Northern Leaf Blight of Maize in New Zealand: Relationship of Drechslera turcia Airspora to Factors Influencing Sporulation, Conidium Development, and Chlamydospore Formation

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ABSTRACT

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Airborne conidia of *D. turcica* trapped above a diseased maize field were most abundant during days after warm nights with long continuous periods (10-12 hr) of high relative humidity (RH) (>90% approx.). Laboratory experiments confirmed that high atmospheric humidity is essential for sporulation. Conidiophores and conidia developed abundantly on young leaf lesions at RH's above 92% and 94%, respectively. On newly formed conidiophores, slightly higher humidities were required for conidium formation than on old conidiophores. Free moisture on leaf lesions was not necessary for sporulation. Conidiophores were able to produce more than one batch of conidia as favorable conditions recurred. Temperature influenced the rate of development of conidia and also their morphology and septation. The lower limit of sporulation was approximately 9 C, the upper limit 30 C, and the optimum range was about 20 to 26 C. At optimum temperatures, conidium development (but not that of conidiophores) was inhibited by light. Exposure of conidiophores to at least 4 hr of continuous darkness (20 C) was required for conidium development. Under normal summer conditions near Pukekohe, New Zealand, *D. turcica* sporulated only on nights when atmospheric humidity was high for a continuous and extended period (approximately 10-12 hr) and then only if temperature was not limiting. Sporulation was lacking during the day because humidities generally were too low and because light inhibited conidium development. Under natural conditions, successive nights with relatively low temperatures (approximately 10 C) appeared to induce the formation of chlamydospores within conidia.

Additional key words: *Helminthosporium*, spore trapping.

In our New Zealand studies (9) on northern leaf blight of maize [which is caused by *Drechslera turcica* (Pass.) Subram. and Jain (= *Helminthosporium turcicum* Pass.)] we examined the effect of certain climatic factors on sporulation and spore liberation. We found (9) that, under natural conditions, spores were released into the atmosphere from leaf lesions by wind, rain, and by forcible discharge initiated by changes of relative humidity (RH) as well as by exposure to red-infrared (IR) radiation (7). The forcible discharge has been termed diurnal release and it may involve electrostatic forces (8). In this article, we describe the relationships of relative humidity, temperature, and light to sporulation under natural and laboratory conditions. Studies on lesion development and the influence of low temperatures on chlamydospore formation within conidia also are briefly reported.

MATERIALS AND METHODS

Monitoring of airborne spores.—Airspora over a diseased maize crop at Pukekohe, N. Z. was monitored continuously for 6.5 mo using a Hirst spore trap. Wind, rain, RH, leaf wetness, and air temperature were recorded continuously over the same period. Details of the plot and instrumentation were presented elsewhere (9).

Sporulation under natural conditions.—Periodic microscopic observations of leaf lesions were made during the spore-trapping study. A minimum of 20 lesions were collected twice weekly between 0900 and 1000 hr, placed in unsealed plastic bags, and examined microscopically, usually within 2-4 hr after collection. The purpose of these collections was to determine the first seasonal occurrence of sporulation as well as to study the production and distribution of conidia on old and young lesions.

To ascertain whether "old" conidiophores would develop new batches of spores, large necrotic lesions well covered with conidiophores, but lacking conidia, were collected during dry periods, incubated in a humidity chamber in darkness at 21 C for 24 hr and then examined microscopically for the presence of spores.

Sporulation under controlled conditions.—Laboratory experiments were conducted to define the relationships of RH, leaf wetness, temperature, and light to sporulation.

The effects of light and temperature, and their

interaction, on sporulation were determined for both in vitro cultures and naturally infected young leaf lesions incubated on temperature-gradient plates (5). Temperature gradient plates (8 C to 36 C; 110 × 30 cm) were monitored at 10-cm intervals using thermocouples and a multichannel recording potentiometer. One temperature-gradient plate was kept in darkness, the other was exposed either to continuous light (two 40 W "cool-white" daylight fluorescent lamps and two 40 W NUV-emitting BLB "Black Light" fluorescent lamps), or to an alternating 12-hr cycle of light and darkness using the same arrangement of lamps. Replicated (three reps) cultures were grown on 2% malt extract agar (15 ml MA per 60 × 15 mm plastic petri dish; pH 5.0) for 11 days under the three light regimes, then measured for radial growth, examined microscopically, and rated for sporulation (sporulation index: 0 = no sporulation; 1 = sparse; 2 = moderate; 3 = abundant; 4 = profuse). Replicated (eight reps) 15-mm diameter disks cut from young nonsporulating leaf lesions were treated in a similar manner. Lesion disks were placed in moist chambers (60 × 15-mm pyrex petri dish containing one moist Whatman No. 1 filter paper) and incubated for 48 hr. Although the same combination of lamps was used for colony and lesion studies, the lamps were suspended 30 cm above the cultures and 60 cm above the lesions.

