Pertanika 13(3), 321 - 325 (1990)

Infection of Chilli by *Cercospora capsici*

SARIAH MEON
Department of Plant Protection
Faculty of Agriculture, Universiti Pertanian Malaysia
43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

Key words: chilli, infection, *Cercospora capsici*.

ABSTRAK

C. capsici *didapati selalu bersekutu dengan bintik daun cili, menurunkan aktiviti fotosintesis tanaman yang dijangkiti dan membawa kepada kerugian hasil. Simpton awal terdiri daripada lesion nekrosis yang kecil pada permukaan daun, yang kemudian bercantum menghasilkan lesion yang tidak sekata dan hawar dengan pengeluaran konidia yang banyak. Pensporulaan patogen tinggi pada julat 20-30° C tetapi rendah pada 10° C. Tiada pensporulaan pada 40°C. Pensporulaan pada lesion tinggi pada kelembapan tepu tetapi menurun dengan menurunnya potensi air sehingga -6mPa. Suhu yang elok untuk percambahan konidia berjulat 20-30° C dan menurun dengan menurunnya potensi air. Ujian kepatogenan dan kajian cara penjangkitan* C. capsici *pada daun cili menunjukkan kulat masuk melalui stomata meyakinkan bahawa ia adalah patogen primer.*

ABSTRACT

C. capsici *was shown to be consistently associated with leaf spot of chilli, reducing appreciably the photosynthetic activity of infected plants leading to losses in yield. Incipient symptoms consisted of small, necrotic lesions on the surface of the leaves, which later coalesced giving an irregular and blighted appearance with the production of abundant conidia. The pathogen sporulated abundantly at 20-30° C but poorly at 10° C. At 40° C, no sporulation occurred. Sporulation on lesions was greatest near moisture saturation but declined with decreasing water potential to -6 MPa. The best temperature for germination was in the range of 20-30° C and declined with decreasing water potential. Pathogenicity tests and studies on the mode of infection of* C. capsici *on chilli leaves revealed that the fungus entered through the stomata, indicating that it was a primary invader.*

INTRODUCTION

Cercospora capsici Heald & Wolf causes a foliar disease of chilli (*Capsicum annuum*, L) known as leaf spot. Both young and old leaves of chilli can be severely infected. Leaf spots are distinct on both surfaces of the leaves. Spots are circular when young, becoming oval or somewhat elongated as the spots age, with light grey centres and each spot delineated by a dark brown ring. Sometimes spots are surrounded by a diffuse yellow necrotic zone. Two or three spots may coalesce as they expand. Under favourable conditions, the number of lesions increases rapidly and their coalescence causes extensive necrosis of leaf tissue and defoliation.

Leaf spot was not serious disease on chilli in Malaysia until recently as the chilli varieties grown were the more resistant, locally selected and adapted varieties. As new introduced varieties began to be grown they were more susceptible to leaf spot by *C. capsici*. This together with the indiscriminate use of fungicides led to a situation whereby the disease reached epidemic proportions during certain seasons of the year. This paper reports the causal organism, its pathogenicity and mode of infection of chilli by *Cercospora capsici*.

MATERIALS AND METHODS

An isolate of *C. capsici* obtained from naturally infected chilli leaves collected from a field in Universiti Pertanian Malaysia, was used throughout this study. The fungus was maintained on one to two month old chilli plants grown in 15 cm diameter pots in a greenhouse.

Morphological Characteristics

Diseased leaf specimens from infected chilli plants were collected for examination. The causal pathogen from the leaf spots was carefully studied with a light microscope and a scanning electron microscope (SEM). Samples for SEM observations were fixed in 6% buffered glutaraldehyde at 4° C overnight and post-fixed with 1% buffered osmium tetroxide for 2 h. They were then dehydrated in acetone series (30%-100%), dried in a critical point drier, mounted on stubs, coated with gold in a sputter coater and examined with a JEOL JSM JSC scanning electron microscope.

Effect of Temperature and Humidity on Sporulation

Chilli leaf discs containing a 3-4 mm diameter lesion (lesion discs) were excised with a 5 mm-diameter cork borer immediately before use in controlled temperature or moisture studies. Discs were placed in petri dishes containing moist filter paper and incubated at 10, 15, 20, 25, 35 or 40° C. The plates were kept away from direct sunlight to delay senescence. After 4 days, conidia were counted and lesions measured. Sporulation was quantified as conidia/lesion area of three lesion discs (a replicative unit comprised three discs). Lesion discs supporting conidia were placed in vials (three discs per vial) containing 2 ml of water amended with 3 ml Tween 20 per 100 ml of water. Conidia were dislodged by rapid stirring for 1 min and numbers of conidia per vial were based on an average of six haemocytometer counts. Lesion area was calculated from lesion radius using the equation for the area of a circle.

