Pertanika 11(3), 341-344 (1988)

Seed-borne Infection and Development of *Colletotrichum capsici* in Naturally Infected Chili Seed

SARIAH MEON & W. ZAINUN W. NIK
Department of Plant Protection
Faculty of Agriculture
Universiti Pertanian Malaysia
43400 UPM, Serdang, Selangor, Malaysia

Key words: ***Colletotrichum capsici;*** **chili seed; site of infection.**

ABSTRAK

Pengujian biji benih menggunakan kaedah kertas serap dan agar dekstros menunjukan penjangkitan Colletotrichum capsici *yang jelas di dalam dan di permukaan luar biji benih cili. Hirisan microtome menunjukkan miselium didapati dalam lapisan luar dan dalam kulit dan dalam endosperma. Pembentukan aservulus dimulakan di bawah kulit biji benih dan juga di kawasan endosperma dan keluar dengan memecahkan kulit biji. Tisu-tisu parenkima juga bertukar bentuk. Patogen akhirnya hidup di atas permukaan biji benih.*

ABSTRACT

The testing of seeds using both the blotter and potato dextrose agar showed infection of Colletotrichum capsici *to be well established both within and on the external surfaces of chilli seeds. Microtome sections showed that mycelia were present in the outer and inner layers of the testa and in the endosperm region. Formation of the acervulus was initiated below the seed coat and also in the endosperm and emerged to the surface after disrupting the seed coat. Parenchymatous tissues were also distorted. The pathogen finally grows on the seed surface.*

INTRODUCTION

Anthracnose or ripe fruit rot of chillies *(Capsicum annuum,* L) caused by *Colletotrichum capsici* (Syd.) Butler & Bisby has been found to be a serious disease in the chilli growing areas of Malaysia. The disease attacked the leaves, stems, flowers and was most damaging on mature ripe fruits. Disease symptoms were occasionally observed on green fruits as well. The first indication of the disease is the appearance of small, elliptical or oblong straw–coloured, slightly sunken lesions on the surface of the mature fruit. This is followed by the development of black acervuli arranged in concentric rings, giving a target board appearance. Current observations in Peninsular Malaysia shows that the reduction in yield of marketable fruits due to anthracnose generally ranges from 10%–60% depending on certain seasons of the year (Mah, 1987).

The pathogen persists in the soil, infected crop residues and weeds (Saxena *et al.* 1982). Several workers reported that *Colletotrichum capsici* was seed-borne (Higgins, 1930; Grover and Bansod, 1970; Mordue, 1971; Rout and Rath, 1972; Holliday, 1980). Hence, the use of anthracnose infected seeds can give rise to weak seedlings which then become the primary inoculum source in a chilli field. From the primary inoculum source, spread of the disease can be through wind-borne spores or through rain splash. Although, control of *C. capsici* affecting foliage, branches and fruits of chillies had been well documented, no attention had been paid so far to the determination of the seed-borne nature of *C. capsici.* Hence this study is initiated to determine the site of infection and subsequent development in the infected seed.

MATERIALS AND METHODS

Chilli seeds used in this study were collected from diseased fruits of the variety MC–4 obtained from an experimental field in Universiti Pertanian Malaysia. Four hundred seeds were used for each method of isolation. External seed-borne infection was determined by directly plating the seeds on moist blotter. Internal seed-borne infection was determined by surface – sterilising the seeds with 10% chlorox for 3 minutes, followed by repeated washings with sterilised water and plating on potato dextrose agar. The plates were incubated for seven days at 25 C in alternating cycles of 12hr near ultraviolet-light (NUV) and darkness, and examined for fungal infection using a stereo and a compound microscope. The experiment was arranged in a completely randomised design and the mean percentage of isolation was based on 400 seeds.

On the fourth day of incubation, naturally-infected seeds were fixed in 3% glutaraldehyde in 0.025M phosphate buffer and vacuum extracted for half an hour. They were then dehydrated in eight graded alcohol series (30, 40, 50, 60, 70, 80, 90 and 100 % ethanol) and embedded in gelatin capsules with JB–4 embedding mixture (Polysciences, Inc. , Warington, PA 18976). Sections were cut on an ordinary rotary microtome and stained with 0.1% toluidine blue in 0.1% sodium borate (O' Brien *et al,* 1964).

