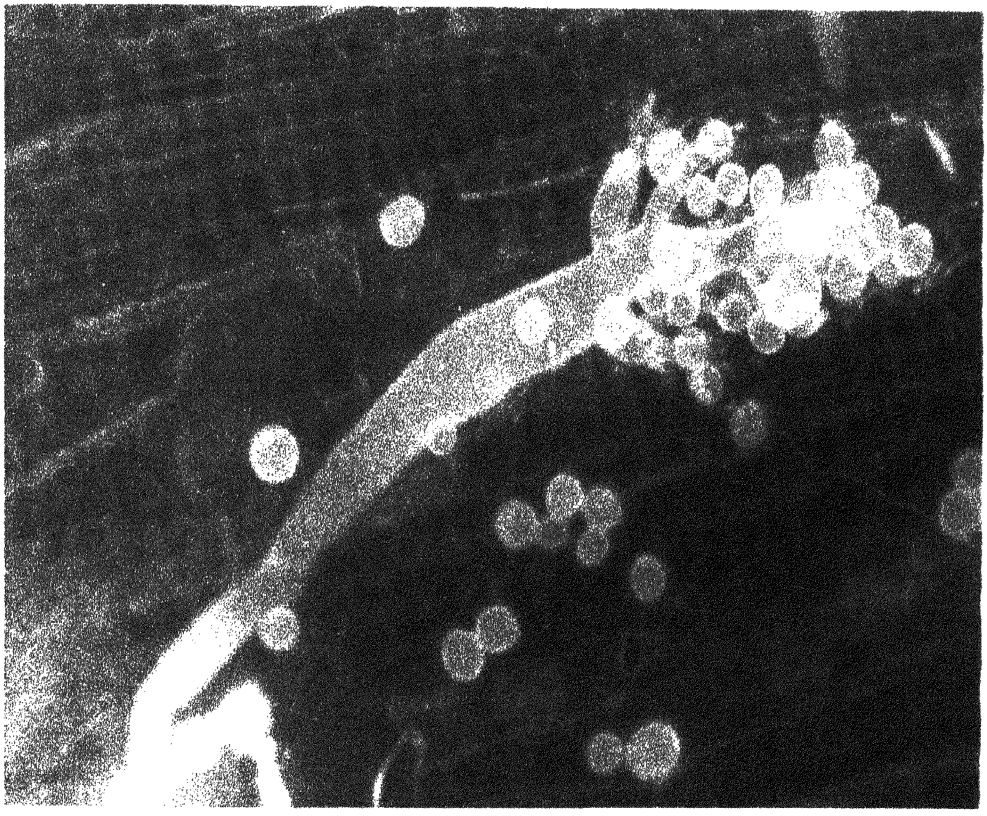

Plate 12. Micrograph of pearl millet leaf sample showing detached sporangia from sporangiophores.

Table 2. Number of stomata with sporangiophore primordia at 0 h and sporangiophore production at 3 and 8 h after incubation of infected pearl millet leaves at different temperatures.

Temp (°C)	0 h of incubation time SF	3 h after incubation		8 h after incubation	
		NSP	NSP SF^{-1}	NSP	NSP SF^{-1}
10	18.46	0	0	15.13	0.81
14	16.44	0	0	20.43	1.24
18	16.25	25.26	1.55	27.00	1.66
20	15.01	25.53	1.70	35.64	2.37
22	16.28	34.69	1.79	36.73	1.90
25	15.81	26.62	1.68	34.92	2.20
30	14.23	11.93	0.83	16.00	1.12
35	25.22	0	0	0	0
Mean	17.58	15.5	0.94	23.23	1.41

Temp - Temperature.
SF - Stomata with sporangiophore primordia $2mm^{-2}$ leaf area.
NSP - Number of productive sporangiophores $2mm^{-2}$ leaf area.

On an average, pearl millet leaves used in these experiments contained 25-28 stomata $2mm^{-2}$ area. Across all temperatures, the number of productive sporangiophores (NSP) that developed in stomata that initially contained sporangiophore primordia (SF), increased as incubation time progressed from 3 h (0.94) to 8 h (1.41). The NSP SF^{-1} also increased as incubation temperature increased from 10°C (0) to 22°C (1.79) at an incubation period of 3 h, and from 10°C (0.81) to 20°C (2.37) at 8 h of incubation period. Increasingly more NSP were produced from 10°C to 22°C; further increase in temperature (25°C-35°C) resulted in a gradual reduction of

NSP. Productive sporangiophores were not produced at 35°C.

4.1.2. Effect of temperature and incubation time on the number of productive sporangiophores per unit leaf area.

The observations were recorded at 160x magnification. Sporangiophores were formed at all the temperatures studied except at 35°C. The maximum temperature limit for sporangiophore formation was 30°C, while optimum observed was 22°C. Increase in temperature hastened the sporangiophore formation and reduced the time taken for sporangiophore formation. The data on the incubation temperature and sporangiophore number per unit leaf area is presented in Table 3 and Fig. 1.

ble 3. Effect of temperature on the number of productive *Sclerospora graminicola* sporangiophores formed per it leaf area of pearl millet at 160 x magnification*.

ıcuba- on(h) ime	Temperature(°C)								
	10°C	14°C	18°C	20°C	22°C	25°C	30°C	35°C	Mean
	1.22^{r} (0.00)	1.22^{r} (0.00)	1.22^{r} (0.00)	1.22^{r} (0.00)	1.22^{r} (0.00)	1.22^{r} (0.00)	1.22^{r} (0.00)	1.22^{r} (0.00)	1.22^{r} (0.00)
.5	1.22^{r} (0.00)	1.22^{r} (0.00)	3.07^{op} (9.40)	3.35^{mno} (10.80)	4.12^{hijk} (16.53)	3.32^{mno} (10.80)	1.22^{r} (0.00)	1.22^{r} (0.00)	2.34^{f} (6.44)
0	1.22^{r} (0.00)	1.22^{r} (0.00)	3.80^{klm} (14.61)	4.59^{efg} (20.60)	5.06^{bcd} (25.13)	3.54^{lmno} (11.96)	1.73^{q} (2.66)	1.22^{r} (0.00)	2.79^{e} (9.74)
.5	1.22^{r} (0.00)	1.22^{r} (0.00)	4.91^{bcde} (23.86)	4.65^{defg} (21.23)	5.38^{bcd} (28.53)	4.55^{def} (20.43)	2.81^{p} (7.53)	1.22^{r} (0.00)	3.28^{d} (13.46)
0	1.22^{r} (0.00)	1.22^{r} (0.00)	5.06^{bc} (25.26)	5.09^{bcd} (25.53)	5.84^{a} (34.69)	5.16^{bc} (26.62)	3.51^{lmno} (11.93)	1.22^{r} (0.00)	3.59^{cd} (16.37)
0	1.22^{r} (0.00)	4.23^{ghij} (17.53)	4.93^{bcde} (23.87)	5.06^{bcd} (25.13)	5.84^{a} (34.10)	5.81^{a} (33.06)	3.89^{ijkl} (14.66)	1.22^{r} (0.00)	4.02^{b} (18.79)
0	3.95^{ijkl} (15.13)	4.56^{def} (20.43)	5.23^{bc} (27.00)	5.97^{a} (35.64)	6.09^{a} (36.73)	5.91^{a} (34.92)	4.05^{hijk} (16.00)	1.22^{r} (0.00)	4.51^{a} (22.21)
4.0	3.74^{klmn} (14.46)	3.79^{klmn} (14.56)	4.50^{fgh} (20.26)	4.51^{fgh} (20.26)	5.62^{bcd} (31.58)	4.31^{ghi} (18.20)	3.26^{op} (10.20)	1.22^{r} (0.00)	3.86^{bc} (16.31)
lean	1.87^{d} (4.44)	2.33^{c} (7.19)	4.11^{b} (18.4)	4.30^{b} (20.02)	4.85^{a} (25.53)	4.22^{b} (19.62)	2.71^{c} (8.12)	1.22^{s} (0.00)	

	Temperature	Incubation time	Interaction
	0.0845	0.0904	0.2391
) at 5%	0.1657	0.1771	0.4686

*Leaf area visible at 160 x magnification was 2 mm^2

*Values in the parentheses are actuals.

*Values of similar superscript do not differ significantly at 5% probability.

It was observed that with increase in incubation time, the number of productive sporangiophores per field increased from 6.44 at 1.5 h to 22.21 at 8 h. An increase in mean number of sporangiophores was also seen with increase in temperature from 10°C (4.44) to 22°C (25.53). The sporangiophore number was maximum at 22°C after 8 hr of incubation (36.73 sporangiophores per field). While the number of sporangiophores increased with increase in incubation time up to 8 hours, but decreased after 24 h of incubation. The under-developed sporangiophores (unbranched sporangiophores) were not taken into account.

4.1.3. Effect of temperature and incubation time on sporangiophore length and diameter

In the past, the size of the asexual propagule and its morphology was considered as one of the criteria for classification of pathogen. However, it is observed that the propagule size is greatly influenced by the environmental factors. Hence to study their (temperature and incubation time) effects these experiments were carried out.

In general, increase in length of the sporangiophore was observed with increase in temperature (Table 4 and Fig. 2). At 10°C of incubation temperature the sporangiophores were stout, dwarf and branched at the apex, and the branches were few. As the temperature increased from 10 to 25°C, the sporangiophores were elongated and branches were more and widely spread. The mean length of sporangiophores at 10°C and 22°C were 45.64µm and 187.75µm respectively. But at 30°C, the sporangiophores were few in number and were thin and much more elongated (Plate 13). After 8 h of incubation time the length of sporangiophores at 10°C, 22°C and 30°C were 178.22 µm, 214.32 µm and 250.58 µm respectively. Thus an increase in temperature (up to 30°C) favours the growth of sporangiophore.

Table 4 Effect of temperature on sporangiophore length (µm) of *Sclerospora graminicola* in pearl millet at 80 x magnification

Incubation time	Temperature (°C)								
	10	14	18	20	22	25	30	35	Mean
	1 22^{d} (0 00)	1 22^{d} (0 00)	1 22^{d} (0 00)	1 22^{d} (0 00)	1 22^{d} (0 00)	1 22^{d} (0 00)	1 22^{d} (0 00)	1 22^{d} (0 00)	1 22^{d} (0 00)
	1 22^{d} (0 00)	1 22^{d} (0 00)	14 01^{ab} (197 40)	14 28^{ab} (203 91)	14 19^{ab} (201 35)	14 54^{ab} (213 60)	1 22^{d} (0 00)	1 22^{d} (0 00)	9 23 (102 5)
	1 22^{d} (0 00)	1 22^{d} (0 00)	14 30^{ab} (204 49)	14 42^{ab} (207 93)	14 38^{ab} (206 78)	14 48^{ab} (209 67)	14 44^{ab} (208 81)	1 22^{d} (0 00)	9 46^{c} (130 0)
	1 22^{d} (0 00)	1 22^{d} (0 00)	14 42^{ab} (207 93)	14 51^{ab} (210 54)	14 48^{ab} (209 67)	14 63^{ab} (214 03)	15 26^{ab} (232 86)	1 22^{d} (0 00)	9 62 (134 7)
	1 22^{d} (0 00)	1 22^{d} (0 00)	14 23^{ab} (202 49)	14 66^{ab} (214 91)	14 59^{ab} (221 71)	14 83^{ab} (219 92)	15 81^{a} (249 95)	1 22^{d} (0 00)	9 76 (138 92)
	1 22^{d} (0 00)	13 80^{ab} (190 44)	14 30^{ab} (204 49)	14 38^{ab} (206 73)	14 55^{ab} (212 57)	14 67^{ab} (215 20)	15 55^{ab} (242 75)	1 22^{d} (0 00)	11 06^{b} (159 26)
	13 35^{b} (178 22)	13 34^{b} (177 95)	14 30^{ab} (204 49)	14 41^{ab} (207 64)	14 64^{ab} (214 32)	14 61^{ab} (213 45)	15 53^{ab} (250 55)	1 22^{d} (0 00)	12 71^{a} (180 91)
	13 45^{b} (188 90)	14 15^{ab} (200 22)	14 00^{ab} (196 00)	14 42^{ab} (207 93)	14 39^{ab} (207 93)	14 67^{ab} (215 20)	14 99^{ab} (224 70)	1 22^{d} (0 00)	12 66^{a} (153 62)
	4 26^{d} (45 64)	5 92^{c} (71 70)	12 59^{ab} (177 25)	12 75^{a} (182 35)	12 84^{a} (184 30)	12 95^{a} (187 75)	11 79^{b} (176 41)	1 22^{d} (0 00)	