The influence of humidity on sporulation was studied by placing leaf discs on metal screen in a 5 mm gap between agar slabs located on the top and bottom of 9 cm diameter petri dishes, a system similar to that described by Harris *et al.* (1970). To achieve various humidity levels, the osmotic potential of the agar was adjusted to -0.5, -3, -5, -6, -8 mPa (corresponding to 99.6, 98.2, 97.2, 96.1 and 94.6% RH, respectively) using sodium chloride concentrations, based on data of Robinson and Stokes (1955). Plates were sealed with parafilm and incubated at 25° C. After 4 days, conidia were counted and lesions were measured.

All experiments were arranged in a completely randomised design with four replications. The experiment was conducted twice and analysis of variance was conducted on pooled data.

Effect of Temperature and Humidity on Conidial Germination

Germination was assessed on glass-slides using conidia suspended in sterile distilled water. In all experiments, 5 µl drops (25-50 conidia per drop) were placed at three sites on each of the three replicate slides.

To determine the influence of temperature on conidial germination, conidia were incubated on slides at 100% RH in the dark at 10, 15, 20, 25, 30, 35, 40° C. After 24 h, conidia were stained with aniline blue in lactophenol, and germination was assessed.

The influence of humidity on germination of conidia of *C. capsici* was studied on slides positioned on metal screens in a 5mm gap between agar slabs as described above. Plates were sealed with parafilm and incubated at 25°C. After 24 h, conidia were stained and germination assessed.

Experiments were completely randomised in design and were conducted twice. Germination assessments were based on 50 conidia per slide. The conidium was considered germinated when the length of germ tube was longer than the width of the conidium.

Pathogenicity and Mode of Infection

Chilli seedlings of 2-4 weeks old were used. Conidial suspension of 2×10^6 conidia/ml plus 2 drops of Tween 80 were sprayed on both the abaxial and adaxial surfaces of the leaves. Control leaves were sprayed with sterile distilled water. Six replicates per treatment were employed. Diseased symptoms were observed at intervals. To study the mode of infection of the fungus, leaf samples (1 cm x 1 cm) taken at intervals were cleared and stained using the method given by Shipton and Brown (1962).

RESULTS

Morphological Characteristics

The stroma of the fungus was small and irregular, consisting of a few huge dark brown cells. Fruiting was amphigenous and more abundant on the lower surface of leaves. Fascicles consisted of 2-10 stalks of conidiophores which were medium brown and simple, erect, some slightly flexuous, septate and measured 40-50 µm x 2.3 µm. The tip of each conidiophore was blunt, with a prominent conidial scar. Conidia were pale olivaceous, straight to slightly curved, indistinctly

multiseptate, conidial length ranged from 3-4 µm x 50-200 µm. The conidial scar at the base was distinct while the tip of the tail was bluntly rounded.

Effect of Temperature and Humidity on Sporulation

There were only small differences in sporulation intensity between individual leaf discs of the same temperature treatment. Conidial production increased from 10° C to 25° C and declined sharply between 30° C to 40°C (*Fig. 1*). Furthermore, conidia produced at 35° C were shorter in length than those produced at other tempratures.

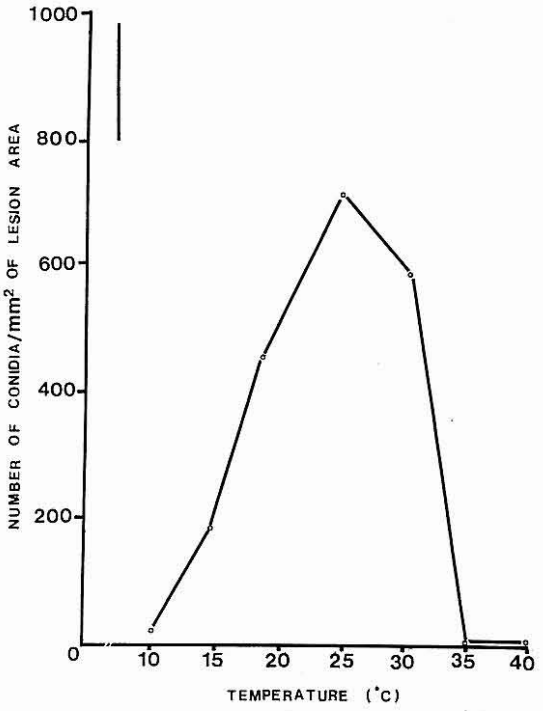

Fig. 1: Sporulation of Cercospora capsici *in excised lesion incubated for 4 days. Vertical line indicates L.D.S. value at p < 0.05.*

