Oil Palm Pestalotiopsis Leaf Spot Disease Endemic In Southeast Asia Is Attributed To A Complex Of Synergisms Between Microbial Pathogens And Not By A Singular Pathogen

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**PESTALOTIOPSIS LEAF SPOT DISEASE in LATIN AMERICA**

**Symptoms:**
Appearance of brown spots with yellowish haloes which quickly turn brown and necrosis spreads over the leaf parenchyma.

**Pathogen(s):**
- *Pestalotiopsis palmarum* (Labarca et al., 2006)
- Escalante et al., (2010) reported that the disease was attributed to a **fungal complex involving** *P. palmarum, P. glandicula, Colletotrichum, Curvularia, Gloesporium and Helminthosporium.**

**Insect vectors** (Lepidoptera insect vectors and Hemiptera (Leptopharsa gibbicarina). Disease severity of Pestalotiopsis damage increased in the presence of pest outbreaks.
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**Impact on FFB yields:**
- Infection can cause defoliation of the canopy and in severe cases can spread to the upper canopy.
- Under such severe conditions, yield reductions from 30 to 5 tonnes FFB/ha/yr over a 4-year period were reported, reviewed by Martínez & Plata-Rueda (2013).
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**Insect vectors** (Lepidoptera insect vectors and Hemiptera *(Leptopharsa gibbicarina)*. Disease severity of *Pestalotiopsis* damage increased in the presence of pest outbreaks.

**Control measures:**
“Chemical treatments have to be used against the insects rather than against the pathogen”
- Imidacloprid ± B. T ± Beuveria
- Monocrotophos via trunk injection.

From our own trials testing methamidophos, monocrotophos, dimehypo and acephate distribution in oil palm fronds, chemical distribution is lower in older fronds and also affected by trunk height.

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LEAF SPOT DISEASE OF OIL PALM IN SOUTHEAST ASIA

Symptoms in Southeast Asia

- Symptoms
- Epidemiology
- Pathogen
- Control measures
- Effect on growth and FFB yields
- On-going research
LEAF SPOT DISEASE OF OIL PALM IN SOUTHEAST ASIA
INTRODUCTION

- Leaf spot diseases is **not a fatal disease** of Oil Palm.
- In Southeast Asia, the incidence of leaf spot disease has risen in the last decade.
- It was only in the mid-2000s that AAR started observing its rising incidence, notably worse off in the Southern states of Malaysia, and now commonly sighted throughout East and West Malaysia, Indonesia and reported as well in Thailand and China.
Early symptoms:

- Translucent **circular and elliptical orange spots** with light brown to dark **brown sunken centre** in the centre of the lesion.
- The orange to yellow halo is only obvious against a light source otherwise appears as dark orange spots.
- Easily **mistaken for K-deficiency spotting**

Advanced stage symptoms:

- Severity/number of leaf **spots increases with older fronds**.
- Localised spots coalesce leading to necrotic lesions which eventually die and become dry and brittle.
PESTALOTIOPSIS LEAF SPOT DISEASE in MALAYSIA:

EPIDEMIOLOGY

- Survey of 6 blocks reveal a correlation between elevation and severity of disease.
  - Higher terrain has LOWER disease incidence.
  - Lower/flat terrain has HIGHER disease incidence.
- The main factors are likely (1) below canopy HUMIDITY and (2) below canopy TEMPERATURE which varied with terrain.
OIL PALM LEAF SPOT DISEASE in MALAYSIA: SEARCHING FOR THE PATHOGEN

Thailand: *P. theae*

First report of leaf spot disease on oil palm caused by *Pestalotiopsis theae* in Thailand

- Nakarin Suwannarach - Kanaporn Sejarit - Jatavong Kamla - Rosomam Basabhan - Saisaman Lunnong
- 221

China: *P. microspora*

First Report of *Pestalotiopsis microspora* Causing Leaf Spot of Oil Palm (*Laeis guineensis*) in China

Molecular identification is now commonly used in the identification and classification of *Pestalotiopsis* species but due to its cryptic sequence, i.e. high sequence homology of the ITS gene, inter-specific delineation is unsuccessful.

Taxonomy name is based on the International Code of Nomenclature for algae, fungi and plants but the species named has in the past been named according to their host associations, e.g. *P. mangiferae* named after the host *Mangifera indica* (mango).

