

## **PENICILLIUM FUNICULOSUM ISOLATES ASSOCIATED WITH FRUIT BLEMISHES OF PINEAPPLE (CV MASMERAH) IN PENINSULAR MALAYSIA**

W.H. LIM\*

*Keywords:* Pineapple, *Penicillium funiculosum*, Morphological strains, Leathery pockets, Cork spot, Fruitlet core rot.

### **RINGKASAN**

Kulat yang disisihkan dari buah nenas yang cacat-rupa (blemishes), iaitu, mata dalam, 'cork spot' dan 'fruit core rot' (atau 'black eye') telah dikaji dan dikenalpasti. *Penicillium funiculosum* adalah yang paling kerap disisih diikuti oleh *Fusarium moniliforme*. Enam strain morfologi jenis *P. funiculosum* dikenali berdasarkan pewarnaan, ciri-ciri koloni, penghasilan 'micro-selerotia' dan penumbuhan. Frekuensi penyisihan strain-strain dari tiga jenis cacat-rupa juga diperhatikan.

### **INTRODUCTION**

Three types of fruit blemishes commonly encountered on the Masmerah pineapple in Peninsular Malaysia are leathery pocket (LP), cork spot (CS) and black eye (BE) – also known as fruitlet core rot (LIM, 1980). A fourth type of fruit blemish, interfruitlet corking, is rarely encountered.

Of the three fruit blemishes mentioned above, the most common and serious is leathery pocket (LP) which can cause up to 40 percent losses per harvest (ANON, 1979). Cork spot is also common but because of the superficial nature of the infection, damage to the fruit is negligible (LIM, 1980). Black eye is the least common, and if present, normally affects one or two fruitlets per fruit.

All the aforementioned diseases have been reported from other major pineapple growing countries. The causal agent of fruitlet core rot (or black eye) on cv. Smooth Cayenne in Hawaii, Mexico, South Africa and Queensland has been shown to be *Penicillium funiculosum* and/or *Fusarium moniliforme* (OXENHAM, 1957; LINFORD, 1952; ROHRBACH & PFEIFFER, 1976). *P. funiculosum* is also responsible for leathery pocket (ROHRBACH & PFEIFFER, 1976) and interfruitlet corking (HEPTON &

ANDERSON, 1968) in Hawaii. The existence of morphological strains of *P. funiculosum* was earlier observed by OXENHAM (1962) when he distinguished between a pigmented and non-pigmented strain from fruitlet core rot (black eye) tissues. The strain of *P. funiculosum* associated with interfruitlet corking (HEPTON & ANDERSON, 1968) and leathery pocket (ROHRBACH & PFEIFFER, 1976) are non-pigmented. In Hawaii, three strains of *P. funiculosum* from pineapple tissues, were distinguished but only one, the non-pigmented strain, was able to induce leathery pocket, fruitlet core rot and interfruitlet corking (LIM, 1977; LIM & ROHRBACH, 1980).

This paper reports on the strains of *Penicillium funiculosum* associated with leathery pocket, cork spot and black eye tissues of pineapple in Peninsular Malaysia. Studies on their pathogenicity and infection site will be presented in a subsequent paper.

### **MATERIALS AND METHODS**

Fruits of *Ananas comosus* cv. Masmerah were obtained from various localities in Pontian and Kelang. The fruits were shelled in the laboratory and examined for fruit blemishes, viz. leathery pocket (LP), fruitlet core rot or black eye (BE) and cork spot (CS). Tissues exhibiting those

\*Fruit Branch, MARDI, Jalan Kebun, Selangor.

disease symptoms were surface sterilized in 10 percent Chlorox (R) and plated on potato dextrose agar (PDA) plates. Subsequently, a partially selective medium useful for distinguishing strains of *Penicillium funiculosum* was developed and used. The composition of the medium is as follows:— mycostatin (50,000 units/l), tetracycline (100 ug/ml), streptomycin (100 ug/ml), PCNB (0.01%), Mancozeb (0.05% a.i.), distilled water (1 litre) and PDA (40 gms). The isolates obtained were reisolated onto PDA test tube slants from hyphal tips and subsequently distinguished and classified on the basis of cultural characteristics. Selected isolates were sent to the Commonwealth Mycological Institute, Kew, England for identification and confirmation.