Sporulation in lesions was greatest near moisture saturation but declined with decreasing water potential to - 6mPa (*Fig. 2*). Conidia were not detected at -8mPa.

Effect of Temperature and Humidity on Conidial Germination

Conidial suspensions of *C. capsici* incubated on glass slides gave abundant germination at 20°C-30°C. Means of conidia that germinated at 10, 15, 20, 25 and 30, were 5%, 30%, 92%, 98%, 96%, respectively. No germination occurred at temperatures above 35°C (*Fig. 3*).

Germination after 24 h at 25° C declined with decreasing water potentials from -0.05mPa through -8mPa (*Fig. 4*).

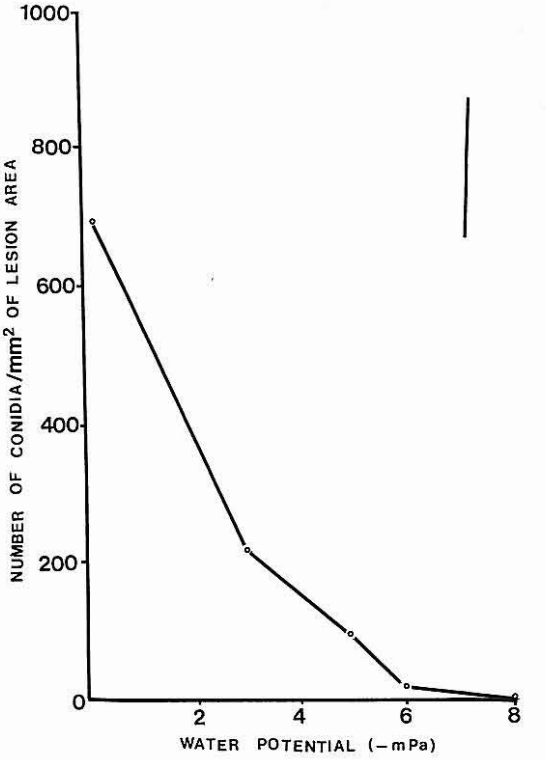

Fig. 2: Effect of water potential on sporulation of Cercospora capsici *in excised lesions. Vertical line indicates L.D.S. value at P < 0.05*

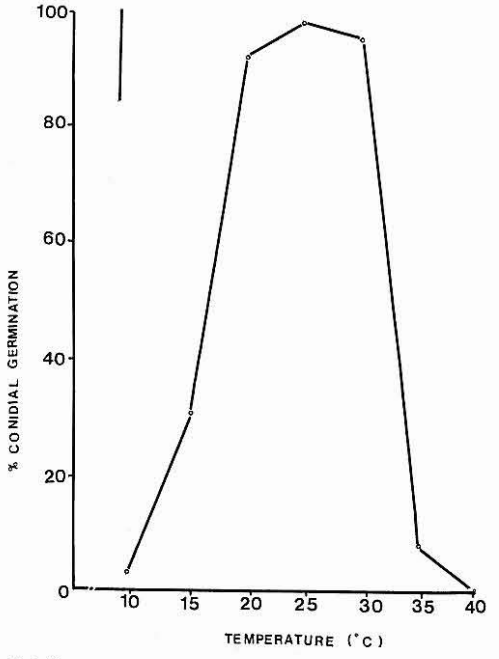

Fig. 3: Influence of temperature on germination of Cercospora capsici *on glass slides. Vertical line indicates L.D.S. value at P < 0.05*