To further resolve taxonomic issues, a combined dataset DNA sequence comprising ITS, β-tubulin and *tef1* gene is recommended, i.e. via a multi-loci approach.

99% similarity to *P. palmarum*

100% similarity to *P. neglecta*
**PESTALOTIOPSIS DIVERSITY IN OIL PALMS ISOLATED FROM DIFFERENT STATES ACROSS MALAYSIA**

Isolation was carried out from diseased lesions of infected oil palm pinnae as well as asymptomatic or healthy tissues. Isolation resulted in several Pestalotiopsis species with distinct morphometric characteristics. So, which are pathogenic?

- **P. olivacea**
- **P. zonata**
- **P. clavispora**
- **P. versicolor**
- **P. mangiferae**
- **P. theae**
- **P. microspora**
- **P. neglecta**

*Thailand*  

*Southern China*  
Shen et al., (2014) APS. 98: 1429.2
CONFIRMING PATHOGENICITY OF PESTALOTIOPSIS ISOLATES/SPECIES

- 3-month old oil palm plantlets were transplanted in sealed plastic containers with acid-washed sand culture.
- Infection can only be induced under humid conditions (55-60% RH) while temperature maintained at 28°C (daylight conditions) and reduced to 22-24°C (dark conditions).
- Each treatment comprises 5 replicates with 6 plants/replicate.
- Infection was observed over a period of 2-3 weeks.

Symptoms of leaf spotting observed while re-isolation of the inoculant was successful.

Symptom differed from leaf spot disease symptoms observed in the field
OIL PALM LEAF SPOT DISEASE in MALAYSIA: IDENTIFYING THE PATHOGEN

In addition to several different *Pestalotiopsis* species isolated from necrotic disease tissue lesions, other fungi were also isolated which included pathogenic fungi (*Curvularia* sp., *Colletotrichum* sp. and *Nigrospora* sp.). However, these were occasionally isolated.

- **Commonly isolated together with Pestalotiopsis**
- **Occasionally isolated**
- **Pestalotiopsis**

*Aspergillus* sp. and *Phomopsis* sp. were commonly isolated together with *Pestalotiopsis* sp.
OIL PALM LEAF SPOT DISEASE in MALAYSIA: IDENTIFYING THE PATHOGEN

- Co-inoculation with other fungal isolates commonly isolated from leaf spot disease pinnae tissues produced more severe symptoms which resembled more closely with field symptoms.

- Inoculation with individual species (*Phomopsis* sp. and *Aspergillus* sp.) did not induce leaf spot disease symptoms.

- All inoculants were re-isolated and thus complying with Koch’s postulates.

Hence, we have associated leaf spot disease symptoms to be attributed to interspecies or complexes of interacting pathogenic fungi involving *Pestalotiopsis* species (*P. theae, P. microspora, P. virgulata, P. versicolor* and *P. crassiuscula*), *Aspergillus* sp. and *Phomopsis* sp.
PESTALOTIOPSIS LEAF SPOT DISEASE of OIL PALM in MALAYSIA:
CONTROL MEASURES

- Semi-commercial fungicide trial on 2002 planting (9 year old Dxp palms on Rengam/Beserah soil series; Tipik Lutualmekuts)
- 3 replicates per treatment.
- 200-250 palms per replicate
- LSD census: n=80 palms per replicate/lote
- Dosage as per the manufacturer's recommendation for oil palm

<table>
<thead>
<tr>
<th>Treat. No.</th>
<th>Active Ingredient</th>
<th>Cost/Ha (RM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>T2 *</td>
<td>Benzimidazole</td>
<td>28.56</td>
</tr>
<tr>
<td>T3 *</td>
<td>Carbendazim</td>
<td>39.17</td>
</tr>
<tr>
<td>T4 #</td>
<td>Thiram</td>
<td>42.43</td>
</tr>
<tr>
<td>T5 #</td>
<td>Cu oxychloride</td>
<td>78.09</td>
</tr>
<tr>
<td>T6 #</td>
<td>Propineb</td>
<td>88.13</td>
</tr>
<tr>
<td>T7 #</td>
<td>Metiram</td>
<td>33.05</td>
</tr>
<tr>
<td>T8 ##</td>
<td>Chlorothalonil</td>
<td>35.20</td>
</tr>
<tr>
<td>T9 ##</td>
<td>Enoxiconazole</td>
<td>251.34</td>
</tr>
</tbody>
</table>

* Contact fungicides
# Systemic fungicides
## Partially systemic fungicides

1 USD = RM4.15
PESTALOTIOPSIS LEAF SPOT DISEASE of OIL PALM in MALAYSIA: CONTROL MEASURES

- All fungicides were capable of lowering disease incidence.