## RESULTS AND DISCUSSION

### Symptoms of leathery pocket (LP), cork spot (CS) and black eye (BE)

The symptoms of the three diseases, viz. LP, CS and BE are illustrated in *Figure 1*. Basically, LP is an infection of the ovary wall (via the stylar canal) while CS is an infection of the nectary ducts. In the case of fruitlet core rot or BE, the fruitlet core or swollen septa of the ovary becomes necrotic. Leathery pocket (LP) tissues are hard and leathery while that for CS and BE are wet but firm.

### Fungi isolated from fruit blemishes

*Penicillium funiculosum* was the predominant fungus species isolated from LP, BE and CS infected tissues, followed by *Fusarium moniliforme*. Other fungi which were occasionally isolated were *F. oxysporum*, *Thielaviopsis paradoxa* and *Trichoderma* sp.

### Strains of *Penicillium funiculosum*

A total of six strains, tentatively labelled Types 1 to 6, were distinguished based on their cultural characteristics on PDA medium. *Table 1* summarises their important characteristics.

*Penicillium funiculosum* characteristically produces red or purple to almost black pigmentation. A description of a typical *P. funiculosum* culture is as follows (THOM, 1930):

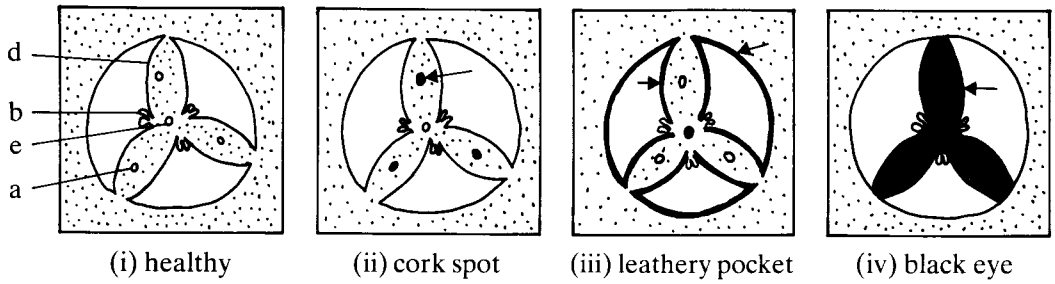
‘Colonies on bean agar are deep green, broadly spreading, surface closely floccose; reverse and medium red or purple to almost black. Conidiophores arise laterally from aerial hyphae, the latter commonly in ropes; occasionally arise directly from the substratum, 20–80 $\mu$  or 100  $\mu$  long. Conidial fructification in three stages, up to 160 long, columbar; phialides 10–14 x 2–3 $\mu$ , in dense, parallel verticils. Conidia elliptical, 3–4 x 2–3 $\mu$ , green smooth’.

The morphology, structure, shape and size of the conidial fructifications and conidia of the six strains fitted closely the information of the type culture of RAPER & THOM, (1968). However, differences in colony colour, pigmentation, sclerotia production, mycelial colour, appearance, and temperature response were observed between the strains (*Table 1*, *Figures 2 & 3*).