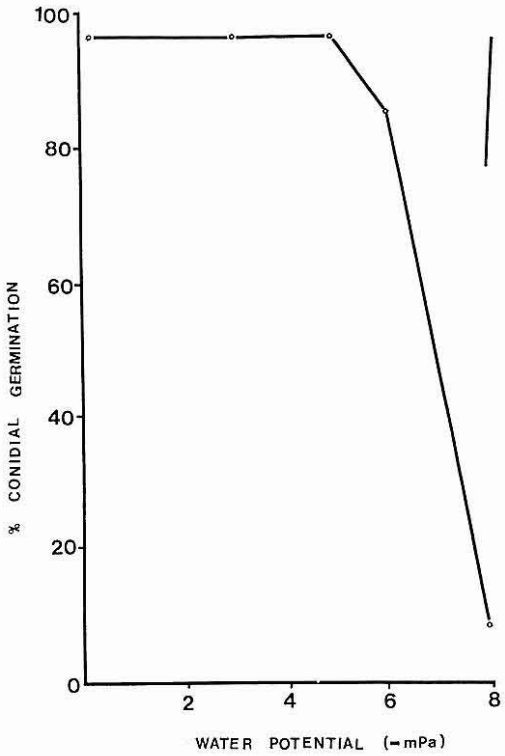

Fig. 4: Influence of water potential on germination of Cercospora capsici *on glass slides at 25°C. Vertical line indicates L.S.D. value at $P < 0.05$*

Pathogenicity Studies

Both young and old leaves of chilli were severely infected. Leaf spots were distinct on both surfaces of the leaves. Spots were circular when young becoming irregular as the spots aged with white centres and delineated by a dark brown ring, surrounded by a diffuse yellow necrotic zone. Abundant conidia were produced on the necrotic areas by the second week of inoculation. None of the inoculated leaves sprayed with sterile distilled water developed any disease symptoms.

Mode of Infection

Conidia of *C. capsici* inoculated on leaves of chilli germinated after 48 h and by 72 h ramified the surface of the lamina. Germ tubes exhibited positive tropism towards stomata before formation of appressoria. Once in the leaf, the hyphae infected the hypodermal and mesophyll cells. By the seventh day the tissues appeared chlorotic and conidia were produced on the dead tissues by 10 - 14 days after inoculation. Sections of the necrotic areas showed the infected mesophyll cells had collapsed and the stomatal cavity filled

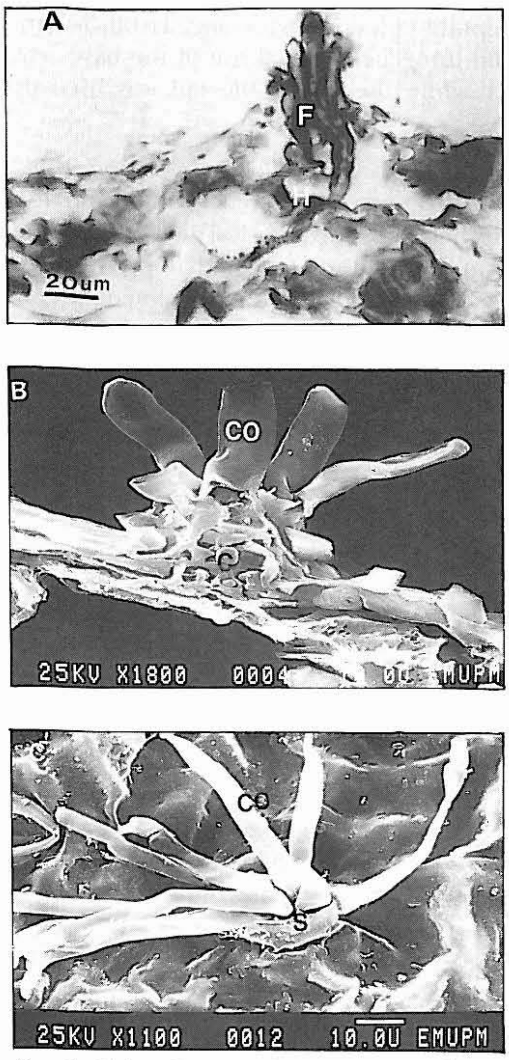

Plate 1: Light and scanning electron micrograph (SEM) of pathogenesis by C. capsici *on chilli leaf.*

A. cross-section of lesion showing necrotic tissues (H) and fascicle primodium (F)

B. SEM of stomatal cavity with collapsed cells (C) and conidiophores (CO)

C. SEM showing conidiophores (CO) emerging through stomata (S).

with fungal stroma. This later gave rise to fasicles of conidiophores which emerged through the stomata bearing the conidia which rapidly diffused over the surface of the leaf (Plate 1 : A,B and C).