Temperature	Incubation time	Interaction
0 4223	0 4515	1 1945
0 8273	0 8849	2 3413

ues in the parentheses are actuals

ues of similar superscript do not differ significantly at 5% probability

The measurement of the sporangiophore diameter (thickness) were made at a magnification of 80X is presented in (Table 5 and Fig 3) Temperature variation influenced the diameter of the sporangiophores, between 10°C and 30°C

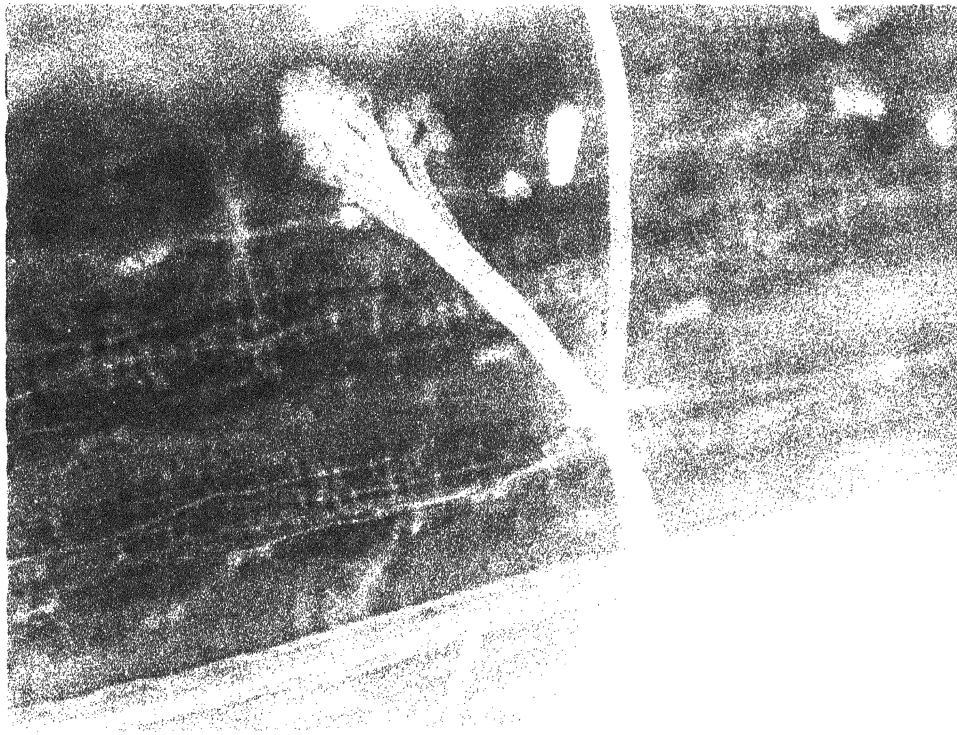

Plate 13. Abnormal elongation of sporangiophore due to temperature (30°C).

Table 5. Effect of temperature on sporangiophore diameter (µm) of *S. graminicola* in pearl millet at 80 x magnification.

Incubation time (h)	Temperature (°C)								
	10	14	18	20	22	25	30	35	Mean
0	1.22^{d} (0.00)	1.22^{d} (0.00)	1.22^{d} (0.00)	1.22^{d} (0.00)	1.22^{d} (0.00)	1.22^{d} (0.00)	1.22^{d} (0.00)	1.22^{d} (0.00)	1.22^{d} (0.00)
1.5	1.22^{d} (0.00)	1.22^{d} (0.00)	4.57^{a} (20.88)	3.45^{b} (10.33)	4.60^{a} (20.73)	2.37^{c} (7.73)	1.22^{d} (0.00)	1.22^{d} (0.00)	2.60^{d} (8.95)
2.0	1.22^{d} (0.00)	1.22^{d} (0.00)	4.54^{a} (20.13)	4.57^{a} (20.88)	4.62^{a} (20.86)	4.64^{a} (21.06)	4.14^{a} (16.13)	1.22^{d} (0.00)	3.18^{c} (12.48)
2.5	1.22^{d} (0.00)	1.22^{d} (0.00)	4.31^{a} (18.07)	4.68^{a} (21.46)	4.47^{a} (19.53)	4.59^{a} (20.53)	4.27^{a} (17.26)	1.22^{d} (0.00)	3.30^{c} (13.05)
3.0	1.22^{d} (0.00)	1.22^{d} (0.00)	4.39^{a} (18.80)	4.66^{a} (21.26)	4.65^{a} (21.20)	4.64^{a} (21.06)	4.25^{a} (17.06)	1.22^{d} (0.00)	3.33^{c} (13.34)
6.0	1.22^{d} (0.00)	4.59^{a} (21.68)	4.55^{a} (20.70)	4.67^{a} (21.40)	4.57^{a} (20.88)	4.64^{a} (21.06)	4.21^{a} (16.96)	1.22^{d} (0.00)	3.76^{b} (16.14)
8.0	4.34^{a} (18.33)	4.55^{a} (20.70)	4.57^{a} (20.88)	4.70^{a} (21.66)	4.67^{a} (21.40)	4.57^{a} (20.40)	4.20^{a} (16.81)	1.22^{d} (0.00)	4.17^{a} (18.43)
24.0	4.39^{a} (19.27)	4.57^{a} (20.88)	4.54^{a} (20.26)	4.65^{a} (21.13)	4.48^{a} (19.60)	4.62^{a} (20.86)	4.28^{a} (17.51)	1.22^{d} (0.00)	4.14^{a} (18.09)
Mean	2.00^{e} (4.76)	2.47^{d} (7.90)	4.08^{ab} (17.46)	4.20^{a} (17.2)	4.16^{a} (18.02)	3.91^{b} (16.58)	3.69^{c} (12.71)	1.22^{f} (0.00)	

	Temperature	Incubation time	Interaction
SE	0.1105	0.1182	0.3127
CD at 5%	0.2167	0.2316	0.6128

*Values in the parentheses are actuals.

*Values of similar superscript do not differ significantly at 5% probability.

The mean diameter of sporangiophore was 4.76 µm at an initial incubation temperature of 10°C. The thickness increased with increase of temperature up to 22°C (18.02 µm). The mean thickness was less at 25°C (16.58 µm) and 30°C (12.71 µm). The reduction in thickness was significant statistically. At 35°C sporangiophore growth was not observed.

4.1.4. Effect of temperature and incubation time on sporulation

An increase in the incubation temperature decreased the incubation time for sporulation (Table 6 and Fig. 4). The minimum time required for sporangial production was 2.5 h at 18-30°C. Sporulation was maximum (4.12×10^5 spores cm^{-2}) at 22°C. Sporangiophore length was maximum, but sporangia production was less (1.43×10^5 cm^{-2}) at 30°C. At 10°C, sporangiophore production was late (more than 8 h) and sporulation was minimum (0.93×10^5 spores cm^{-2}). At 35°C sporulation was not observed.

Table 6. Effect of temperature on sporulation ($x10^5$ number of sporangia cm^{-2} leaf area) of *S. graminicola* in pearl millet.

Incubation time(h)	Temperature(°C)								
	10	14	18	20	22	25	30	35	Mean
0	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{f} (0.00)
1.5	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{f} (0.00)
2.0	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.22^{f} (0.00)
2.5	1.22^{g} (0.00)	1.22^{g} (0.00)	1.33^{e} (0.54)	1.62^{cd} (1.36)	1.61^{cd} (1.34)	1.49^{d} (0.73)	1.24^{fg} (0.13)	1.22^{g} (0.00)	1.36^{e} (0.07)
3.0	1.22^{g} (0.00)	1.22^{g} (0.00)	1.82^{bc} (1.86)	2.01^{b} (2.42)	2.12^{bc} (2.96)	2.23^{a} (3.18)	1.34 (0.58)	1.22^{g} (0.00)	1.92^{d} (1.37)
6.0	1.22^{g} (0.00)	1.22^{g} (0.00)	1.94^{b} (2.28)	2.04^{b} (2.51)	2.28^{a} (3.36)	2.24^{a} (3.22)	1.68^{c} (1.43)	1.22^{g} (0.00)	2.06^{b} (1.60)
8.0	1.22^{g} (0.00)	1.81^{bc} (1.86)	2.31^{a} (3.53)	2.28^{a} (3.36)	2.40^{a} (4.12)	2.02^{b} (2.46)	1.36^{e} (0.61)	1.22^{g} (0.00)	2.23^{a} (1.99)
24.0	1.49^{d} (0.93)	1.44^{d} (0.71)	1.36^{e} (0.64)	1.48^{d} (0.73)	1.30^{e} (0.49)	1.28^{f} (0.21)	1.22^{g} (0.00)	1.22^{g} (0.00)	1.98^{c} (0.46)
Mean	1.23^{f} (0.11)	1.38^{e} (0.32)	1.84^{d} (1.10)	1.92^{c} (1.25)	1.96^{c} (1.53)	2.12^{b} (1.22)	2.19^{a} (0.34)	1.22^{f} (0.00)	

	Temperature	Incubation time	Interaction
SE	0.0323	0.0345	0.0632
CD at 5%	0.0632	0.0676	0.1788

*Values in the parentheses are actuals.

*Values of similar superscript do not significantly differ at 5% probability.

4.2. Effect of RH and incubation time

4.2.1. Effect of RH and incubation time on the number of productive sporangiophores formed per stomata

The sporangiophore production in stomata containing sporangiophore primordia was studied at a RH range of 95 to 100%. Even though the sporangiophore initials were present in the stomata at RH levels of 95 and 96%, they did not develop into sporangiophores (Table 7). At 97% RH the ratio of NSP SF^{-1} was 0.22 at 3 h after incubation and 0.28 at 8 h after incubation. At 98 and 100% RH good (24.26 and 25.00 at 3 h and 28.46 and 33.40 at 8 h respectively) number of sporangiophores were observed and the ratios of NSP/SF at 98% RH was 1.40 at 3 h of incubation and 1.64 at 8 h of incubation. At 100% RH, the ratio was 1.48 at 3 h of incubation and 1.98 at 8 h of incubation. A slight increase in number of sporangiophores/stomata was observed at 100% RH compared to 98% RH.

Table 7. Number of stomata with sporangiophore primordia at 0 h and sporangiophore production at 3 and 8 h after incubation of pearl millet downy mildew leaves at different humidity levels.

Relative humidity (%)	0 h after incubation SF	3 h after incubation		8 h after incubation	
		NSP	NSP/SF	NSP	NSP/SF
95	18.42	0	0	0	0
96	17.98	0	0	0	0
97	17.82	3.95	0.22	5.16	0.28
98	17.26	24.26	1.40	28.46	1.64
100	16.84	25.00	1.48	33.40	1.98
Mean	17.66	10.64	0.62	13.41	0 78

SF - Stomata with sporangiophore primordia in 2 mm^{-2} leaf area.
NSP - Number of productive sporangiophores/unit leaf area.