The relationship of RH to conidiophore and conidium formation was determined by placing 15-mm diameter disks cut from young nonsporulating leaf lesions, into humidity chambers (600-ml screw-cap preserving jars). Eight disks taken from different lesions were placed on perforated plastic platforms within each chamber and incubated at various humidities in darkness for 72 hr at a constant temperature of 20 C. At the end of this period, sporulation (conidiophores and conidia) was rated using the same sporulation index 0-4. Glycerine and water solutions (13) were used in the first series of experiments to provide relative humidities from 60-100% (60, 70, 80, 90, 100%). This was followed by a more precise range of humidities using sulphuric acid and water solutions (1) ranging from 86-100% RH (86, 88, 90, 92, 94, 96, 98, 100%). Similar experiments also were conducted on old leaf lesions (i.e., those already covered with conidiophores) to determine whether the humidity requirements for conidium formation on old and young conidiophores were different.

Conidium development.—Young leaf lesions (15-mm diameter disks) were placed in petri dish moist chambers at 22 C where they were exposed continuously to NUV-blue light (one 40 W BLB type "Black Light" fluorescent lamp at 40 cm). This treatment inhibited conidium development and synchronized spore formation when the specimens were transferred to a 20 C incubator in darkness. The rate of conidium development was followed by microscopic examination of the specimens at 1-hr intervals. Stages of development were drawn and photographed.
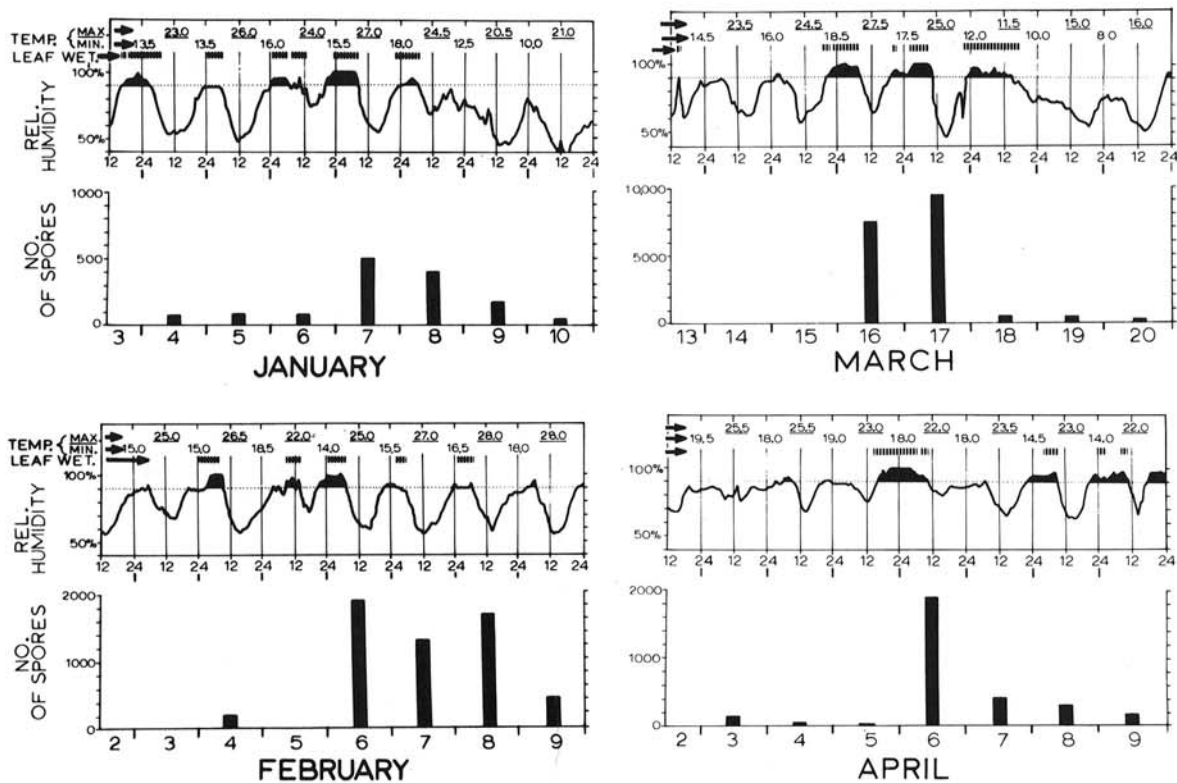
The effect of different temperatures on development

Fig. 1. Relationship of number of airborne spores of *Drechslera turcica* trapped above a diseased maize field during the day, to meteorological conditions [relative humidity (RH), leaf wetness, and air temperature] during the preceding 24 hr (RH 90% is shaded black).

and morphology was studied by synchronizing sporulation in the above manner and incubating disks in darkness at 5, 10, 15, and 20 C.