DISCUSSION

It is apparent that leaf spot disease of chilli caused by *C. capsici* is a common and serious disease of chilli. The present recommended control measure of prophylactic spraying of fungicides is

somewhat inadequate to prevent sudden outbreaks of the disease. Symptom development is distinct and individual lesion development is easily observed. Leaf spots are usually amphigenous, circular 2-10 mm in size, later coalesing to form large patches with dark brown margins. Conidiophores are produced in fasicles mainly on the lower surface of infected leaves. Conidia are hyaline and indistinctly multiseptate.

Field observations indicated that temperatures below 20° C are not favourable for leaf spot development. Poor sporulation was observed at temperatures below 20° C, which is consistent with these observations. Germination was greatest at moderate temperatures of 25-30° C and low at temperatures of 30-35° C.

This optimum range of temperature for sporulation and germination showed a distinct correlation to the annual mean temperatures in the lowlands of this country, which is between 25 C and 28.5 C (Dale 1974). This indicates that ecologically, this fungus is well suited to growth and multiplication in the lowland chilli growing areas in this country, causing severe leaf spot disease whenever conditions are favourable for infection by *C. capsici* spores.

Moderate night temperature could favour sporulation and germination, and germ tube probably continue to elongate during warmer morning or daytime conditions, provided humidities are near saturation.

Conidial development of *C. capsici* was similar to that reported for many *Cercospora* species on other plants (Oso 1972; Beckman & Payne 1983; Alderman & Beute 1986, 1987; Cooperman and Jenkins 1986). Pathogenicity test and studies on the mode of infection of *C. capsici* on chilli leaves revealed that the fungus entered through the stomata indicating that it is a primary invader. Further development occurred in the mesophyll cells and substomatal cavity. Humidity in excess of 95% maybe advantageous to *C. capsici* in maintaining varying stages of conidial development in between periods of high humidity, and are also most favourable for leaf spot development. High ambient humidities would imply that humidities within a chilli canopy, when leaves are transpiring, would be near 100% leaf surfaces. Prolonged periods of high humidities wil favour stomatal tropism and penentration. Conditions under which rain or dew evaporate quickly and humidities decline rapidly, on the other hand, would delay growth of germ tubes and stomatal penetration. Since *C. capsici* penetrates via stomata, infection efficiencies can also be a function of both humidity and stomatal behaviour. These, together with prolonged periods of high moisture and moderate temperature favour disease development in the fields, resulting in extensive defoliation and yield loss.

REFERENCES

ALDERMAN, S.C and M.K. BEUTE. 1986. Influence of Temperature and Moisture on Germination and Germ Tube Growth of *Cercospora arachidicola* of Peanut. *Phytopathology* **76:** 715-719.

ALDERMAN, S.C and M.K. BEUTE. 1987. Influence of Temperature, Lesion Water Potential, and Cyclic Wet-dry Periods on Sporulation of *Cercospora arachidicola* onPeanut. *Phytopathology* **77:** 960-963.

BECKMAN, P.M. and G.A. PAYNE. 1983. Cultural Technique and Conditons Influencing Growth and Sporulation of *Cercospora zeae - maydis* and Lesion Development in Corn. *Phytopathology* **73:** 286-289.

COOPERMAN, C.J and S.F. JENKINS. 1986. Conditions Influencing Growth and Sporulation of *Cercospora asparagi* and *Cercospora* Blight Development in Asparagus. *Phytopathology* **76:** 617-622.

DALE, W.L. 1974. The Rainfall of Malaya. Part I. In Readings on the Climate of West Malaysia and Singapora, ed. Ooi Jin Bee and Chia Lin Sien. Singapore : Oxford University Press.

HARRIS, R.F, W.R. GARDNER, A.A. ADEBATO and L.E. SOMMERS. 1970. Agar Dish Isopiestic Equilibrium Method for Controlling the Water Potential of Solid Substrates. *Appl. Micro.* **19:** 536-537.

OSO, B.A. 1972. Conidial Germination in *Cercospora arachidicola*. *Hori. Trans. Br. Mycol. Soc.* **59:** 167-172.

ROBINSON, R.A., and R.H. STOKES. 1955. *Electrolyte Solutions*. N. York: Academic Press.

SHIPTON, A. and BROWN, J.F. 1962. A Whole Leaf Clearing and Staining Technique to Demonstrate Host-pathogen Relationship on Wheat Stem Rust. *Phytopathology* **52:** 1313.

(Received 28 August, 1990)