In addition, scanning electron microscopy was also used as an additional tool in this study. Seed samples were fixed in 6% buffered glutraldehyde, washed three times with phosphate buffer and post-fixed with 1% osmium tetroxide. The samples were then dehydrated in acetone series and dried in a Polaron Critical Point drying apparatus. The dried specimens were stuck to copper stubs and shadowed with gold in a Polaron Sputter Coater. Micrographs were taken in a JOEL JSM 35C Scanning electron microscope.

RESULTS AND DISCUSSION

Colletotrichum capsici was isolated most frequently both by the blotter and potato dextrose agar, suggesting that the pathogen could be present on the seed surface as well as inside the seed (Table 1). Similar results were reported by Grover and Bansod (1970) and Rout and Rath (1972) on chilli seeds. In addition, eight genera of fungi were also isolated (Table 1). *Rhizopus stolonifer, Chaetomium globosum* and *Pestalotiopsis* sp. were isolated from chilli seeds and represent new records of chilli seed-borne infection.

Transverse sections of seeds naturally infected by *Colletotrichum* showed a number of

TABLE 1

Percentage isolation of fungi from chilli seeds using the blotter method and potato-dextrose agar

	* Percentage isolation *	
	Blotter method	Potato-Dectrose Agar method
Colletotrichum capsici (Syd.) Butler & Bisby	37	29
Fusarium spp.	25	31
Aspergillus spp.	17	3
Drechslera rostrata (Drechsl.) Richardson & Fraser	16	19
Curvularia lunata (Wakker) & Boedijin	3	11
Colletotrichum gloesporiodes (Penz.) sacc	0.3	0
Chaetomium globosum Kze	0.4	0
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind	1.3	0
Pestalotiopsis sp.	0	7

acervuli over the seed surfaces. After infection, acervulus primordia were formed beneath the inner layers of the testa and within the endosperm region *(Plate 1)*. Subsequently, the young acervulus enlarged and caused the testa to bulge outwards by exerting pressure on the testa *(Plate 2)*.

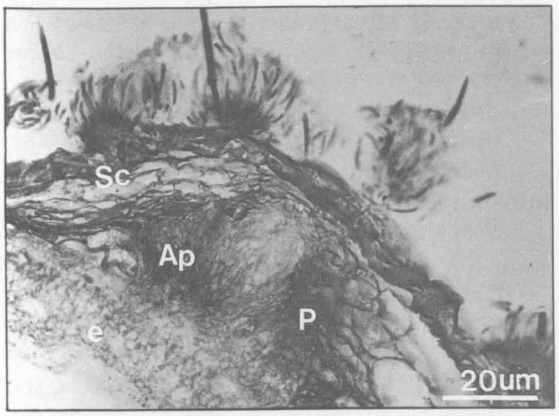

Plate 1. Acervulus primordia (arrowed) beneath seed coat (Sc - seed coat; Ap - Acervulus primordia; P - parenchyma; e - endosperm).

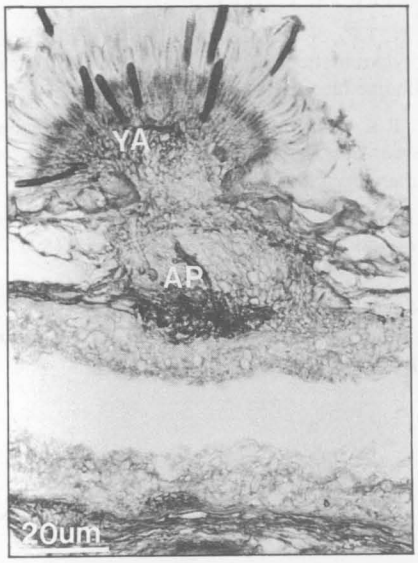

Plate 2. Acervulus primordia emerging through seed coat. (YA - young acervulus; AP - acervulus primordia)

Ultimately, the testa ruptured and the young acervulus emerged. Each mature acervulus bore numerous setae and masses of conida *(Plates 3A, B)*. On bursting, conidia were liberated in the form of ooze. Mycelia were also detected in the endosperm region *(Plates 4A, B)* just below the inner testa. The mycelium could grow directly into the inner layer of the testa surrounding the endosperm, or could remain dormant until the seed germinated. Infected parenchymatous tissues of the seed appeared distorted.