To determine at what stage of conidium development light was inhibitory, conidium formation was synchronized by exposure to light and the specimens were transferred from light to incubators at 5, 10, 15, and 20 C (dark). At 1-hr intervals, replicated specimens were removed from the incubators and returned to the NUV-blue light chamber at 22 C. Conidium development was followed microscopically at 1-hr intervals over the next 24 hr.

Chlamydospore induction.—Near the end of the growing season, following a succession of cool nights, we observed that many airborne conidia contained chlamydospores (9). To determine whether low temperature induced chlamydospore formation, leaf disks (15 mm in diameter) cut from lesions were placed in humidity chambers, and conidium formation was synchronized at 22 C under NUV-blue lamps. Half the specimens were transferred immediately to incubators at 5, 10, 15, and 20 C (dark) before conidium development had begun; the others were placed at 20 C in darkness for 48 hr to allow normal conidium development, and then were transferred to 5, 10, 15, and 20 C. Periodically (12, 24, 48, 72, and 106 hr) specimens were removed from the incubators and examined microscopically for the presence of chlamydospores.

Lesion development.—An ancillary study was made to determine the length of time necessary for lesions to develop under natural conditions. During mid-January, leaf axils of 20 young maize plants were inoculated with spore suspensions obtained from heavily sporulating colonies grown on an agar substrate for 10-14 days. Forty eight hr after inoculation, distinct bands of small chlorotic spots were evident at the bases of leaf laminae. Selected spots were marked with a "felt-point" pen, and these were examined twice weekly over the next 24 days for development of typical northern leaf blight lesions.

RESULTS

Sporulation under natural conditions as reflected by airspora.—Figure 1 shows the incidence of airborne conidia and pertinent weather records for selected periods of January, February, March, and April of 1974. More detailed records of the daily incidence of conidia for this same period already have been reported (9). Continuous recordings were made of air temperature, leaf wetness periods, amounts of rain, and relative humidity, but for the sake of brevity, these records have been greatly condensed (Fig. 1).

The occurrence of spores on leaf lesions under natural conditions together with spore-trapping data suggested that sporulation occurred only at night, was favored by long and continuous periods of high atmospheric humidity (RH > 90% approx.), and was favored by warm nights (Fig. 1). "Free" water (dew and rain) was usually present during nights that favored abundant sporulation. Typical examples of nights favorable for sporulation are shown in Fig. 1 (e.g., the nights preceding 7 and 8 January; 6, 7, and 8 February; 16 and 17 March; and 6 April). All of these nights had at least 7 hr of RH > 90%, most involved considerable longer periods (e.g., 12 hr the

night preceding 7 January; 11 hr 6 February; 15 hr 16 March; and 18.5 hr 6 April). On several nights, RH appeared to favor sporulation, but incidence of trapped conidia the next day was low; e.g., the nights preceding 4 January, 18 March, and 9 April. On these nights temperatures were possibly limiting (13 C minimum on the night preceding 3 January; 12 C on 18 March; and 14 C on 9 April). On nights when the RH significantly fluctuated above and below 90%; e.g., the night preceding 6 January and during the day of 5 February, numbers of trapped spores were low, suggesting that spore development might require continuous periods of high humidity.

During dry periods, young green lesions (2-10 cm long) usually lacked conidiophores or conidia, whereas older necrotic lesions (10-30 cm long) always were covered densely with conidiophores. When both kinds of lesions were placed in humidity chambers in darkness for 12 hr at 22 C, abundant conidia were formed. In young lesions, conidia developed evenly over the entire lesion except for freshly invaded peripheral tissue. In older lesions, sporulation generally was more dense in the older parts of the lesions; however, in a few instances conidiophores at the centers of the lesions failed to produce conidia, suggesting that they were no longer functional. Conidiophores produced several crops of spores as RH fluctuated between favorable and unfavorable over several days. This phenomenon is similar to the "re-sporulation" reported for *D. maydis* (12).