4.2.2. Effect of RH and incubation time on the number of productive sporangiophores per unit leaf area

Studies on the effect of relative humidity on production of sporangiophores were conducted between the range of 95 and 100% RH. Sporangiophore production was not noticed at 95% RH (Table 8 and Fig. 5). A few sporangiophores formed at 96% RH after 8 h and many of them elongated slightly from the sporangiophore primordia and stopped growing (Plate 14). After 24 h of incubation at 96% RH less than 8 productive sporangiophores were found in 2 mm^2 leaf area. At 97% RH the number of sporangiophore was less (3.69) and their growth was slow (4-6 h). Only at

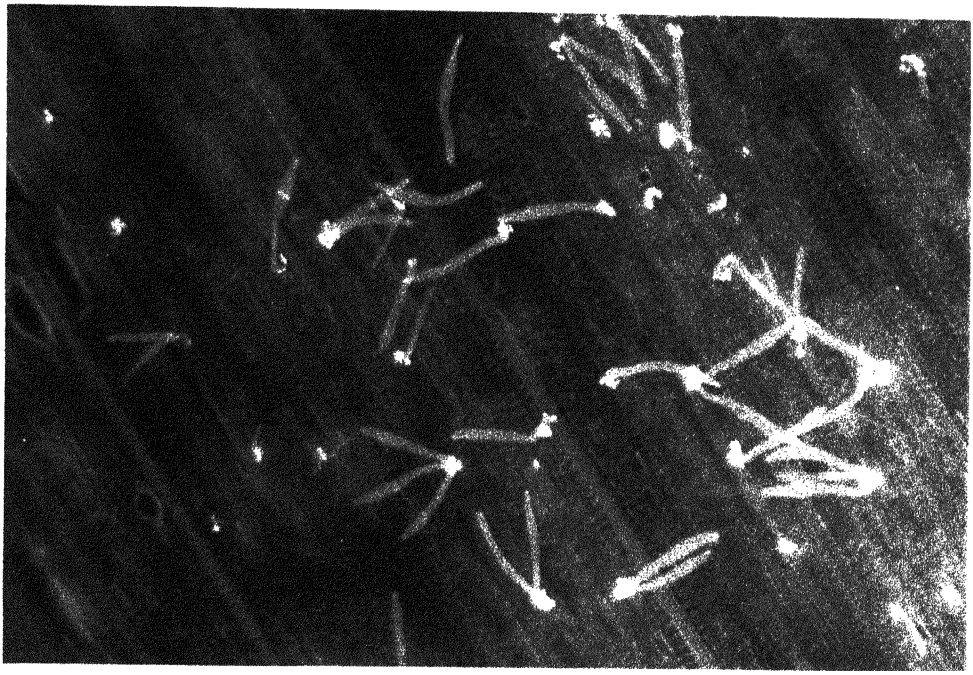

Plate 14. Effect of RH on development of sporangiophore (96% RH).

98 and 100% RH, a good number of sporangiophores were observed after 1.5 h of incubation time. The sporangiophore number per $2mm^2$ leaf area increased with increase in incubation time. At 98% RH there were 4.16 sporangiophores after 1.5 hrs of incubation which increased to 28.46 after 8 h. Similarly, at 100% RH after 1.5 h, the number of sporangiophores per 2 mm^2 leaf area was 21.93 which increased gradually with increase in incubation time up to 8 h. However the number of sporangiophore were fewer at 24 h both at 98 and 100% RH compared to 8 h. Maximum number of sporangiophores (33.46/microscopic field) was recorded at 100% RH after an incubation period of 8 h.

Table 8. Effect of relative humidity on the number of productive sporangiophores formed per 2 mm^2 leaf area.

Incubation time (h)	Relative humidity (%)					
	95	96	97	98	100	Mean
0	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{e} (0.00)
1.5	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.92 (4.16)	4.73^{abcd} (21.93)	2.06^{d} (5.21)
2.0	1.22^{h} (0.00)	1.22 (0.00)	1.22^{h} (0.00)	4.53^{bcd} (20.06)	4.87^{abc} (23.26)	2.61^{c} (8.66)
2.5	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	5.00^{abc} (24.53)	4.99^{abc} (24.53)	2.75^{bc} (9.81)
3.0	1.22^{h} (0.00)	1.22^{h} (0.00)	2.02^{fg} (3.95)	4.96^{abc} (24.26)	5.04^{ab} (25.00)	2.97^{ab} (10.64)
6.0	1.22^{h} (0.00)	1.22^{h} (0.00)	2.20^{fg} (4.86)	5.17^{ab} (26.33)	5.22^{ab} (26.86)	3.04^{ab} (11.61)
8.0	1.22^{h} (0.00)	1.22^{h} (0.00)	2.06^{fg} (5.16)	5.38^{a} (28.46)	5.86^{a} (33.46)	3.00^{ab} (13.41)
24.0	1.22^{h} (0.00)	2.62^{f} (7.42)	3.98^{d} (15.56)	3.77^{e} (14.06)	4.24^{cd} (17.63)	3.16^{a} (10.93)
Mean	1.22^{d} (0.00)	1.44^{d} (0.92)	1.89^{c} (3.69)	3.96^{b} (17.73)	4.45^{a} (21.58)	

	RH	Incubation time	Interaction
SE	0.1372	0.1735	0.3880
CD at 5%	0.2728	0.3451	0.7717

*Values in the parentheses are actuals.

*Values of similar superscript do not significantly differ at 5%

4.2.3. Effect of RH and incubation time on the length and diameter of sporangiophore

The observations on the length and diameter of the sporangiophore were made at 80 x magnification. Mean length of sporangiophore was 21.03 µm at 96% RH and 185.75 µm at 100% RH (Table 9 and Fig. 6). This difference was due to delayed growth of sporangiophore at 96% RH. Only after 24 h of incubation period sporangiophores were observed at 96% RH (168.26 µm). The maximum length (213.40 µm) of sporangiophore was observed at 100% RH after 2.5 h of incubation period. In general, RH did not have any effect on length of sporangia-bearing sporangiophores at 97 to 100% RH (data not presented).

Table 9. Effect of RH on sporangiophore length (µm) of pearl millet downy mildew at 80 x magnification. field.

Incubation time (h)	Relative humidity (%)					
	95	96	97	98	100	Mean
0	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{d} (0.00)
1.5	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	13.36^{ab} (176.48)	14.58^{a} (212.33)	6.32^{c} (77.76)
2.0	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	14.5^{a} (209.25)	14.55^{a} (211.33)	6.54^{c} (84.11)
2.5	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	14.45^{a} (207.80)	14.62^{a} (213.40)	6.54^{c} (84.24)
3.0	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	14.56^{a} (210.99)	14.60^{a} (212.70)	6.56^{c} (84.73)
6.0	1.22^{b} (0.00)	1.22^{b} (0.00)	13.08^{ab} (168.14)	14.5^{a} (209.25)	14.59^{a} (212.43)	8.92^{bc} (117.96)
8.0	1.22^{b} (0.00)	1.22^{b} (0.00)	14.60^{a} (213.30)	14.6^{a} (211.16)	14.61^{a} (213.03)	9.25^{ab} (127.51)
24.0	1.22^{b} (0.00)	13.11^{a} (168.26)	14.4^{a} (208.43)	14.52^{a} (210.26)	14.53^{a} (210.80)	11.55^{a} (159.55)
Mean	1.22^{c} (0.00)	2.70^{c} (21.03)	6.02^{c} (73.74)	12.71^{a} (179.39)	12.91^{a} (185.75)	

	RH	Incubation time	Interaction
SE	1.0466	1.3239	2.9604
CD at 5%	2.0818	2.6333	5.8882

*Values of similar superscript do not differ significantly at 5% probability.
*Values in the parenthesis are actuals.

The effect of RH on the sporangiophore diameter was presented in (Table 10 and Fig. 7).

The mean diameter of sporangiophore was 1.34 µm and 19.23 µm at 96% and 100% RH

respectively. The sporangiophore diameter was minimum at 96% RH (10.78 µm) after 24 h of incubation and maximum (23.53 µm) at 100% RH after 3 h of incubation.

Table 10. Effect of RH on sporangiophore width (µm) of pearl millet downy mildew at 80 x magnification.

Incubation time (h)	Relative humidity (%)					
	95	96	97	98	100	Mean
0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)
1.5	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	3.36^{ab} (11.24)	4.61^{ab} (21.25)	2.32^{b} (6.49)
2.0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	4.68^{ab} (21.45)	4.73^{a} (21.86)	2.61^{b} (8.66)
2.5	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	4.66^{ab} (21.26)	4.70^{ab} (21.60)	2.60^{b} (8.57)
3.0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	4.65^{a} (21.21)	4.81^{a} (23.53)	2.62^{b} (8.94)
6.0	1.22^{c} (0.00)	1.22^{c} (0.00)	3.57^{ab} (15.06)	4.71^{ab} (21.73)	4.70^{ab} (21.60)	3.08^{ab} (11.67)
8.0	1.22^{c} (0.00)	1.22^{c} (0.00)	3.44^{ab} (11.46)	4.70^{ab} (21.60)	4.77^{a} (22.75)	3.07^{ab} (11.16)
24.0	1.22^{c} (0.00)	3.22^{b} (10.78)	4.68^{ab} (21.45)	4.63^{ab} (20.96)	4.66^{a} (21.26)	3.68^{a} (14.89)
Mean	1.22^{c} (0.00)	1.47^{c} (1.34)	2.22^{b} (5.99)	4.07^{a} (17.43)	4.27^{a} (19.23)	

	RH	Incubation time	Interaction
SE	0.2749	0.3478	0.7776
CD at 5%	0.5468	0.6917	1.5467

*Values in the parentheses are actuals.

*Values of similar superscript do not differ significantly at 5% probability.

The mean sporangiophore diameter was 6.49 µm at 1.5 h and 14.89 at 24 h of incubation time.

4.2.4. Effect of RH and incubation time on the asexual sporulation process

Sporulation was not noticed at 95 and 96% RH (Table 11 and Fig. 8). At 97% RH after 8 h of incubation, sporulation was 0.09 x 10^5 spores cm^{-2} leaf area. Sporulation after 2.5 h of incubation was 0.54 x 10^5 spores and 0.73 x 10^5 spores/cm^2 at 98% and 100% RH respectively. At higher RH levels (98% and 100%) the sporangial production increased with increase in incubation time up to 8 h (2.42 x 10^5 spores and 3.26 x 10^5 spores/cm^2, respectively). Number of sporangia decreased at 24 h of incubation time at high RH levels (2.31 x 10^5 spores and 1.56 x 10^5 spores/cm^2, respectively).

Table 11. Number of sporangia ($x10^5$ cm^{-2} leaf area) of *Sclerospora graminicola* on pearl millet leaves at different RH.

Incubation time (h)	Relative humidity (%)					
	95	96	97	98	100	Mean
0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)
1.5	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)
2.0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)
2.5	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.33^{c} (0.54)	1.49^{d} (0.73)	1.29^{d} (0.25)
3.0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.46^{de} (0.71)	1.84^{c} (2.02)	1.39^{c} (0.54)
6.0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.22^{c} (0.00)	1.92^{bc} (2.21)	2.03^{b} (2.50)	1.52^{ab} (0.94)
8.0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.24^{c} (0.09)	2.01^{b} (2.42)	2.24^{a} (3.26)	1.58^{a} (1.14)
24.0	1.22^{c} (0.00)	1.22^{c} (0.00)	1.28^{c} (0.21)	1.98^{bc} (2.31)	1.73^{c} (1.56)	1.48^{b} (0.82)
Mean	1.22^{d} (0.00)	1.22^{d} (0.00)	1.23^{c} (0.04)	1.54^{b} (0.97)	1.62^{a} (1.30)	

	RH	Incubation time	Interaction
SE	0.0279	0.0352	0.0788
CD at 5%	0.0554	0.0701	0.1567

*Value in the parentheses are actuals.

*Values of similar superscript do not differ significantly at 5% probability.

The effect of RH on the sporangial size was studied in respect of the sporangial length and sporangial diameter. The mean length of sporangium was 5.5 µm at 97% RH and 13.12 µm at 100% RH (Table 12 and Fig. 9). At 2.5 h of incubation the mean length of sporangium was 8.74 µm and at 24 h of incubation period it was 13.00 µm. The effect of RH on sporangium length was statistically significant. The sporangium length was minimum (20.12 µm) at 6 h of incubation period at 100% RH and maximum (22.13 µm) at 98% RH after 2.5 h of incubation period.

Table 12. Effect of RH on sporangium length (µm) of *Sclerospora graminicola* in pearl millet downy mildew observed at 80 x magnification.