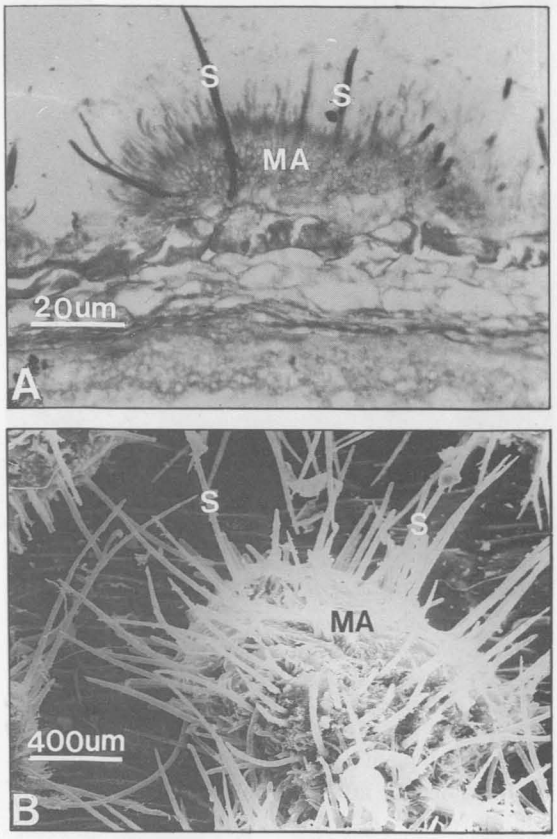

Plate 3. (A) Tranverse section, (B) Whole mount (SEM micrograph) of mature acervulus showing numerous setae. (S - setae; MA - mature acervulus)

It is evident from these studies that *C. capsici* was borne intraembryonal in chilli seeds. Disruption of seed tissues could be due to the activity of cellulolytic and pectionlytic enzymes produced by *C. capsici* (Sariah, 1980). Spores oozing out from acervuli can serve as a primary inoculum source for the spread of the disease.

The fungus can then spread from the seed to the placenta of the fruit. The pathogen could then penetrate the developing ovules, or young seed with unlignified testa at any point on their surface. Infection of seeds could also occur directly from

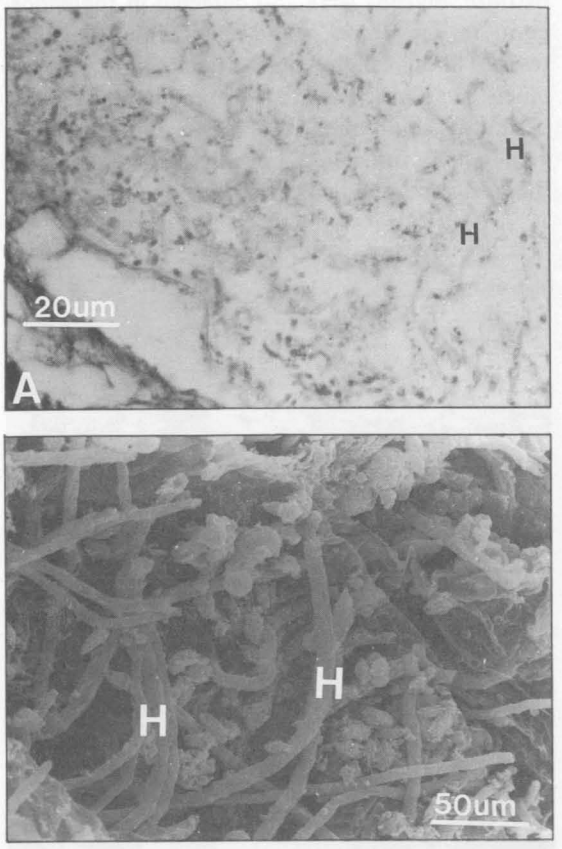

Plate 4 Transverse section of infected seed showing the presence of hyphae in the endosperm region (A) light microscopy and (B) SEM micrograph (H - hyphae).

the mother plant: through the pedicel, funiculus or integuments into the developing ovule. Also, conidia of *C. capsici* could also be mechanically attached to the surface of the testa, remaining dormant until the seed germinated.

With seed-borne inocula, the way in which the inoculum was carried within or on the seeds influenced the type of control measure used (Neergaard, 1979). Therefore, with internal seed-borne infection as was the case here, systemic fungicides will provide a more practical and effective control measure. Besides anthracnose infected seeds should not be used for planting.

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(Received 29 April, 1988)