Influence of relative humidity on sporulation.—Results of our field studies suggested that the principal environmental factors affecting sporulation were atmospheric humidity, free water, temperature, and (possibly) light. In the first series of laboratory experiments in which young, green, nonsporulating leaf lesions were placed in humidities of 60, 70, 80, 90, and 100% RH for 72 hr at 20 C, sporulation occurred only at 100% RH. In subsequent experiments to test a narrower range of humidities (86, 88, 90, 92, 94, 96, 98, and 100% RH), maximum conidiophore development occurred between 94 and 100% RH, but a few formed at 88% (Fig. 2-A). Conidium development required a slightly higher humidity (94-100% RH). On lesions already bearing
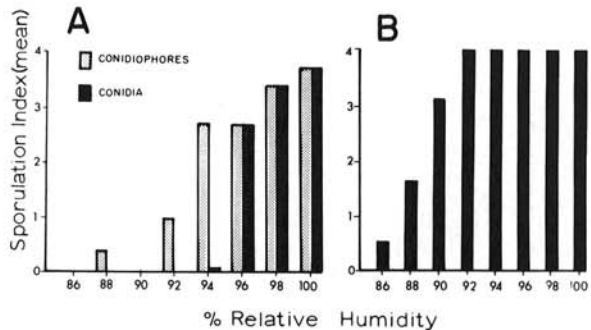

Fig. 2-(A,B). Influence of atmospheric humidity on sporulation of *Drechslera turcica*. **A**) Conidiophore and conidium formation on young leaf lesions (i.e., nonsporulating) after 72 hr in humidity chamber at 20 C. **B**) Conidium formation on old leaf lesions (i.e., conidiophores already present) after 24 hr in humidity chamber at 20 C. (Sporulation index: 0 = no sporulation, 4 = profuse sporulation).

conidiophores when placed in the humidity chambers, conidia formed over a wider range of humidities (86-100%). Free water was not present in these experiments, indicating that sporulation can occur in the absence of free water.

Interaction of light and temperature on growth and sporulation.—The range of temperature for optimum radial growth of *D. turcica* on MA was between 20 C and 30 C (Fig. 3). The lower limit was 8-10 C and the upper limit was 34-35 C. Radial growth was not influenced significantly by the three different dark and light regimes that were tested (Fig. 3).

Sporulation occurred in darkness at temperatures from 9 to 27 C on 2% MA, and from 9 to 30 C on leaf lesions (Fig. 3). The optimum range for sporulation in darkness was approximately 20 to 26 C. Conidia were unable to develop on mature conidiophores held in darkness at 5 C but they did develop at 10 C (Fig. 5). Spores formed at 10 C on mature conidiophores, required almost twice as long to develop to maturity as those grown at 20 C (Fig. 5). Incubation temperature also had a profound effect on the morphology and septation of conidia. Spores developed at 20 C were mostly slightly curved and had six to seven septa in contrast to those produced at lower temperatures which had fewer septa, were shorter, and mainly straight (Fig. 5-6).
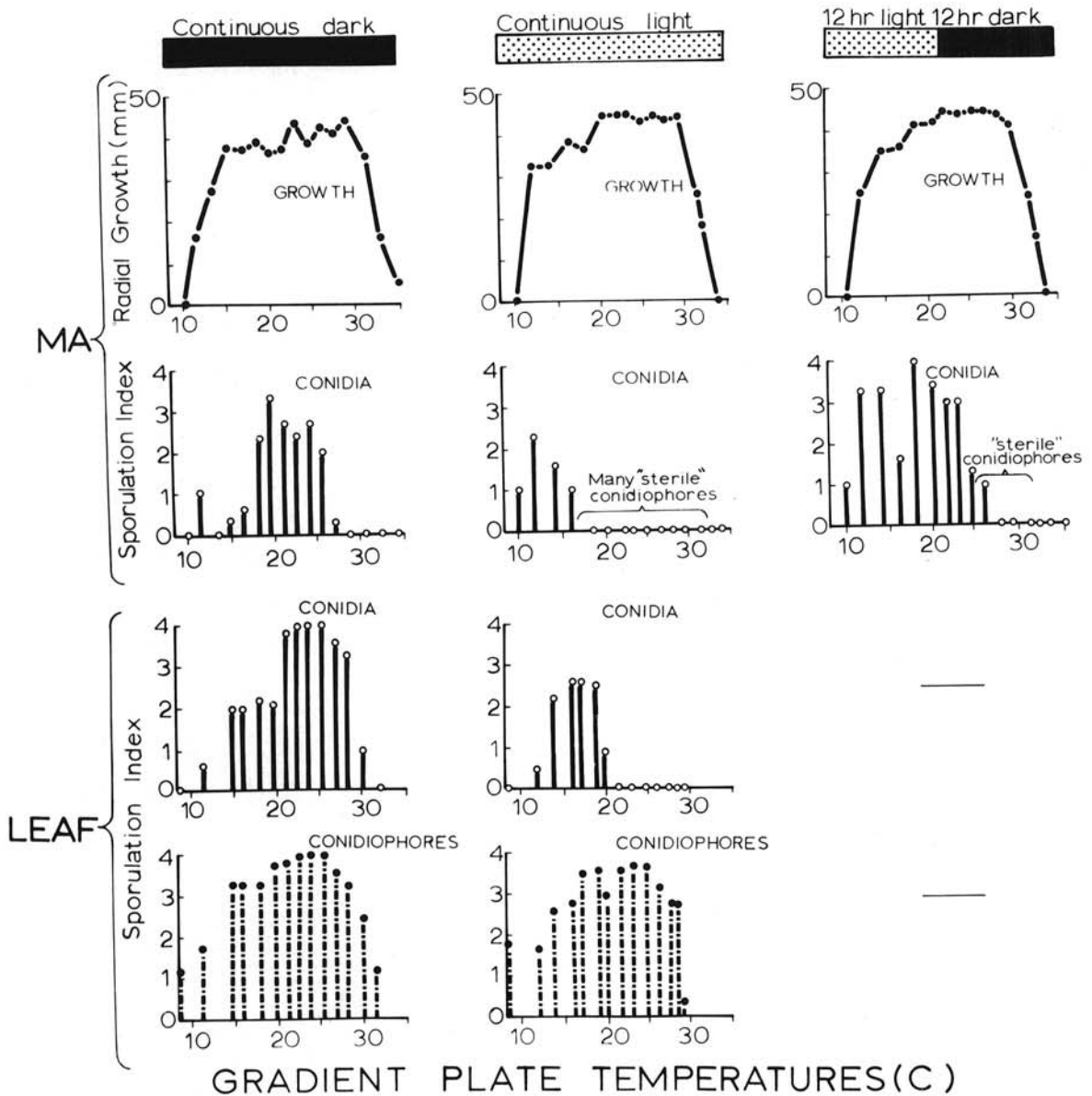