Incubation time (h)	Relative humidity (%)					
	95	96	97	98	100	Mean
0	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{d} (0.00)
1.5	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{d} (0.00)
2.0	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{d} (0.00)
2.5	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	4.75^{a} (22.13)	4.69^{a} (21.53)	2.62^{c} (8.74)
3.0	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	4.68^{a} (21.43)	4.66^{a} (21.38)	2.60^{c} (8.6)
6.0	1.22^{h} (0.00)	1.22^{h} (0.00)	1.22^{h} (0.00)	4.03^{ab} (19.86)	4.38^{a} (20.12)	2.42^{c} (8.2)
8.0	1.22^{h} (0.00)	1.22^{h} (0.00)	4.72^{a} (21.86)	4.56^{a} (20.62)	4.72^{a} (21.86)	3.28^{a} (12.8)
24.0	1.22^{h} (0.00)	1.22^{h} (0.00)	4.75^{a} (22.10)	4.62^{a} (21.04)	4.68^{a} (21.43)	2.82^{b} (13.00)
Mean	1.22^{c} (0.00)	1.22^{c} (0.00)	2.09^{b} (5.5)	3.29^{a} (13.22)	3.34^{a} (12.12)	

	RH	Incubation time	Interaction
SE	0.1562	0.1976	0.4418
CD at 5%	0.3107	0.3930	0.8787

*Values in the parentheses are original.
*Values of similar superscript do not differ significantly at 5% probability.

The mean diameter of sporangium at 97% RH was 4.40 µm and at 100% RH it was 11.36 µm (Table 13 and Fig. 10). There was no significant variation in the diameter of sporangium

between 98% RH (11.40 µm) and 100% RH (11.36 µm). The mean diameter of sporangium at 2.5 h of incubation time was 7.6 µm and at 8 h of incubation time it was 11.00 µm. The minimum diameter of sporangium observed was 15.23 µm at 100% RH after 6 h of incubation time and maximum observed was 19.86 µm at 97% RH after 8 h of incubation time.

Table 13. Effect of RH on sporangium width (µm) of *S. graminicola* of pearl millet downy mildew observed at 80 x magnification.

Incubation time (h)	Relative humidity (%)					
	95	96	97	98	100	Mean
0	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{d} (0.00)
1.5	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{d} (0.00)
2.0	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{d} (0.00)
2.5	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	4.26^{a} (18.83)	4.44^{a} (19.01)	2.47^{c} (7.6)
3.0	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	3.98^{a} (15.44)	4.46^{a} (19.13)	2.42^{c} (6.9)
6.0	1.22^{b} (0.00)	1.22^{b} (0.00)	1.22^{b} (0.00)	4.41^{a} (18.96)	3.91^{a} (15.23)	2.39^{c} (6.85)
8.0	1.22^{b} (0.00)	1.22^{b} (0.00)	4.51^{a} (19.86)	4.22^{a} (18.62)	4.29^{a} (18.76)	3.09^{b} (11.3)
24.0	1.22^{b} (0.00)	1.22^{b} (0.00)	4.06^{a} (16.96)	4.36^{a} (19.02)	4.33^{a} (18.94)	3.45^{a} (11.00)
Mean	1.22^{c} (0.00)	1.22^{c} (0.00)	1.98^{b} (4.40)	3.11^{a} (11.40)	3.13^{a} (11.36)	

	RH	Incubation time	Interaction
SE	0.1459	0.1845	0.4125
CD at 5%	0.2901	0.3670	0.8206

*Values in the parentheses are actuals.

*Values of similar superscript do not differ significantly at 5% probability.

4.3. Longevity of sporangiophore under intermittent high and low RH regimes.

In experiments (mentioned previously) conducted to study the effect of RH and incubation time on the sporangiophore and sporangial production, it was observed that the sporangiophores were formed after 1.5 h of incubation at 98% RH and the sporulation occurred (i.e. sporangial formation) after 2.5 h of incubation.

Two experiments were conducted to understand the sporulation process in nature where alternate periods of high (night) and low (day) humidity occur. Often, the periods of low and high humidity vary. In these experiments, high moisture conditions were provided in either dew chambers (for wet conditions) or single plant chambers (for maintaining variable levels of high humidity). Low humidity conditions were provided in a lighted incubator (E 15) in which 60% RH was maintained.

4.3.1. Effect of intermittent wet and dry conditions on the longevity of sporangiophore.

In the first experiment, the level of high humidity and low humidity was kept constant, but the number and duration of intermittent exposures to low humidity were varied. High humidity was maintained by providing wetness in dew chambers and low humidity (dry) was maintained in E-15.

At first, leaves were exposed to 60% RH for 1 h, then in dew chambers at 100% RH for 1.5 h to induce sporangiophore formation. Subsequently, they were exposed to low humidity for 10, 30 and 60 min followed by incubation in the dew chamber again for 1.5 h. The cycle of low and high humidity was repeated up to four times in different sets of plants. The number of sporangiophores and sporangia were counted at the end of 1.5 h wet period before the plants were exposed again to 60% RH (Plate 15). After the final exposure to the low humidity period for all treatments, plants were continuously, incubated in the dew chamber for 6 h to induce sporangia formation. Sporangial counts were taken 3 and 6 h after initiating final wet period to allow formation of sporangia. Plants in the control treatment was incubated continuously in the dew chamber and therefore did not receive any low RH exposure.

After exposure to the first wet period (i.e., before initiating the first dry period, the number of sporangiophores in different treatments were almost similar and ranged from 23.5 to 30.2 $2mm^{2}$ leaf area (Table 14 and Fig. 11). The similarly the number of sporangiophores in different treatments is not surprising because all the plants were grown under similar conditions until then. In all treatments except one (three low RH exposures of 10 min each), the number of sporangiophores increased significantly when the plants were exposed to the second wet period after receiving the first low RH period. The increase in sporangiophore number ranged from 32-51% over the previous period. Subsequent exposures to intermittent wet and dry regimes did not provide significant increases in the number of sporangiophore. A maximum of 42.3 sporangiophores $2mm^{2}$ leaf area was observed when plants were exposed to four low RH exposures of 60 min each. Exposure period to low RH did not have much influence on the number of sporangiophore since sporangiophore production was almost similar at each stage irrespective of the duration of low RH.

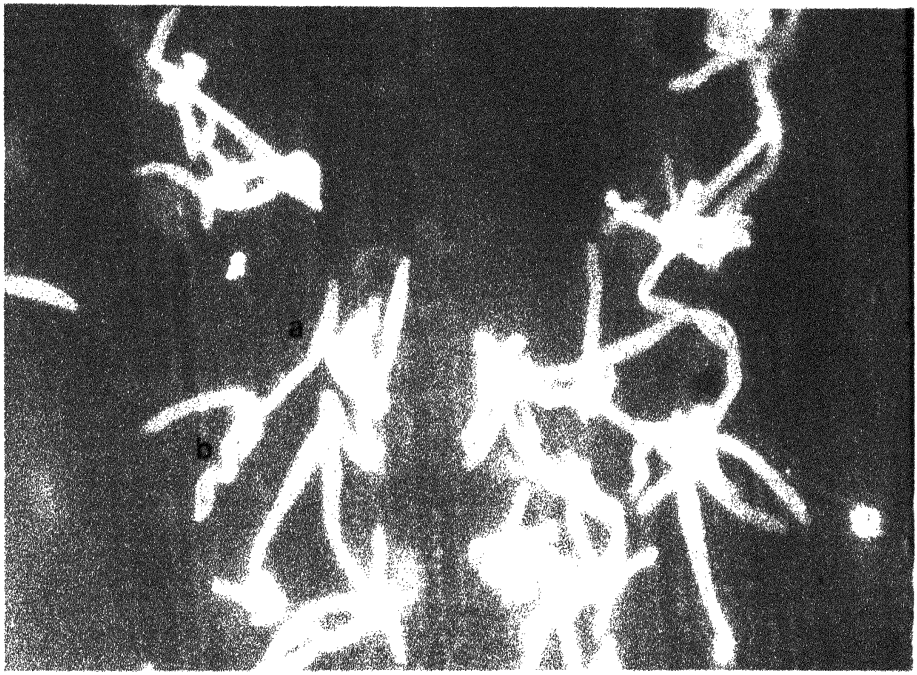

Plate 15. Effect of [illegible] on [illegible] of sporangiophore.

a. Newly formed sporangiophores.
b. Deteriorated sporangiophores.

There was not much difference in observations between different time intervals (60, 30 and 10 min).

Table 14. Effect of high (100% RH) and low (60% RH) humidity regimes on sporangiophore production on pearl millet leaves by *Sclerospora graminicola*

Time period of low RH (min)	No. of low RH exposure	Number of sporangiophores 2mm^{-2} leaf area before initiating			
		1st dry period	2nd dry period	3rd dry period	4th dry period
60	0	28.0 (±2.04)			
	1	28.2 (±2.35)			
	2	24.0 (±2.55)	36.2 (±2.13)		
	3	25.7 (±1.03)	38.3 (±1.71)	41.2 (±2.13)	
	4	29.7 (±0.65)	40.9 (±0.70)	41.6 (±1.38)	42.3 (±2.14)
30	0	30.5 (±2.20)			
	1	26.0 (±2.11)			
	2	28.2 (±1.62)	37.1 (±2.59)		
	3	26.7 (±0.60)	40.0 (±3.12)	41.4 (±4.23)	
	4	25.0 (±0.72)	33.0 (±2.19)	36.6 (±2.97)	40.1 (±3.32)
10	0	30.2 (±2.22)			
	1	29.5 (±1.99)			
	2	26.0 (±1.74)	38.3 (±1.91)		
	3	29.2 (±1.40)	31.5 (±2.35)	39.3 (±3.08)	
	4	23.5 (±1.42)	35.2 (±1.69)	39.6 (±2.09)	40.6 (±2.15)

*Standard errors are given in parentheses.

Indeed, sporulation was observed on leaves only when the infected plants were exposed to high RH (100%) continuously for 3 h or more. Sporulation was not observed when the infected plants were exposed to low RH (60%) even for 10 min after production of sporangiophores, and then for 1.5 h at high RH (Table 15). Four exposures of leaves to low RH for as little period as 10 min each were detrimental to sporangiophores since no sporulation occurred on leaves after 3 h wet period which is normally sufficient to induce sporulation.

Table 15. Effect of high (100% RH) and low (60% RH) humidity regimes on sporangia production on pearl millet leaves by *Sclerospora graminicola*

Time period of low RH (min)	No. of low RH exposure	Sporangia number ($x10^3$ spores cm^{-2} leaf area)					
		Before initiating				After (h) final dry period	
		1st dry period	2nd dry period	3rd dry period	4th dry period	3h	6h
60	0					2.33 (±0.13)	4.16 (±0.48)
	1					1.95 (±0.38)	2.59 (±0.15)
	2	0	0			1.62 (±0.14)	2.27 (±0.13)
	3	0	0	0		0.91 (±0.19)	1.18 (±0.07)
	4	0	0	0	0	x	0.05 (±0.06)
30	0					2.81 (±0.33)	3.85 (±0.25)
	1	0				2.67 (±0.35)	3.21 (±0.14)
	2	0	0			1.53 (±0.16)	2.14 (±0.18)
	3	0	0	0		1.02 (±0.09)	1.49 (±0.17)
	4	0	0	0	0	x	0.04 (±0.12)
10	0					2.8 (±0.39)	4.1 (±0.12)
	1	0				2.07 (±0.14)	2.86 (±0.22)
	2	0	0			1.22 (±0.17)	1.91 (±0.20)
	3	0	0	0		1.00 (±0.08)	1.16 (±0.48)
	4	0	0	0	0	x	0.08 (±0.01)

*Standard errors are given in parentheses.
(-) Observations not recorded.
(x) Observations could not be taken.

Irrespective of the duration of low RH exposures, an increase in sporulation was observed with decrease in the number of low RH exposures. When leaves were exposed for 60 min in low RH for four times sporulation after 6 h of final dry period was 0.05 x 10^5 spores cm^{-2} leaf area. The corresponding level without any exposure to low RH was 4.16 x 10^5 spores cm^{-2} leaf area. Similarly in the 30 min duration of low RH exposures the figures were 0.04 x 10^5 and 3.85 x 10^5 and in the 10 min duration of low RH exposure the figures were 0.08 x 10^5 and 4.1 x 10^5 spores/sqcm leaf area respectively.

