Introduction

Pitaya (Hylocereus spp.) is one of the important newly emerging fruit crops in Taiwan. However, the fruit production is limited by pitaya canker and wet rot, which were found in 2009 (Chuang et al. 2012) and 2012 (Lin et al. 2014), respectively. The pathogen identification and management of these two diseases are introduced in this paper.

Pitaya Canker

Pitaya canker is now considered to be the most severe disease of pitaya in Taiwan in recent years. The disease could be found in nearly 80% of the commercial planting areas. At the early stage of the disease, the symptoms on the stem are small, circular, orange sunken spots, and then develop into cankers. At the late disease stage, stems subsequently rot, and pycnidia could be observed on the surface of the cankers (Fig. 1A-C). The similar symptoms were also visible on the infected fruits (Fig. 1D-F). The pathogen could also infect the fruits after harvest. The infected fruits showed brown to black water-soaked rot symptom (Fig. 1G), and eventually dried with numerous pycnidia erumpent from the surface.

Fig. 1. Neoscytalidium dimidiatum caused canker symptoms on stems (A-C), young fruits (D-F) and harvested mature fruit of pitaya (G).
Identification of the pathogen of pitaya canker

Tissues adjacent to cankers were placed on acidified potato dextrose agar (PDA) and incubated at room temperature. After culturing for 5-7 days, fungal colonies grew from the disease tissues with dark gray-to-black aerial mycelium. Under the light microscope, hyphae were branched, septate, and pale brown, and easily disarticulated into 0- to 1-septate arthrospores (Fig. 2A). Conidia (12.79 ± 0.72 × 5.14 ± 0.30 μm) produced in pycnidia, were one-celled, ovate and hyaline (Fig. 2B). Dark, septate and 3-celled conidia were not observed on agar media but in the diseased samples (Fig. 2C). The extracted DNA of the cultured mycelium was amplified by PCR reaction with the primer pair ITS1 and ITS4 for the internal transcribed spacer (ITS) region. The ITS (ITS1-5.8S-ITS2) sequence (GenBank Accession No. HQ439174) showed 99% identity to Neoscytalidium dimidiatum (Penz.) Crous & Slippers (GenBank accession No. GQ330903). On the basis of morphology and nucleotide-sequence identity, the isolates were identified as N. dimidiatum.

The taxonomic classification of N. dimidiatum is as follows (Crous et al. 2006).

Fungi
Ascomycota
Dothideomycetes
Botryosphaeriales
Botryosphaeriaceae
Neoscytalidium

Fig. 2. Arthroconidia (A) and young (B) and mature (C) pycnidal conidia of Neoscytalidium dimidiatum in vitro (A-B) and in diseased samples of field (C). Bar = 10 μm.

Pathogenicity and epidemic study of Neoscytalidium dimidiatum

Disease development was observed in detail by inoculating conidia of N. dimidiatum on pitaya stems with wound treatment in the field. After inoculation, small, circular, orange sunken, orange spots appeared and then developed into cankers 2 weeks later. Black pycnidia were observed on the surface of the diseased tissue 6 weeks after inoculation, and the lesion tissue fell off and form shot-hole symptom on the stem 12 weeks after inoculation. The infection could extend from the shot-hole edge to the healthy tissue and resulted in the collapse of a large area of stem tissue 18 weeks after inoculation.

So far, we have observed that the primary inoculum source of pitaya canker in the orchard are from the diseased seedlings and spreads through water and rain. It has also been observed that the pathogen can spread and infect the tissue by arthrospores and pycnidial conidia in the field only in the existence of free water under high humidity.

Besides, according to the experiments in vitro, the optimal temperatures for mycelial growth and pycnidial conidia germination of N. dimidiatum were 25–35°C (Fig. 3) and 20–40°C in free water (Fig. 4), respectively.

In summary, these evidences might be able to explain that the symptoms of canker of pitaya are observed nearly 1 to 2 months after raining and the disease spreads faster in the hot and raining seasons, which are spring and summer in Taiwan.
Chemicals selection for the control of pitaya canker

According to the plant protection manual in Taiwan, there are 9 synthetic chemicals could be used legally on pitaya. The tests of effects of these 9 chemicals on N. dimidiatum were conducted on agar media. Mycelial growth was effectively inhibited in vitro by cyprodinil + fluoxonil, azoxystrobin + difenoconazole and tebuconazole at 10 ppm a.i. Pycnidial conidia germination of N. dimidiatum in vitro was inhibited by metiram, trifloxystrobin, pyraclostrobin, azoxystrobin, azoxystrobin + difenoconazole and iminoctadine at 10 ppm a.i. These fungicides were therefore recommended to control canker of pitaya in the field in Taiwan. Further tests should be conducted in the field before these chemicals are recommended to control the disease (Figs. 5-6) (ref).