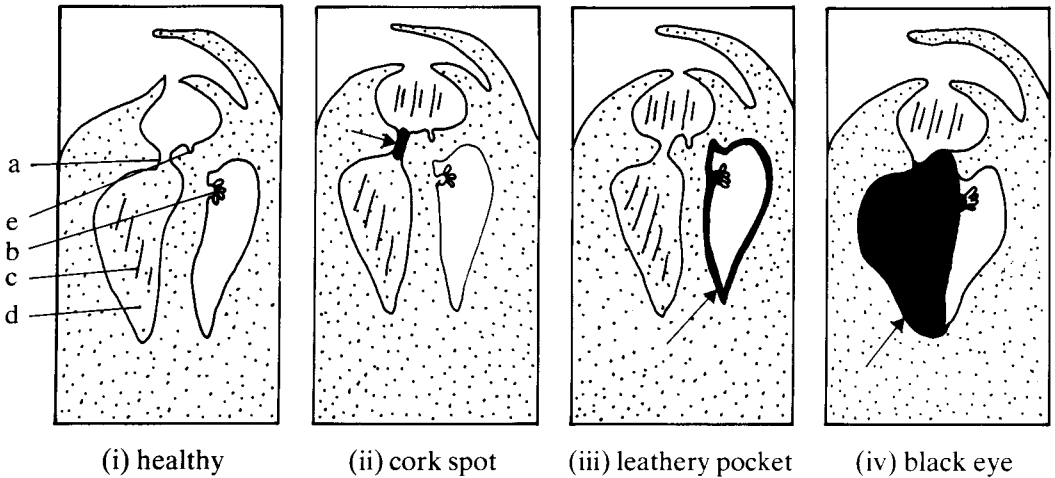
On PDA, strains 1 and 5 do not normally produce red pigmentation, although the former may produce yellow to brown pigmentation. Strains 2, 3, 4 and 6 produce varying degrees of red pigmentation (*Figure 3*). Strain 2 is similar to strain 3 with the exception that the latter produces numerous small black microsclerotia ranging in diameter from 0.2 to 0.5 mm. Strain 4 is distinguished from the rest by its yellow mycelium and by the presence of coremia. Strain 5 is characterised by its pinkish-orange mycelium and the presence of short stout pillars of loose erect mycelium measuring 2 mm wide by 2–4 mm high. It is interesting to note that the non-pigmented (red) strains 1 and 5, are relatively slower growing than the pigmented strains on PDA (*Table 1*).

All the six strains were confirmed by the Commonwealth Mycological Institute to be *P. funiculosum*.

The semi-selective medium used in the



**Transverse section of fruitlet**



**Longitudinal section of fruitlet**

Figure 1. Diagrams of pineapple fruitlets to illustrate the common fruit blemishes of pineapple

- a = nectary duct
- b = ovules
- c = nectary gland
- d = fruitlet core (septum)
- e = styler canal

study was found useful in aiding the differentiation of strains and in suppressing bacteria and many other fungi. Growth of the *P. funiculosum* isolates was slower and sporulation was delayed. The strains were distinguished readily by the following cultural characteristics:—

Strain 1 — colony white, dome-shaped, mycelium very dense with long funiculose strands. (Figure 4).

Strain 2 or 3 — colony white, low and sparse floccose mycelium (Figure 4).

Strain 4 — colony white and yellow, center green, dense floccose mycelium not dome-shaped.

Strain 5 — colony dirty green, mycelium low and sparse; fastest growing of the six strains (Figure 4).

Strain 6 — colony light pink, mycelium low and densely floccose.

TABLE 1: CULTURAL CHARACTERISTICS OF SIX STRAINS OF *PENICILLIUM FUNICULOSUM* (ON PDA) ISOLATED FROM PINEAPPLE FRUITS

Strains	Cultural characteristics					
	Sporulation/ colony colour	Aerial mycelium	Mycelium colour	Red pigmentation (reverse)	Micro- sclerotia	Diameter (mm) (10 days @ 28°C)
1	Abundant/ deep green	floccose, numerous long funiculose strands (up to 10 mm)	White	absent (yellow to brown (pig. may be present))	absent	50
2	Abundant/ deep green	widely spreading, few short funiculose strands (2-3 mm long)	white	present	absent	80
3	Sparse/ deep green	closely floccose, few short funiculose strands (2-3 mm long)	white	present (faint)	present	78
4	Abundant/ deep green	closely floccose, coremia present	white/ yellow	present	absent	75
5	Abundant/ deep green	closely floccose, few funiculose strands (2-3 mm long)	white	absent (old cultures slightly pink at centre)	absent	47
6	Sparse/ deep green	numerous short thick pillar-like mycelial strands (2-4 mm long x 2 mm wide)	white/ pink orange	present	absent	77