4.3.2. Effect of variable RH regimes on the viability of sporangiophore in single plant controlled chambers.

Leaves were exposed to five levels of low RH (70, 80, 90, 95 and 98%) for certain periods (60, 30 and 10 min) and then at high RH (98%) for 2 h to induce sporangiophore formation (Table 16). The number of sporangiophores and sporangia were counted at the end of 2 h period before plants were exposed again to the five levels of RH. A second cycle of five RH levels followed by 98% RH was given. At the end of 98% RH exposure the leaves were examined again for sporulation.

Table 16. Effect of high and low RH regimes on sporangiophore and sporangia production in *Sclerospora graminicola* using single plant controlled chambers.

Time period of low RH exposure (hrs)	RH	No. of exposures	Sporangiophore production before		Sporangial production ($x10^3$ spores cm^{-2} sqcm leaf area after			
							Final period at <98% RH	
			1st period at <98% RH	2nd period at <98% RH	1st period at <98% RH	2nd period at <98% RH	3h	6h
	70	2	25.53 (±1.23)	35.26 (±2.12)	0	0	1.39 (±0.24)	2.18 (±0.35)
	80	2	24.73 (±2.45)	32.41 (±2.24)	0	0	1.58 (±0.09)	2.47 (±0.19)
	90	2	26.26 (±2.28)	36.42 (±1.46)	0	0	1.28 (±0.33)	1.98 (±0.18)
	95	2	28.2 (±2.26)	37.2 (±1.72)	0	0	1.32 (±0.25)	2.14 (±0.23)
	98	0	26.22 (±2.01)	x	1.18 (±0.07)	1.96 (±0.14)	2.42 (±0.38)	3.76 (±0.18)
	70	2	27.64 (±1.66)	37.32 (±1.03)	0	0	0.92 (±0.42)	1.45 (±0.31)
	80	2	25.84 (±2.21)	32.86 (±2.74)	0	0	1.12 (±0.28)	2.21 (±0.12)
	90	2	23.56 (±1.33)	32.30 (±2.14)	0	0	1.20 (±0.27)	2.36 (±0.18)
	95	2	28.8 (±2.64)	x	0.62 (±0.09)	1.98 (±0.46)	2.28 (±0.32)	3.01 (±0.26)
	98[a]	0	26.86 (±1.23)	x	0.74 (±0.22)	2.04 (±0.31)	2.66 (±0.14)	3.46 (±0.21)
	70	2	28.26 (±2.12)	34.62 (±2.04)	0	0	1.34 (±0.48)	2.06 (±0.31)
	80	2	24.23 (±1.84)	28.48 (±1.08)	0	0	0.86 (±0.07)	1.48 (±0.18)
	90	2	27.43 (±2.42)	36.68 (±2.23)	0	0	1.28 (±0.39)	2.12 (±0.08)
	95	2	28.42 (±1.92)	x	0	1.19 (±0.12)	2.23 (±0.11)	2.98 (±0.09)
	98	0	30.23 (±2.18)	x	0	1.25 (±0.26)	2.14 (±0.12)	2.48 (±0.08)

[a]Pots were maintained continuously at 98% RH and served as controls. Data presented to show production of sporangiophores and sporangia at different time corresponding to observations taken in other treatment.

The number of sporangiophores increased when plants were exposed to 98% RH following incubation at 70-95% RH for 10, 30 and 60 min with increase in number of low RH exposures. Considering all treatments at 70-95% RH, increase in the sporangiophores number were in the range of 4.25-10.24 considerating all duration tested. At the time of 1st dry period the number of sporangiophores produced in 60 min duration at 70-95% RH exposure was in the range of 24.73-28.2 and in the 30 min and 10 min duration of low RH exposures they were in the range of 25.84 to 28.8 and 24.23-30.23 respectively.

Sporulation was not observed in any of the 70-95% RH exposures after 1st and 2nd dry periods in 60 min duration. Only in control (98% RH) sporulation was observed at regular intervals (same time at which 1st and 2nd dry period completed in 70-95% RH exposures). Sporulation after 3 h of final dry period was in the range of 1.28-1.58 x 10^5 spores cm^{-2} leaf area in 70-95% RH exposures treatments in 60 min duration, where as at the same duration of incubation time the sporulation in control was 2.42 x 10^5 spores cm^{-2} leaf area. Similarly at 6 h after final dry period the sporulation in 70-95% RH exposure treatments was in the range of 1.98-2.47, and in control it was 3.76 x 10^5 spores cm^{-2} leaf area.

In the 30 min duration, sporulation was observed at 95% RH exposure treatment after 1st (0.6 x 10^5 spores cm^{-2} leaf area) and 2nd (1.98 x 10^5 spores cm^{-2} leaf area) dry periods where as no sporulation was observed after 1st and 2nd dry periods in other low RH exposure treatments.

Similarly the sporulation was also observed at 95% RH in 10 min duration after 2nd dry period (1.19 x 10^5 spores cm^{-2} leaf area) where as in treatments other than control no sporulation occurred during dry periods.

DISCUSSION

CHAPTER V

DISCUSSION

Asexual reproduction producing short lived sporangia occur outside the host plant and is therefore governed by the external environment. The effects of various environmental factors on sporulation of the fungus (*Sclerospora graminicola*) was studied in the past by several workers (Safeeulla and Thirumalachar, 1956; Nene and Singh, 1976; Shetty and Ahmed 1981; Singh et al. 1987). Weston (1924) reported asexual sporulation in *S. graminicola* to be nocturnal. Safeeulla and Thirumalachar (1956) concluded that nocturnal production of sporangia is a result of coincidence of factors and there was no relationship with the life cycle of the fungi. They could obtain sporulation at will by collecting leaves at certain day light hours after some hours of bright sunlight and then holding them at 100% RH in darkness. The experiments in this study were also conducted during the day time.

In the present study, the seedlings were inoculated at 2 leaf stage (5-6 day old seedlings). They were incubated in a separate dew chamber at 100% RH and 20°C. This was congenial for infection. This is in agreement with Subramanya et al. (1981) who described that the main stalk and the tillers are susceptible to the downy mildew infection in their vegetative phase and the degree of susceptibility reduces gradually as the main stalk or the tiller ages, indicating the possible build up of natural resistance. Similar observations were made by Leu and Chu (1959) who recorded 100% infection when one week old seedlings of maize were inoculated with *Sclerospora sacchari*, but no infection was observed on four week old plants.

Seedlings used in the present study were freshly infected and had not sporulated earlier. After expression of first symptoms (chlorosis) and before the occurrence of sporulation they were maintained at a low (~60%) RH in the growth cabinets (Plate 2). This enabled us to get accurate results in the study. Only four uniformly infected plants of same size were selected in each pot to enable proper handling of plants during experiment.

Infected seedlings used for the experiment was 25 days old and were of same size (approx. one foot in height) and also having uniformly infected leaves. The seedlings below this age were very tender and difficult to handle in single plant chambers. The seedlings above this age were more than one foot in height and started tillering which was not required for the experiment.

5.1. Effect of temperature and incubation time on the sporangiophore formation

The sporangiophores were formed at all the tested temperatures (10, 14, 18, 20, 22, 25 and 30°C) except at 35°C (Fig. 1). It was observed that maximum temperature at which sporangiophores (productive) formed was 30°C. It was also observed that as the temperature and incubation time increased the formation of productive sporangiophores was increased from 10 to 22°C. The maximum number (36.73) of sporangiophores formed at 22°C after 8 h of incubation time. The formation of productive sporangiophores started only after 2 h of incubation time but due to their continuous production, maximum number was observed at 8 h of incubation time. Sporangiophore formation was delayed (6-8h) at lower temperatures and at 24 h of incubation time

a decreased number of productive sporangiophores was observed. Safeeulla and Thirumalachar (1956) reported that at 15°C and below, the sporangial formation was delayed extending up to 24 to 36 hrs. This may be due delayed production of sporangiophores. The decrease in sporangiophore production at 24 h of incubation time may be due to exhaustion of energy in leaves as they were exposed continuously to dark period.

The extent of sporulation is partly governed by the number of productive sporangiophores. Hence the knowledge on distribution of productive sporangiophores is essential in epidemiological studies.

In a particular infected leaf area not all the stomata give rise to sporangiophores. Some stomata contain only sporangiophore initials and some contain ill-formed sporangiophores which are not productive. The present study considered formation of differentiated sporangiophores (NSP) out of stomata containing sporangiophore promordia (SF). It was recorded as the ratio of the (Number of productive sporangiophores per stomata containing sporangiophore primordia or NSF/SF). NSF/SF showed an increase with increased temperature and also with increased incubation time. The maximum ratio of NSP/SF was 1.79 at 22°C after 3 h of incubation time and maximum 2.20 at 25°C after 8 h of incubation time (Table 2). Earlier reports are not available on similar studies.

In the past, the size of the asexual propagule and its morphology was considered as one of the criteria for classification of pathogen. However, it is observed that the propagule size is greatly influenced by the environmental factors.

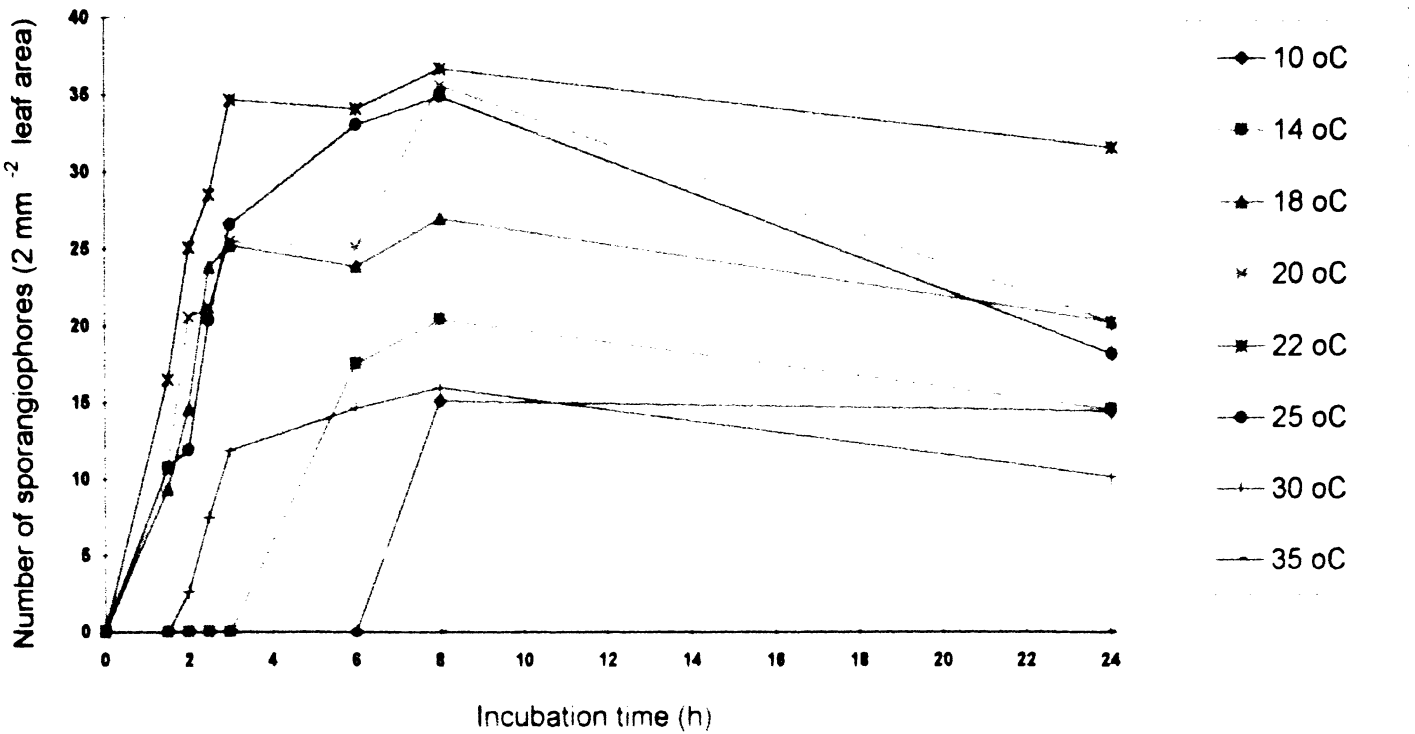

Fig 1: Effect of temperature on sporangiophore production by ***Sclerospora graminicola*** at different time intervals

A wide variation was observed in the sporangiophore size, particularly in length, at different temperatures (178.22 µm at 10°C to 250.58 µm at 30°C) after 8 h of incubation time (Fig. 2). These results are different from results obtained by Singh et al. (1987) who observed decreased sporangiophore length with increased temperature. But these results are in agreement with the observations of Safeeulla and Thirumalachar (1956) who reported that at 10°C, sporangiophores were stout and dwarf with complete suppression of branches and at 15°C the branches were shortened and all the sporangia appeared to be clustered at the top. They observed elongation and proliferation of sporangiophore under excessive moisture conditions.