Fig. 3. The effect of the interaction of light and temperature on the growth and sporulation of *Drechslera turcica* on 2% malt extract agar (incubated 11 days; mean of three replications) and maize leaf lesions (infected leaves incubated 2 days; mean of eight replications).

Light had a marked effect on conidium formation, but no observable effect on the development of conidiophores. Continuous exposure to light totally inhibited conidium formation at temperatures> 16 C in colonies grown on MA, and at temperatures > 20 C for leaf lesions (Fig. 3). Exposure of colonies to a 12-hr alternating regime of light and darkness was most favorable for sporulation and conidia were produced over a wider range of temperatures than for the other treatments (Fig. 3). When conidiophores were grown under continuous light at 20 C and then transferred to various temperatures in darkness before being returned to light (Fig. 5), it was evident that approximately 4 hr of continuous darkness was necessary for conidium development. The precise length of darkness necessary for conidium development was temperature-dependent. Conidiophores transferred from light to darkness at 20 C remained visually unchanged for the first 4 hr (Figs. 4-A and B; Fig. 5); however after approximately 5 hr in darkness, a tiny light refractive spot appeared at the tip of each conidiophore and it was through this region that the young conidium was extruded. After 6 hr in darkness, young conidia were approximately one-fifth the size of mature spores (Fig. 4-C). By 8 hr, conidia had elongated considerably and were the size and shape of mature spores, but lacked septa. Septum formation was observed first during the 10th hr of darkness, at which time most spores had three septa, a few had six or seven septa. By the 12th hr spores were mature, slightly olivaceous in color, and mainly seven-septate (Fig. 4-G).

Chlamydospore formation.—In conidia produced at 20 C and then incubated for up to 106 hr at lower temperatures (5, 10, and 15 C), after 24 hr the cell contents rounded-off, became granular in appearance (Fig. 7) and closely resembled the chlamydospores previously observed in conidia trapped above a diseased maize field (9). These changes became more pronounced the longer the conidia were kept at the lower temperatures.

Lesion development under natural conditions.—Artifical inoculation of leaf axils of young plants in the field, resulted in bands of small chlorotic spots that were evident within 24-48 hr. Ten days later none of these spots had expanded, but after 14 and 24 days, 65% and 85%, respectively, had enlarged into typical lesions of NLB. The remaining chlorotic spots failed to expand into lesions during the next 30 days.