Furthermore, for management of organic farming, the tests of effect of Bordeaux mixture (formulas: 2-2-1 and 4-4-1; copper sulfate (g) - lime (g) - water (L)) on pathogen was also conducted on agar media. The result showed that these materials effectively inhibit both mycelial growth and arthrospore germination of N. dimidiatum in vitro. What’s more, it was proved that disease severity was reduced with the application of Bordeaux mixture in greenhouse (Fig. 7) and the field. So far, Bordeaux mixture has been recommended to control the disease in Taiwan.

Management of pitaya canker in Taiwan (Fig. 8)

1. For preventative management, using disease-free cuttings is suggested since it had been found that propagative material were the major primary inoculum in the orchard.
2. To irrigate, avoid spraying the water from the top of the pitaya.
3. To reduce the inoculum, cankered stems and fruits should be pruned and removed out of the orchard.
4. To eradicate or reduce the disease, in the cold and dry seasons, disease management should be taken action more frequently, since the development and spray of conidia of N. dimidiatum is slowing down and reduced due to cold temperature and less raining respectively.
5. 7-10 days before and 1 day after pruning, spray the chemicals mentioned above subsequently.
6. As Bordeaux mixture can leave a blue-green discoloration on the surface of the tissue, the application of the material is not advised at fruiting season unless the fruits are bagged.

PITAYA WET ROT

Pitaya wet rot caused by Gilbertella persicaria (Eddy) Hesseltine severely affected flowering, fruiting and postharvest storage of pitaya in Taiwan (Lin et al. 2014). In the disease survey of pitaya orchards, the infection occurred mainly during rainy season. Diseased flower buds (Fig. 9A), petals (Figure 9B) and young fruits (Fig. 9C) showed brown and small water-soaked lesion. Most of the infected fruits in the orchard failed to develop normally and dropped prematurely. Mature fruits were infected when harvested or during storage and appeared water-soaked and soft rot on the fruit appearance (Fig. 9D). Wet rot of fruits usually initiated from the stem-ends, occasionally from the middle of fruits or scales, and could develop into completely fruit rot 2-4 days after infection. After harvest, fruits could be infected through the wounds and become rotted during storage or transportation. Brown to black sporangia of G. persicaria could be observed producing on the surface of infected tissues, especially under humid conditions.

Identification and differences of Gilbertella persicaria var. pitaya

According to the description by Benny (1991), the taxonomic classification of G. persicaria is as follows:

- **Fungi**
  - **Zygomycota**
  - **Zygomycetes**
  - **Mucorales**
  - Gilbertellaceae

The morphology of G. persicaria var. pitaya (Figure 10) was similar with the description of the holotype of G. persicaria (CBS 190.32, isolated from rot peach, G. persicaria var. persicaria) (Table 1). However,
interestingly, in *G. persicaria* var. *pitaya*, the number of longitudinal sutures on the sporangial wall were found no less than 1 and splitting the sporangial wall into 2-7, usually 4, equal pieces, while there was only 1 suture in the holotype; the number of the appendages at the ends of the sporangiospores of the pitaya isolates were 0-3 while 2-7 in the holotype.

Table 1. Morphological characteristics of *Gilbertella persicaria* var. *persicaria* and *G. persicaria* var. *pitaya*.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporangiothorpe (branched or rarely branched; bent when young, upright at maturity)</td>
<td>19-33 μm in diam.</td>
<td>18-50 μm in diam.</td>
</tr>
<tr>
<td>Sporangia (yellow to dark brown, more or less globose; covered with calcium oxalate crystals)</td>
<td>45-170 μm in diam.;</td>
<td>60-115 μm in diam.</td>
</tr>
<tr>
<td>Columella (with basal collar; obpyriform, oboboid to cylindrical)</td>
<td>36-81 μm in diam. at the widest</td>
<td>25-60 μm in diam. at the widest</td>
</tr>
<tr>
<td>No. of longitudinal suture (on the surface of sporangia wall)</td>
<td>1</td>
<td>1-5</td>
</tr>
<tr>
<td>No. of separated sporangial wall</td>
<td>2</td>
<td>2-7 (avg. 4)</td>
</tr>
<tr>
<td>Sporangiospores (globose, ellipsoid to ovoid)</td>
<td>5.1-17.8 × 3.8-12.7 μm</td>
<td>5.5-11.8 × 4.5-8.8 μm</td>
</tr>
<tr>
<td>No. of hyaline appendages (up to 24 μm, at the ends of sporangiospores)</td>
<td>2-7</td>
<td>0-3</td>
</tr>
<tr>
<td>Chlamydospores (globose to irregular; light brown, smooth-walled)</td>
<td>15-29 × 10-16 μm</td>
<td>18.9-25. × 14.9-23.8 μm</td>
</tr>
</tbody>
</table>

Occasionally, field survey also showed that *Rhizopus stolonifer* could be found in the petal of withered flowers and might be confused with *G. persicaria* var. *pitaya*. These fungi can be distinguished by morphology and maximum temperature. The differences are listed in the Table 2.