The present study shows that dew along with high temperature may have been responsible for excessive elongation of sporangiophore at 30°C. The mean diameter of sporangiophore decreased at 30°C (12.71 µm) compared to 22°C (18.02 µm) (Table 5 and Fig. 3). This indicates that the elongation of sporangiophores suppressed the diameter.

5.2.Effect of temperature and incubation time on sporulation

Sporulation was observed at a temperature range of 10 to 30°C. At 35°C we observed no sporulation due to lack of growth of sporangiophores from its primordials. Similar results were obtained by Singh et al. (1987). Safeeulla (1976) reported that sporulation occur between 14°C and 30°C. However, Suryanarayana reported that maximum temperature at which sporulation occur was 28°C. In the present study sporulation started after 2.5 h of incubation time at a temperature range of 18°C (0.54×10^5 spores/cm^2) to 30°C (0.13×10^5 spores/cm^2) (Fig. 4). An increased sporulation

was observed with increased incubation time from 2.5 h to 8 h. This is in agreement with Subramanya et al. (1981) who reported that with increase in exposure duration, the number of sporangia produced per unit area also increased. As has been explained by him, photosynthates produced during the period of light exposure could be utilized for actual phase of sporulation which needs high nutrition for the process of wall and protoplast synthesis. When the means were compared maximum sporulation (1.53×10^5 spores/cm^2) was observed at 22°C, but sporulation was less at other temperatures. These results are in agreement with Shetty (1987) who reported maximum sporulation at 23°C and 100% RH, and at temperature lower than 23°C the time required for sporangia production was longer than at 23°C. But he reported that at temperature <12°C no sporulation was observed. These results are in agreement with Suryanarayana (1965) and Singh et al. (1987) who reported that temperature as low as 10 C was favourable for sporulation. The production of sporangia in 2.5 h was also in agreement with the work of Shetty (1987) who reported that inductive period i.e. initiation of sporangiophore in the substomatal region was almost same at all the RH profiles, but at 100% RH the formative phase i.e. process of sporulation was as short as 2 h 25 min. Our studies on temperature were made at 100% RH.

5.3. Effect of RH and incubation time on sporangiophore formation

Under field conditions the processes from sporulation to penetration occurs between midnight and sunrise (Singh and Gopinath, 1990). Temperatures during this period generally are less than 30°C during the rainy season and are not a limiting factor for any of the processes. The RH on the other hand may be a limiting factor. Butler et al. (1995) have constructed single plant chambers that help to maintain a predetermined humidity (up to 99% RH) level accurately, (± 0.2%

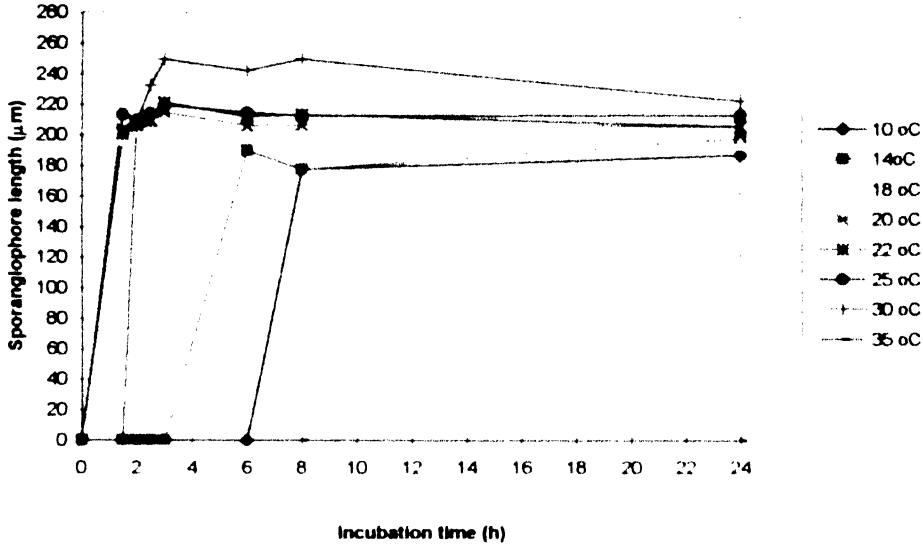

Fig 2: Effect of temperature and incubation time on sporanglophore length of *sclerospora graminicola*

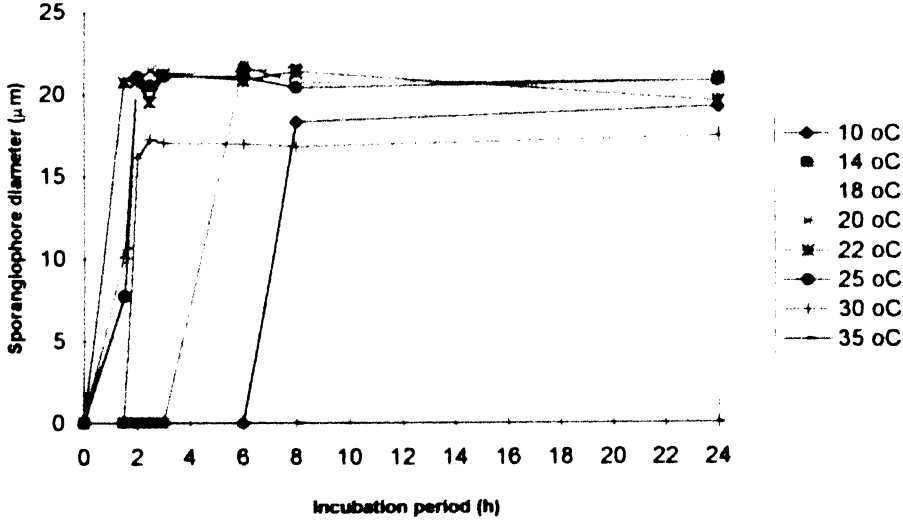

Fig 3: Effect of temperature and incubation time on sporanglophore diameter of *Sclerospora graminicola*

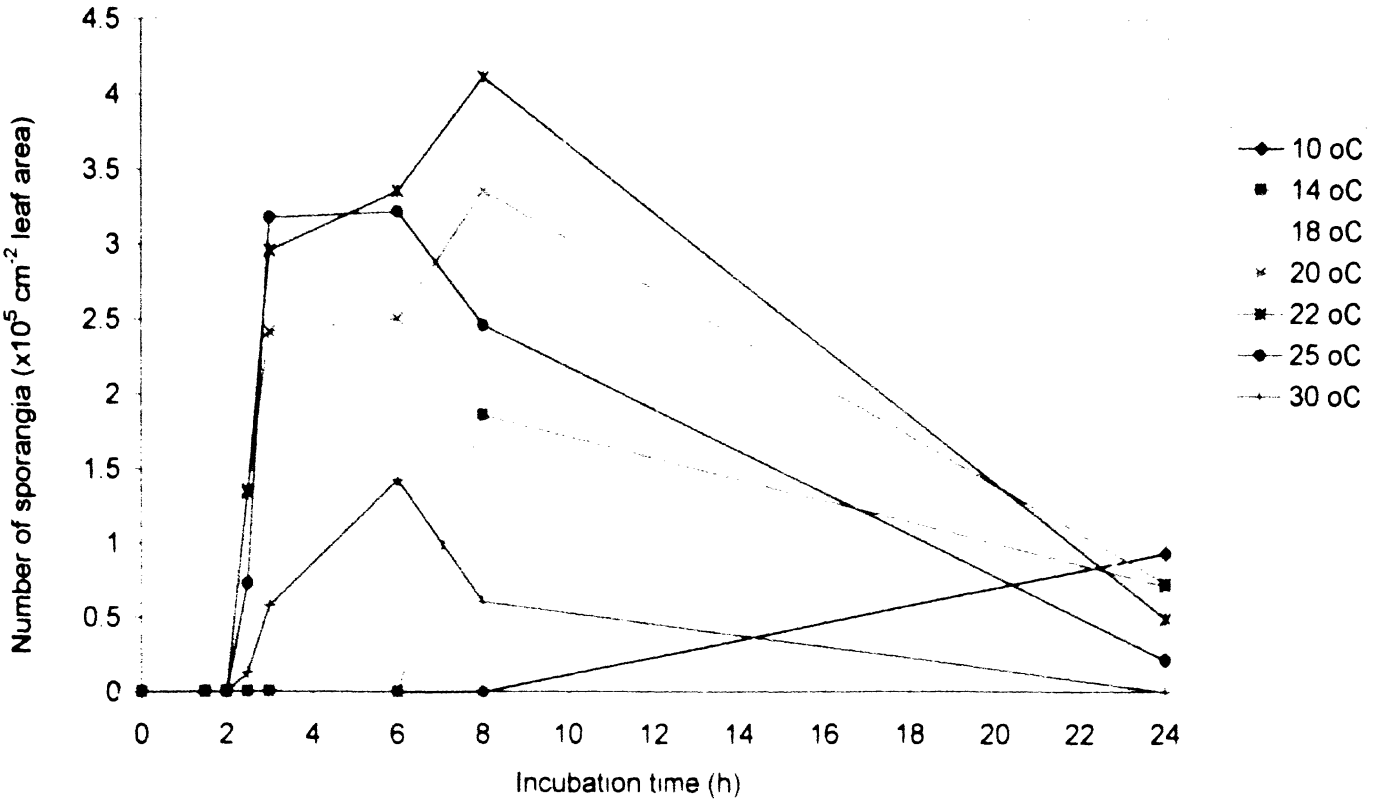

Fig 4: Effect of temperature and incubation time on sporangial production by ***Sclerospora graminicola*** causing pearl millet downy mildew

RH).

Sporangiophores formed at 95% RH were not productive and only negligible (0.92) number of sporangiophores were productive at 96% RH after 24 h of incubation time. At 97% RH productive sporangiophores started to form at 3 h (3.95) and maximum number (15.56) was observed at 24 h of incubation time (Fig. 5). Good number of sporangiophores occurred at 98% (17.73) and 100% RH (21.58) respectively. Earlier reports are not available on this aspect.

The ratio of NSP/SF was increased with increase in RH from 97% to 100%. At 97% the ratio was 0.22 and at 100% the ratio was 1.48 after 3 h of incubation (Table 7). The ratio also increased with increased incubation time from 3 h to 8 h. The increase might be due to continuous production of sporangiophores for several hours, and also due to utilisation of high nutrition, produced during the period of light exposed (Subramanya et al., 1981).

5.4. Effect of RH on sporangiophore size

The mean increase in sporangiophores length was observed from 96% RH (21.03 µm) to 100% RH (185.75 µm) (Table 9 and Fig. 6). The apparent increase in the mean length was due to the delayed growth (24 h) of sporangiophore at 96% and fast growth (1.5 h) at 100%. The mean increase in diameter of sporangiophore was also observed from 96% RH (1.34 µm) to 100% RH (19.23 µm) (Table 10 and Fig. 7). There were no reports on this aspect. The sporangiophore length was observed in the range of 168.26 µm to 213.40 µm. We observed no abnormal increase of sporangiophore size as observed in temperature experiment at 30°c.