DISCUSSION

These experiments have demonstrated that relative humidity, temperature, and light, both individually and interacting, exert a pronounced effect on sporulation of
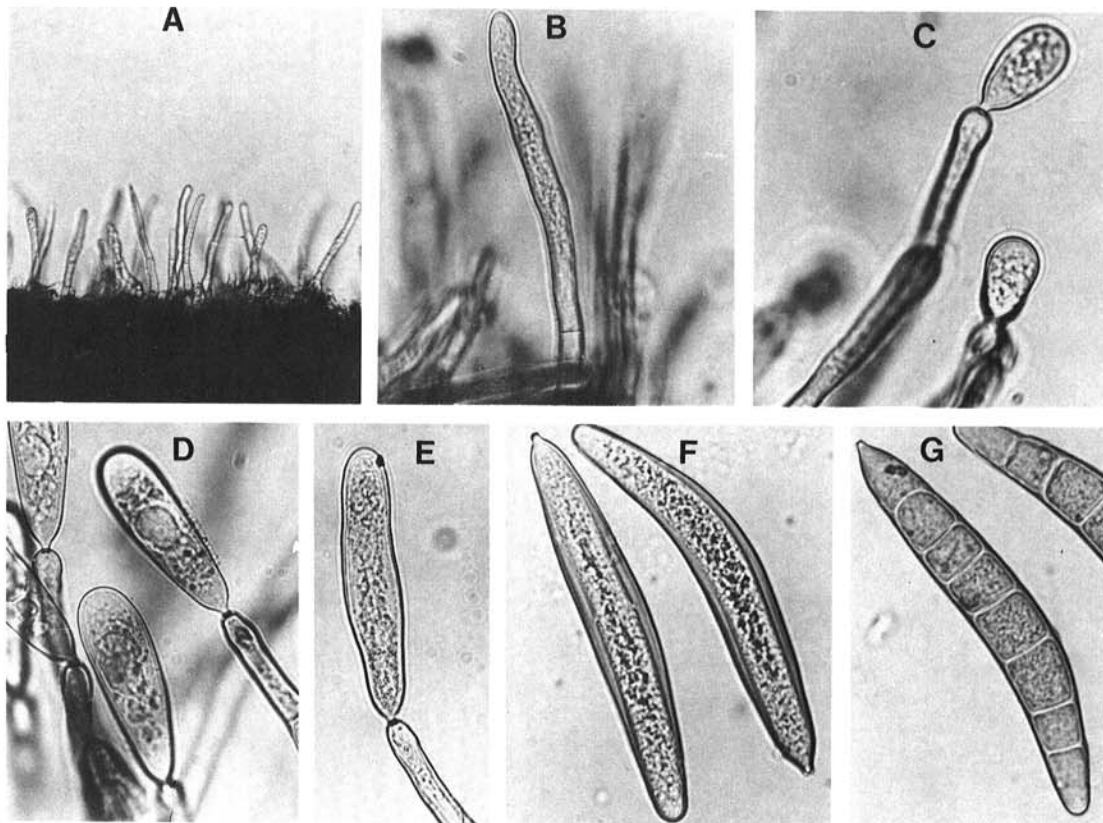

Fig. 4-(A to G). Stages of conidium development of *Drechslera turcica* sporulating on leaf lesions at 20 C. A) Conidiophores grown in light to prevent conidium development (×150). B) Conidiophores transferred to darkness to induce conidium development. C) Conidia after 6 hr in darkness. D) 6.5 hr. E) 7 hr. F) 11 hr - beginning of septum formation in one spore. G) 12 hr - mature conidium (magnification ×440 B-G).

D. turcica both in the laboratory and under natural conditions.

Sporulation occurred only when RH was high. On young lesions, conidiophores developed at slightly lower RH (min. 88%) than conidia (min. 94%). On older lesions with conidiophores already formed, conidia formed at slightly lower relative humidities (> 86%). Because the daytime RH at Pukekohe, N. Z. rarely was above 86%, formation of conidia during daylight hours was rare even when other environmental factors were not limiting. Free water was not necessary for sporulation. The influence of atmospheric moisture on sporulation by *D. turcica* is similar to that reported for *D. maydis* (4), although Nelson and Tung also considered dew to be important in spore production by *D. maydis* (10). They reported that when one dew period was not long enough for conidium development, another dew period the next night could have an additive effect which allowed the conpletion of sporulation. We have not ascertained whether a similar relationship might pertain to periods of high RH on conidium development by *D. turcica*.