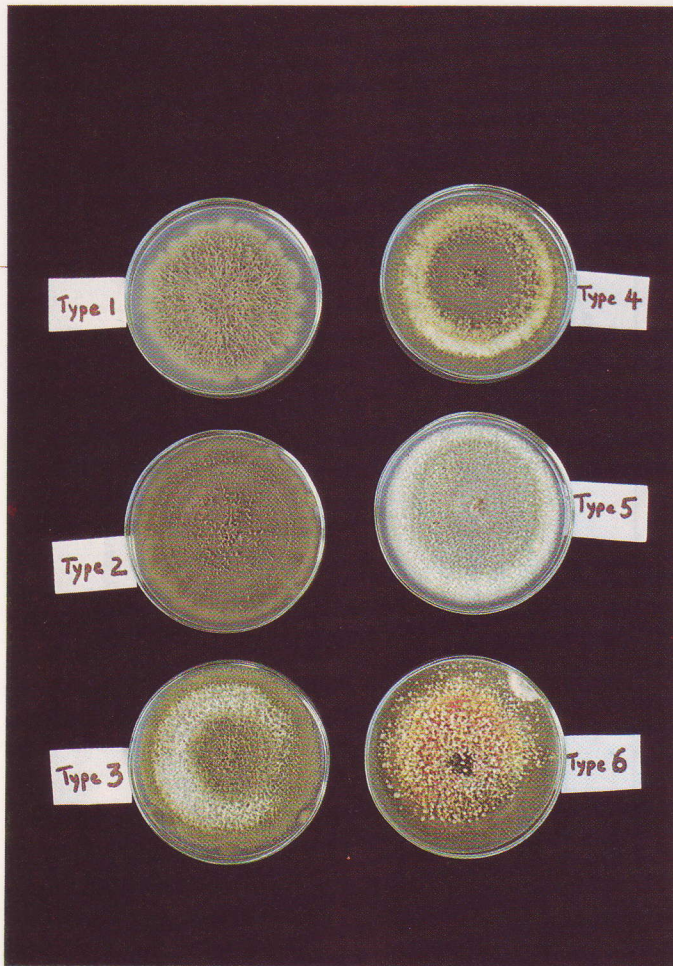


Figure 2. Obverse view of six morphological strains of *P. funiculosum* cultured on PDA.

#### Frequency of *P. funiculosum* strains and *F. moniliforme* associated with fruit blemishes

Table 2 lists the frequency with which the various strains of *P. funiculosum* and *F. moniliforme* were isolated from the three fruit blemishes studied. Strain 1 was obtained from all three fruit blemishes but was most frequently isolated from LP tissues. Similarly, strain 3 was found on all three blemishes but was most predominant on CS tissues. In contrast, strain 6 was only isolated from black eye tissues. Strains 2 and 3 were less frequently isolated than the other

*P. funiculosum* isolates. *F. moniliforme* was isolated from all three blemishes, but was most frequently obtained from LP tissues.

#### CONCLUSION

This paper discusses the strains of *P. funiculosum* and *F. moniliforme* associated with pineapple fruit blemishes. As a result of the study, six morphological strains of *P. funiculosum* were identified. Earlier studies done in Hawaii had shown the existence of only three strains (LIM, 1977).