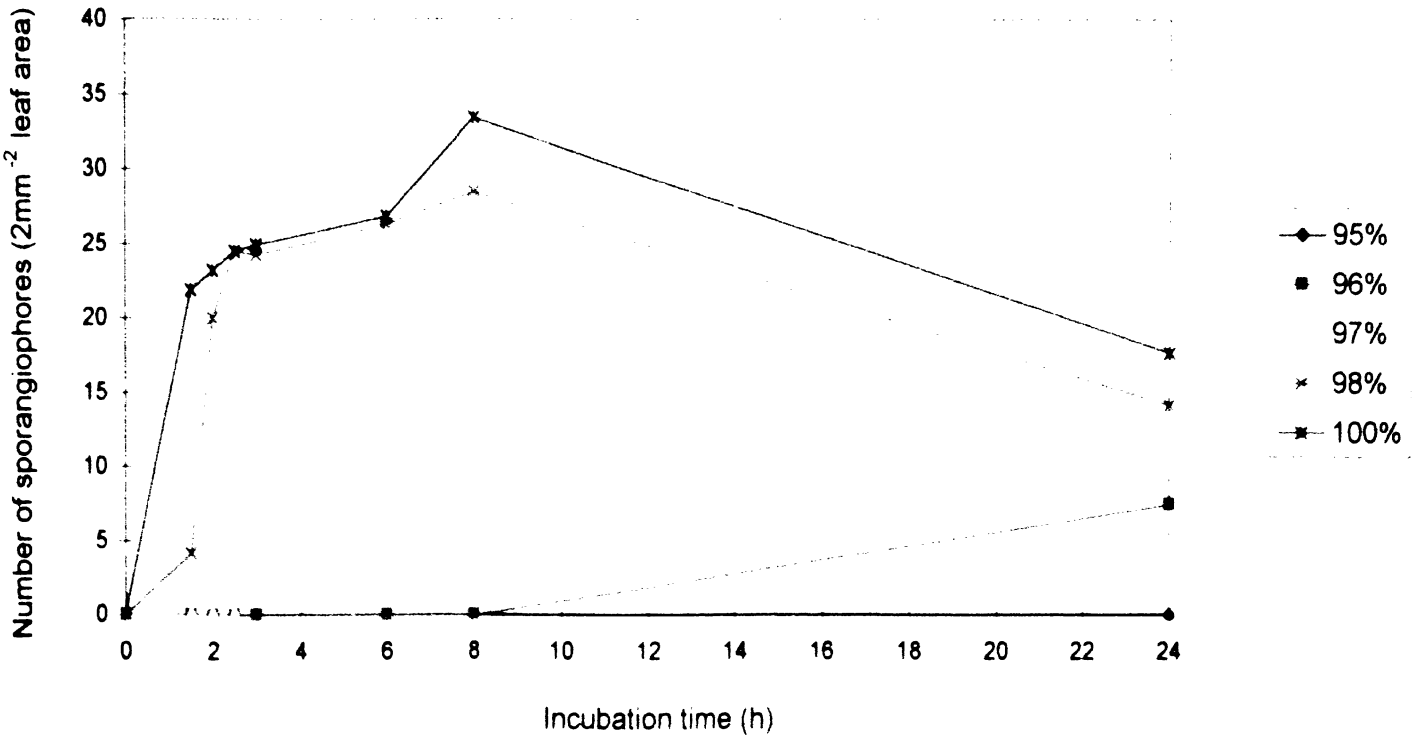

Fig 5: Effect of RH and incubation time on sporangiophore production at different time intervals by ***Sclerospora graminicola***

5.5. Effect of RH on sporulation processes

Sporulation was observed only from 97% RH onwards (Table 11). At 96% RH only small (0.03 x 10^5 spores cm^{-2} leaf area) amount of sporangia was observed after 24 h of incubation time compared to 100% RH where 3.26 x 10^5 spores/cm^2 leaf area was observed after 8 h of incubation time. Thus, sporulation at 96% was more than 100 times less than at 100% RH. A decrease in sporulation was observed at RH of 98% and 100% after 24 h of incubation time compared to 8 h of incubation (Fig. 8). This may be due to longer exposure to continuous darkness and decrease in production of sporangiophores.

Maximum sporulation was observed in these studies was in the range of 3.26 x 10^5 spores cm^{-2} (100% RH at 8 h of incubation) to 4.12 x 10^5 spores cm^{-2} (22°C at 8 h incubation). These results are in contrast with results of Safeeulla (1976) where he reported that under favourable conditions, 35000 sporangia cm^{-2} were produced in infected pearl millet crop. This difference may be due to more (12 h of artificial light) exposure of plants to preincubation light which gives more nutrition to plants. Singh et al. (1987) reported that number of sporangia produced were 6.5x10^5, 6.4x10^5 and 6.0x10^5 sporangia cm^{-2} leaf area in florescence light, NUV light and darkness respectively. Further, Singh et al. (1993) reported that under optimum conditions of temperature and RH, approximately 1.5 x 10^5 sporangia cm^{-2} can be produced during one night. The quantum of sporulation probably depends on the nutrition availability which is the result of number of hours of light exposure in preincubation period.

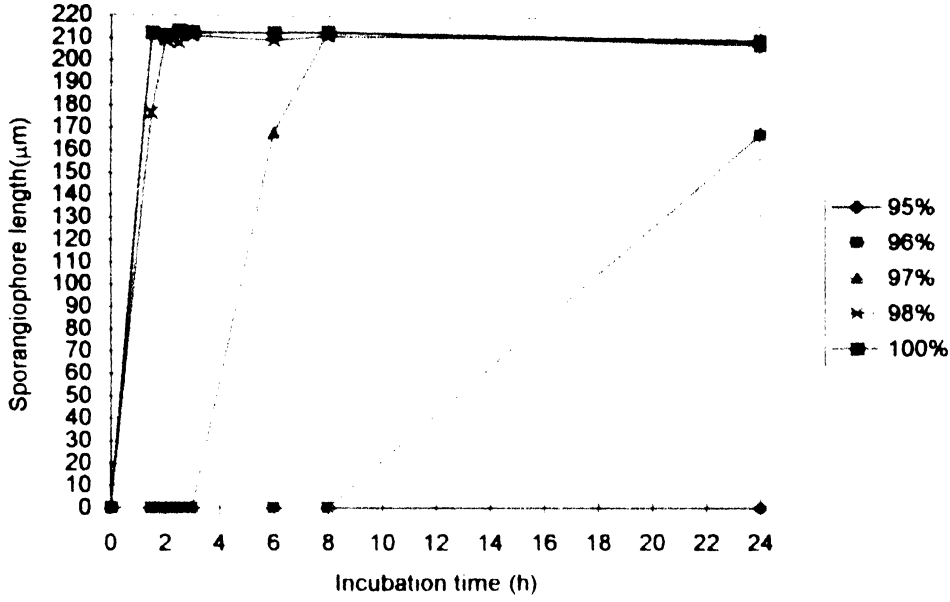

Fig 6 Effect of RH and incubation time on the sporangiophore length by ***Sclerospora graminicola*** causing pearl millet downy mildew

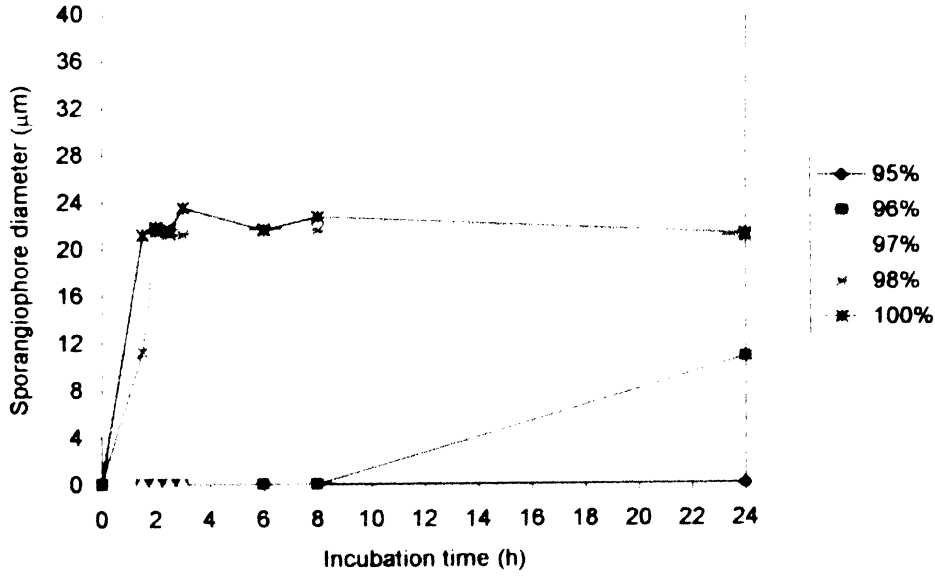

Fig 7 : Effect of RH and incubation time on sporangiophore diameter by ***Sclerospora graminicola*** causing pearl millet downy mildew

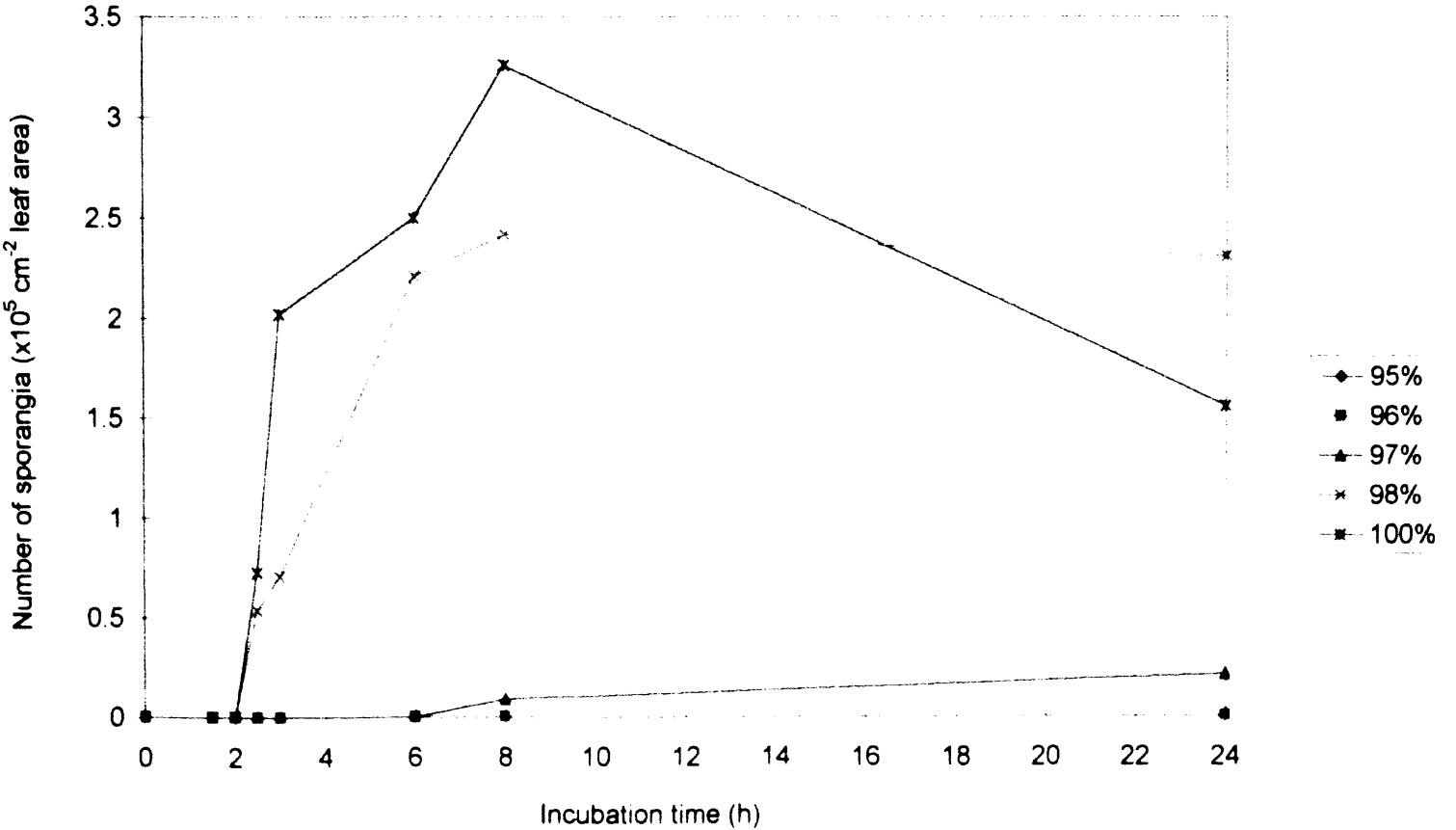

Fig 8 : Effect of RH and incubation time on the sporangial production by ***Sclerospora graminicola***

5.6. Effect of RH and incubation time on sporangium size

Sporangium size in these studies was in the range of 14.26-22.13 x 12.40-19.86 µm (Fig. 9 and Fig. 10). They are in accordance with Singh (1995) who reported sporangium size range as 15-22 x 12-21 µm. Kenneth (1966) reported the sporangium size as 19-31.6 x 15.8-23.7 µm.