Sporulation by *D. turcica* is strongly influenced by temperature. The cardinal temperatures that we have determined for sporulation and growth agree with those reported by Nisikado (11), but differ slightly from those reported by Hilu and Hooker (3). Spores developed to maturity in 12 hr at 20 C, but required twice as long at 10 C and no conidia were produced at lower temperatures. Because conidium development is so much slower at the lower temperatures, periods of continuous high RH must also be increased at lower temperatures if sporulation is to proceed. This can be seen in Fig. 1 during several nights when the RH was very favorable for sporulation (12 to 18 hr) but numbers of trapped spores were low, presumably because of the low night temperatures (12-15 C).
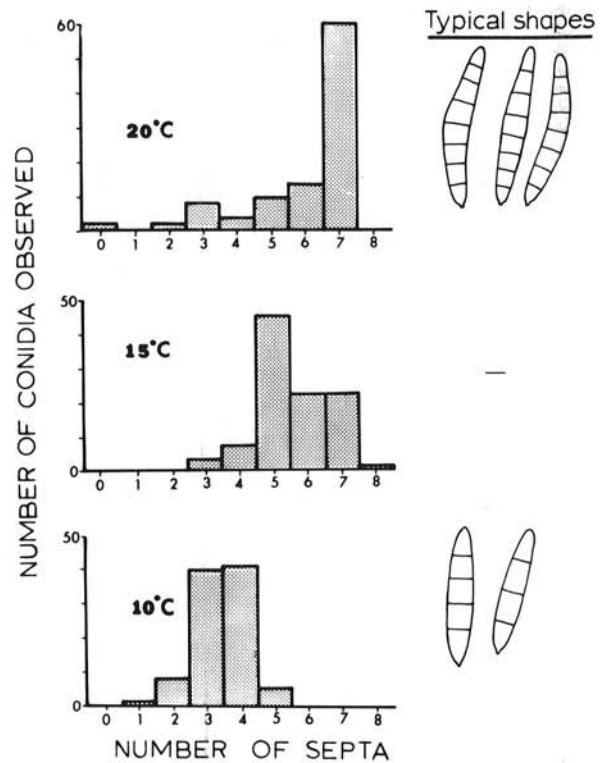

Fig. 6. The influence of temperature during sporulation on the septation and morphology of conidia of *Drechslera turcica* produced on leaf lesions (drawing of 15 C conidium not shown).
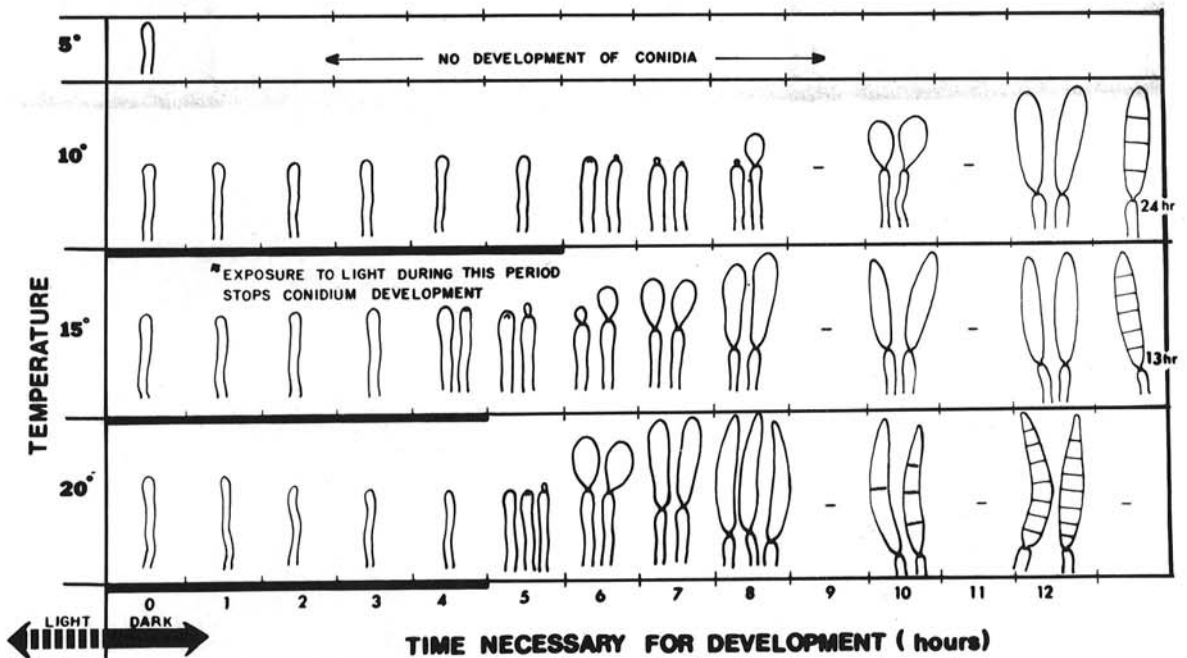