Table 2. Differences of diagnostic characters between *Rhizopus stolonifer* and *Gilbertella persicaria*.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>Rhizopus stolonifer</em> (Schipper and Stalpers. 1984; Liou et al. 2007)</th>
<th><em>Gilbertella persicaria</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporangiospore with appendages</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Max. growth temp.</td>
<td>33 °C</td>
<td>38 °C</td>
</tr>
<tr>
<td>Rhizoid</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sporangiothorpes</td>
<td>Branchless; erect</td>
<td>Branched or rarely branched; bent or upright</td>
</tr>
<tr>
<td>Sporangia</td>
<td>Up to 200-300 μm</td>
<td>Less than 180 μm</td>
</tr>
<tr>
<td>Sporangial wall</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
In addition to morphological characteristics, identity of \textit{G. persicaria} var. \textit{pitaya} was also confirmed by ITS (ITS1-5.8S-ITS2) sequence analysis. The identity between \textit{G. persicaria} var. \textit{pitaya} and the holotype of \textit{G. persicaria} was 98.4\%, which with 6 additional and 5 different nucleotides in the \textit{G. persicaria} var. \textit{pitaya} (Lin et al. 2014).

We also inoculated fruits of pitaya with the \textit{G. persicaira} var. \textit{persicaria} (CBS190.32, the holotype, and F209130). The water-soak lesions caused by these isolates were smaller and browner than those of \textit{G. persicaria} var. \textit{pitaya}.

In summary, these data suggested the \textit{G. persicaria} var. \textit{pitaya} was different from the holotype of \textit{G. persicaria} in regards of the sporangial wall, ITS sequence and virulence to fruits of pitaya.

\textbf{Pathogenicity of Gilbertella persicaria var. pitaya}

Our data showed the optimal temperatures for \textit{G. persicaria} hypha growth were 24-36$^\circ$C (Fig. 11), maximum at 38$^\circ$C. In order to understand the effect of temperature on the disease development of pitaya wet rot, healthy mature fruits were collected from the field for artificial inoculation with sporangiospores of the pathogen. The tests results showed that wounding and higher temperatures (24-36$^\circ$C) resulted in severe infection, indicating that wounding and temperature were important factors affecting the disease severity (Lin et al. 2014).

Results of artificial inoculations also showed that \textit{G. persicaira} var. \textit{pitaya} were pathogenic on fruits of peach and wounded fruits of apple, mango, persimmon, plum and tomato, but they were non-pathogenic on banana and kiwifruits. These results suggested that \textit{G. persicaria} var. \textit{pitaya} might be a potential pathogen for these sensitive fruits in the field or upon marketing and affect the storage periods in the market.

\textbf{Chemicals selection for the control of wet rot}

Synthetic chemicals with different inhibition mechanism were selected to test the inhibition to \textit{G. persicaira} var. \textit{pitaya} on agar media, although some chemicals, like fluazinam, difenoconazole, tridemorph and prochloraz-Mn have not been yet authorized to be used on pitaya. The data showed that the mycelial growth was completely inhibited by cyprodinil + fludinonoxin at 1 ppm a.i., and pyraclostrobin, prochloraz-Mn, tridemorph, difenoconazole and tebuconazole at 100 ppm a.i.. The sporangiospore germination was completely inhibited by fluazinam at 1 ppm a.i., and cyprodinil + fludinonoxin, difenoconazole, metiram, tebuconazole, tridemorph, prochloraz-Mn and pyraclostrobin at 10 or 100 a.i..

The tests of effect of some control agents for organic farming on pathogen were also conducted on agar media. The data showed that neutralized phosphorous acid and lime sulfur solution could inhibit sporangiospores germination at 1000 and 500 dilution rate respectively while Bordeaux mixture with formulas of 2-2-1 and 4-4-1 (copper sulfate (g)- lime (g)- water (L)) could inhibit both mycelial growth and sporangiospores germination. As for the plant extracts, both cinnamon oil products - Hey-Show-Lo and Tan-Wu-Zang - could inhibit mycelial growth and sporangiospores germination at 1000 and 4000 dilution rate respectively, lemongrass oil product - Mei-Tsow - at 1000 and 500 dilution rate respectively, and chinese gall extract product -Hwo-Li-Nen- at 500 dilution rate.

\textbf{Management suggestions of wet rot}

The disease severity of pitaya wet rot is closely related with the occurrence and frequency of the raining based on the epidemic investigation. In addition, the sporangia would emerge from the diseased tissue quickly under high humidity, and the sporangiospores could spread out by rain drops and wind.

Based on the epidemic and pathogenic studies so far, advice are given as follows.

1. To reduce the inoculum, flowers and fruits with wet rot symptoms should be excised, and then packed and removed out of the orchards immediately.
2. For crop protection in the flowering period in the field, sporangiospores germination inhibiting chemicals and mycelia growth inhibiting chemicals are recommended to be used before and after raining, respectively.
3. To reduce fruit infection, harvesting the fruits on the raining day should be avoided, and the wounds and the surface of the fruits should be dried before packaging.

\textbf{REFERENCE}