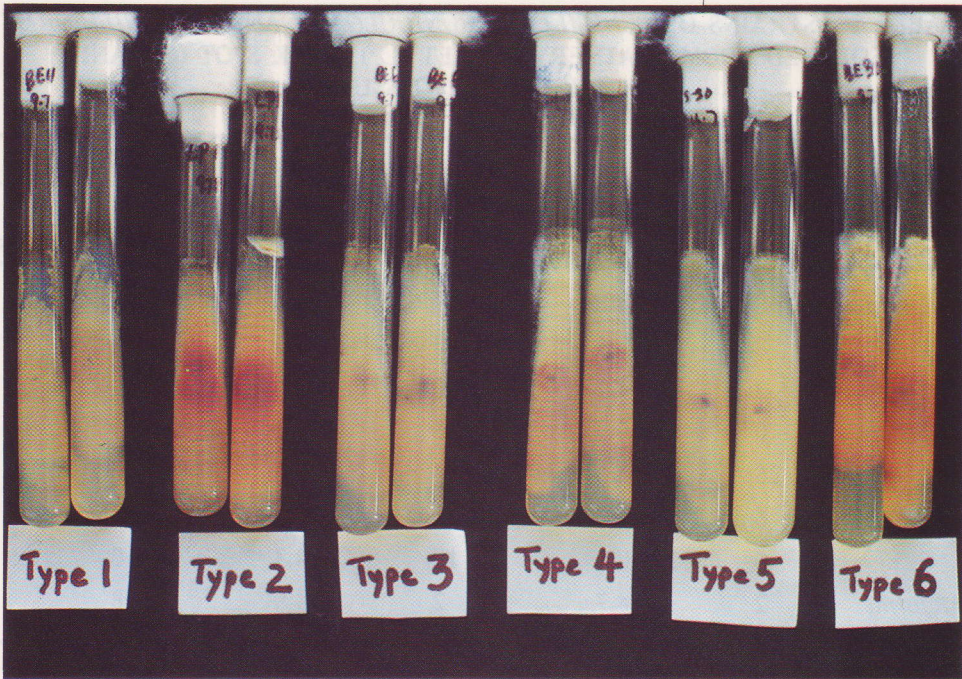


Figure 3. The reverse of six morphological strains of *P. funiculosum* cultured on PDA test tube slants.

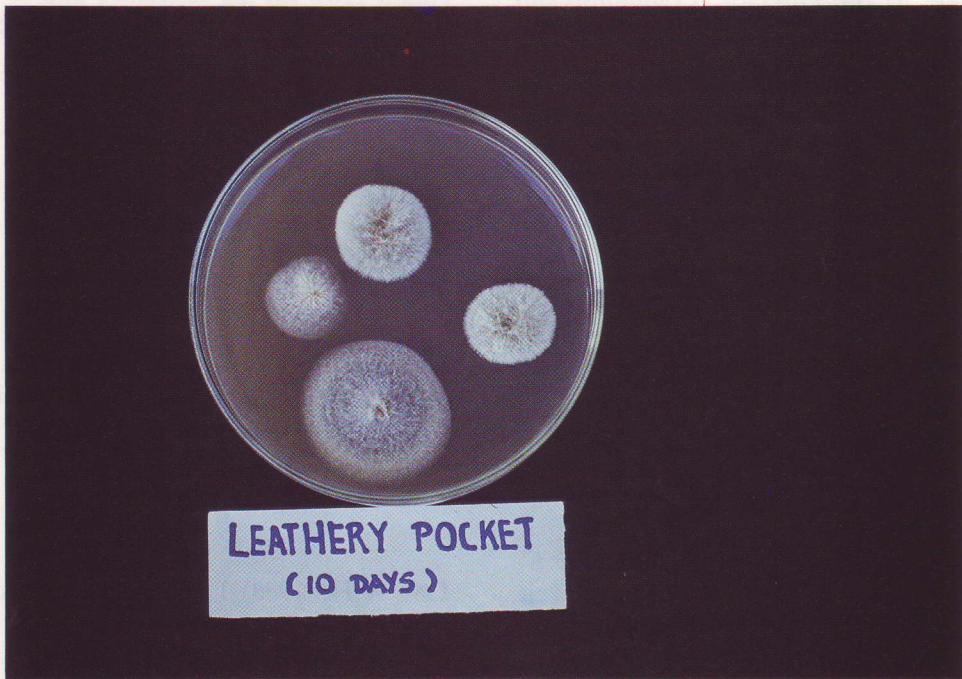


Figure 4. *P. funiculosum* isolates on the amended PDA medium. Strain 1 (top and centre right), Strain 5 (bottom) and Strain 2 (centre left).

TABLE 2: STRAINS OF *PENICILLIUM FUNICULOSUM* AND *F. MONILIFORME* ISOLATED FROM LEATHERY POCKET (LP), CORK SPOT (CS) AND BLACK EYE (BE) TISSUES

	CS		BE		LP	
	No. isolates	% frequency	No. isolates	% frequency	No. isolates	% frequency
<i>P. funiculosum</i>						
Strain 1	8	18.5	20	48.8	33	50.7
2	2	4.7	0	0	5	7.7
3	0	0	5	12.2	3	4.6
4	25	58.1	4	9.8	3	4.6
5	6	14.0	0	0	3	4.6
6	0	0	10	24.3	0	0
<i>F. moniliforme</i>	2	4.7	2	4.9	18	27.7

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#### SUMMARY

Fungi isolated from pineapple fruit blemishes, viz. leathery pocket, cork spot and fruitlet core rot (or black eye) were examined and identified. *Penicillium funiculosum* was most frequently isolated followed by *Fusarium moniliforme*. Six morphological strains of *P. funiculosum* were distinguished, based on pigmentation, colony characteristics, micro-sclerotia production and growth. The frequency of isolation of the strains from the three types of blemishes was also studied.

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