Fig. 5. Differences in rate of development and morphology of conidia of *Drechslera turcica* formed at four different temperatures.
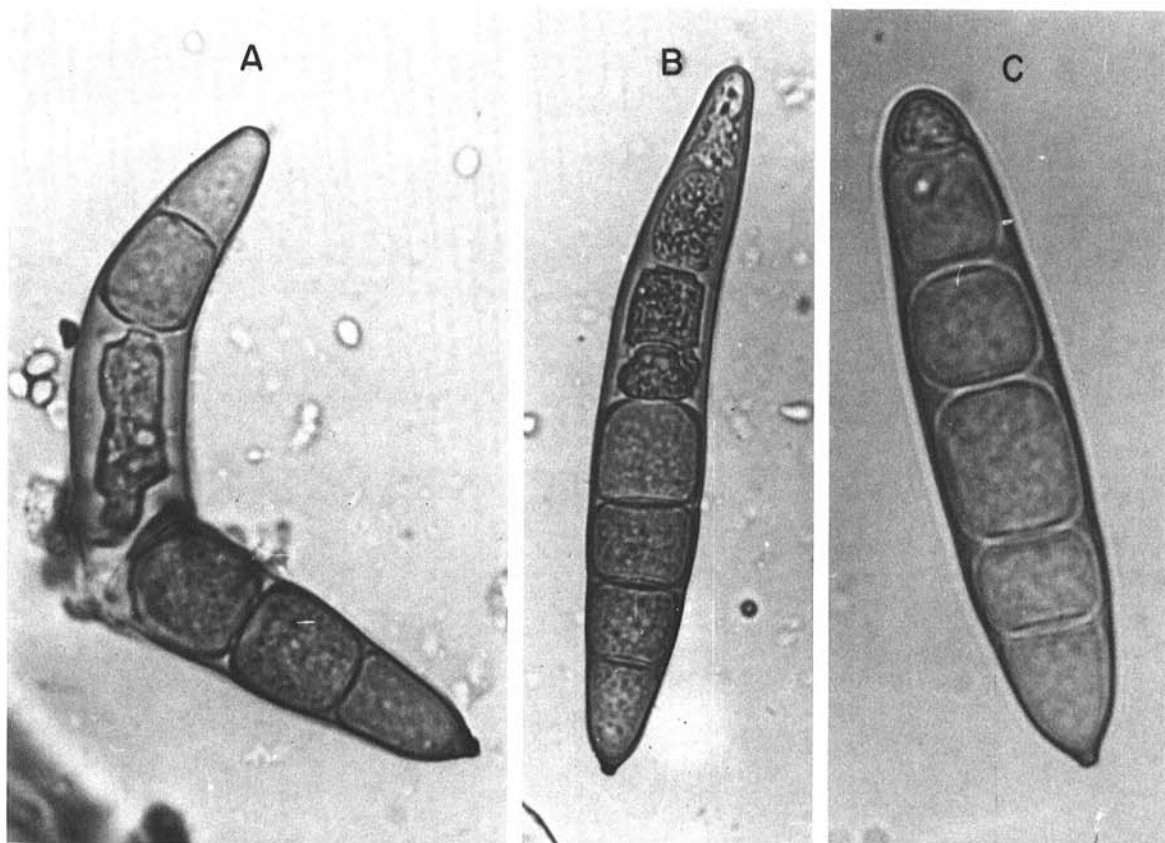
Fig. 7-(A,B). Chlamydospores within conidia of *Drechslera turcica* (×220). A) Artificially induced by incubating conidia at 10 C for 72 hr. B) Conidium trapped over diseased maize in the field.

Immature spores of *D. turcica* (capable of germination) were commonly trapped over diseased maize (9). In our laboratory studies, comparable immature spores took 8 hr to develop on conidiophores at 20 C. We have not determined whether these spores infect leaves, but if they do, then the length of the high-humidity period that limits spore development should be based on the requirements for immature spores rather than those for mature spores.

In the laboratory, temperature not only influenced the rate of development and abundance of spores produced, but also their morphology. These observations on morphological changes confirmed Nisikado's earlier findings (11) and provided an explanation for the differences of spore types commonly trapped above diseased maize in the field. When spore trap slides showed a predominance of elongate, curved, and many-septate conidia, it was evident from our temperature records that the preceding nocturnal temperatures had been relatively warm; short, straight conidia with few or no septa indicated that the preceding night temperatures had been relatively low.

Many fungi are effected by light (6) and *D. turcica* is no exception. In the laboratory, conidium development was inhibited by light at higher temperatures (Fig. 3) but only partially inhibited at lower temperatures. The wavelengths of light inhibitory to spore formation were not determined, but it is likely that they are similar to other fungi that have been classified as "diurnal sporulators" (5, 6). In these fungi, near-ultraviolet radiation stimulates conidiophore formation while near-ultraviolet and blue wavelengths inhibit development of conidia. Such fungi are adapted to the daily changes of night and day coupled with fluctuations of temperature that are so characteristic of nature. Various workers (2, 10, 12) have noted a similar reaction of *D. maydis* to light although its response is not quite as pronounced as that of *D. turcica*. Thus, under field conditions because of the inhibitory effects of light, little sporulation would be expected during the day even when temperature and humidity were favorable.

These and other studies (7, 9) have confirmed the importance of temperature, RH, and light in sporulation and spore release in *D. turcica*. Still needed is a better understanding of the relationship of environmental conditions to spore germination, infection, and lesion development.

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