5.7. Effect of humidity stress on longevity of sporangiophore

In the present study sporangiophores were viable up to 30 min and RH $\geq$ 95%. These studies on the fluctuations in RH showed that the sporangiophores lost their ability to produce sporangia, when they were exposed to 70-90% RH even for 30 min. A continuous period of high RH (>95%) is essential for formation of sporangiophores and for production of sporangia. Singh et al (1987) and Safeeulla (1976) reported that after sporulation, sporangiophores collapsed leaving a bed of sporangia on the leaf surface and formation of new sporangiophores due to continuous process of sporulation (Fig. 11). However, they have not reported the fate of earlier formed sporangiophores after exposure to RH fluctuation. Significant changes in weather parameters can occur in the short-term due to several factors under field conditions. Fluctuations in RH during the 1.5 to 2 h period after the production of sporangiophores can have profound influence on sporangial production in *S. graminicola*. From the results of our studies it can be concluded the sporangiophores are very sensitive and earlier formed sporangiophores desiccate and die even with a slight decrease of RH $\leq$90% for 30 min. Only new crop of sporangiophores form and sporulate on revival of favourable RH conditions.

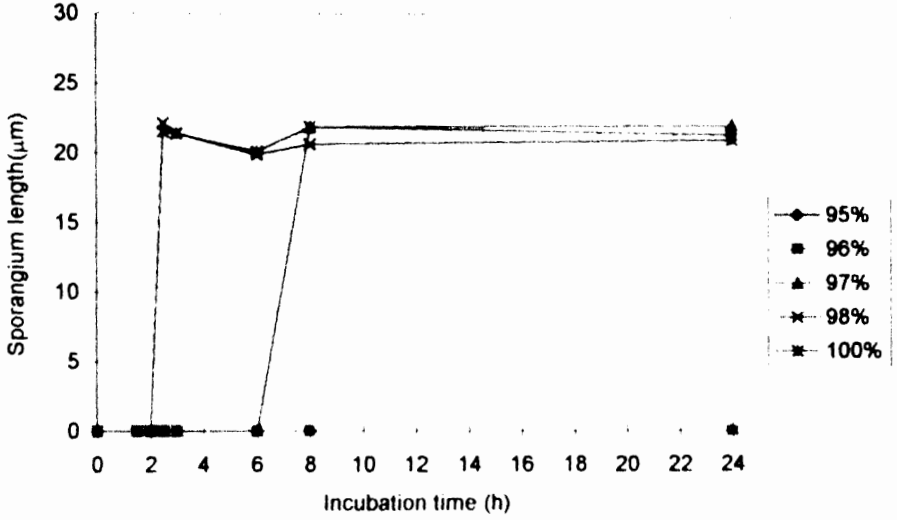

Fig 9: Effect of RH and incubation time on sporangium length by ***Sclerospora graminicola***

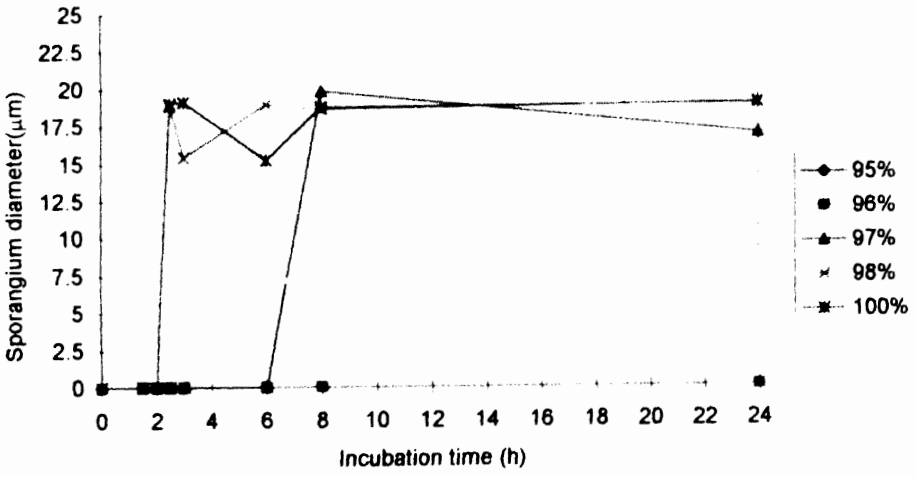

Fig 10 : Effect of RH and incubation time and sporangium diameter by ***Sclerospora graminicola***

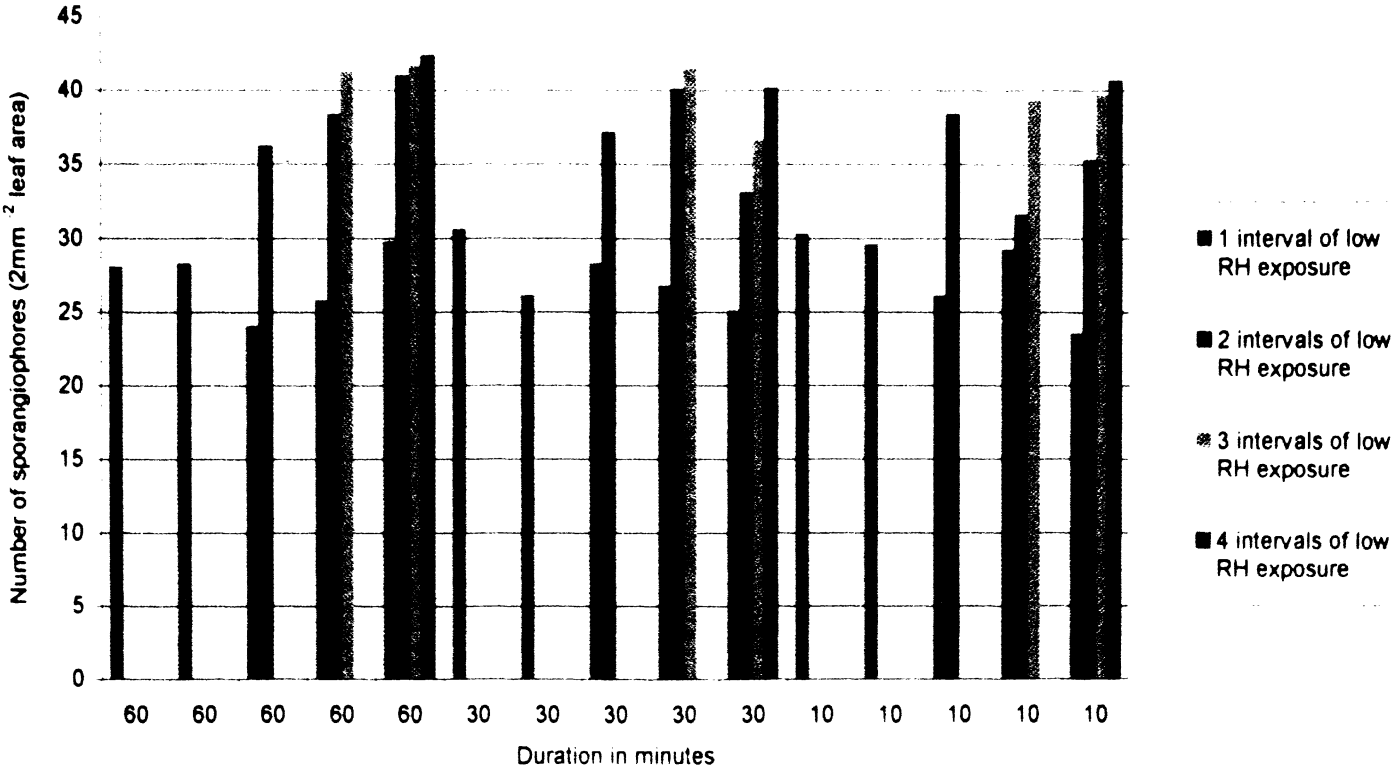

Fig 11: Effect of high (100% RH) and low (60% RH) humidity regimes on sporangiophore production by *Sclerospora graminicola*. Number of sporangiophores observed at the end of every high RH exposure

SUMMARY AND CONCLUSIONS

CHAPTER VI

SUMMARY AND CONCLUSIONS

The green ear disease caused by *Sclerospora graminicola* is one of the important and widespread disease of pearl millet in India and is a limiting factor to bajra production. The leaves infected by this disease produce a large number of sporangiophores and sporangia under favourable moisture and temperature conditions. Though much work has been done in this field, there exists important gaps in knowledge which should be filled to understand the epidemiology of the disease.

Some aspects such as the effect of temperature, relative humidity and incubation time on the sporangiophore distribution per stomata, and per unit leaf area, sporangiophore dimensions, sporulation process and the longevity of sporangiophore under humidity stress were covered in this study.

In the time course study of sporangiophore growth and sporulation it was observed that sporangiophore started branching after 1.5 h of incubation and sporangia initials formed after 2 h 15 min of incubation. Fully developed sporangia were seen after 2 h and 30 min of incubation. An increase in sporangia number was observed with increase in incubation time.

In the studies on the effect of temperature and incubation time on the distribution of sporangiohores per stomata, it was observed that only 1-2 sporangiophores were productive out of 5-6 sporangiophore primordials at a given point of incubation time. Their number increased with temperature (10-22°C) and incubation time (0-8h).

In the studies on the effect of temperature and incubation time on sporangiophore number in an unit leaf area, it was observed that not all the stomata contain sporangiophore primordia, and not all the stomata containing sporangiophore primordia give rise to productive sporangiophores. Sporangiophore number increased with an increase in temperature from 10 to 22°C, and at 25 and 30°C sporangiophore number was less. At 35°C sporangiophores were not observed. The number of sporangiophores also increased with an increase in the incubation time up to 8 h. At 24 h, a decrease in their number was observed.

An increase in the length of sporangiophore was observed with increase in temperature from 10 to 30°C. An abnormal increase in length at 30°C was observed. Sporangiophore diameter was not much affected by temperature.

Sporangia production started after 2.5 h of incubation at temperature of 18-25°C, where as at other temperatures delayed production of sporangia was observed.

In the studies on the effect of RH and incubation time on the sporangiophore distribution per stomata, it was observed that at 95% RH though the leaves contained stomata, they did not produce productive sporangiophores in spite of containing sporangiophore primordials. Productive sporangiophores were formed only after 1.5 h of incubation at 98 and 100% RH. Sporangiophore number increased with incubation time at 98 and 100% RH. At 96 and 97% RH, delayed (6-8h) production of productive sporangiophores was observed. Sporangiophore size was not affected by relative humidity.

Sporangia production started after 2.5 h incubation at 98 and 100% RH. However, sporangia were seen only after 8 h of incubation at 97% RH. The quantum of sporulation increased with RH and incubation time.

Sporangia size was not affected by RH.

In the studies on the viability of sporangiophore under variable RH regimes, it was observed that after full development the sporangiophores were viable for at least 30 min at RH $\geq$ 95%. So it can be concluded that if the RH falls to 90% or below even for 10 min after production of sporangiophores, the sporangiophores will collapse and lose the ability to produce sporangia.

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***Originals not seen.**