- The effective duration of each fungicide application round increased with each application:
  - 1st application: \( p > 0.05 \)
  - 2nd application: \( p < 0.05 \) with 4-5 months effective control duration
  - 3rd application: \( p < 0.05 \) with 7 months effective control duration
PESTALOTIOPSIS LEAF SPOT DISEASE of OIL PALM in MALAYSIA:
IMPACT ON OIL PALM GROWTH AND FFB YIELDS

- VGM was carried out at 0 and 12 MAT. 10% of palms within each plot were selected for VGM measurements.

- Fungicide treated palms which exhibited a reduction in disease incidences ($p<0.05$) had higher VGM values.
PESTALOTIOPSIS LEAF SPOT DISEASE of OIL PALM in MALAYSIA: IMPACT ON OIL PALM GROWTH AND FFB YIELDS

- Fungicide treated palms had higher FFB yields compared to the untreated control plot. FFB yield difference between treated and control plots ranged from 7.8-24.7%, averaging at 16.3%.

- Fungicide treated palms which exhibited a reduction in disease incidences \((p<0.05)\) had higher VGM values as well as higher FFB yields, indicative that if left untreated, severe leaf spot disease reduce oil palm VGM and FFB productivity, attributed to:
  
  a. Reduction in photosynthesizing leaf tissue.
  
  b. Reduction in total carbohydrate content in control vs. fungicide treated palms.

% Increase in FFB yields (Fungicide treated vs. control)

Average T2-T9: 16.3% increase in FFB yields

Control / untreated plots \((n=3\text{ replicates}; \bar{x} = 247\text{ palms per rep})\)

Fungicide treated plots \((n=3\text{ replicates}; \bar{x} = 247\text{ palms per rep})\)
LEAF SPOT DISEASE OF OIL PALM IN SOUTHEAST ASIA

Symptoms in Southeast Asia

Symptoms
Epidemiology
Pathogen
Control measures
Effect on growth and FFB yields
On-going research
PESTALOTIOPSIS LS DISEASE of OIL PALM in MALAYSIA: ON-GOING RESEARCH

CURATIVE CONTROL MEASURES VIA FUNGICIDES

1. Screening fungicides reportedly with higher efficacy to control Ascomycete fungal pathogens

   **Objective:** By increasing fungicide efficacy, can we reduce spraying rounds?

PREVENTIVE CONTROL MEASURES

1. Evaluation of Si as a preventive control measure

   **Objective:** To identify sustainable measures to treat diseases by reducing agro-chemical usage in oil palm cultivation. Furthermore, fungicides will also affect the natural balance of fungal biodiversity in oil palm which also comprises beneficial fungi.

2. Screening for tolerant planting materials

   **Objective:** To identify tolerant materials against leaf spot disease amongst AAR commercial planting materials.
PESTALOTIOPSIS LEAF SPOT DISEASE of OIL PALM in SOUTHEAST ASIA: LIFE CYCLE

- Frond
- Frond heaps (decomposing fronds)
- Conidiogenous cells
- Pycnidium
- Perithecium
- Invade leaf tissue

Able to isolate the pathogen but did not observe conidiogenous cells

- Pycnidium and conidiogenous spores were frequently observed on decomposing fronds, i.e. frond heaps which serve as the main source of inoculum.

- Increase in disease severity with frond age.
- Under severe conditions, infection can lead to lower frond desiccation.

Infected fronds
Frond #4
Frond #12
Frond #20
Frond #28
Frond #36
Frond heaps (decomposing fronds)
ON-GOING RESEARCH:

1. Screening of fungicides to determine fungicides with higher efficacy to control leaf spot diseases based on their IC\textsubscript{50} values (Half Maximal Inhibitory Concentration of fungicides (mg/L)).
2. DMI fungicides have lower IC\textsubscript{50} values and thus capable of inhibiting fungal growth at lower dosages.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Pathogenicity</th>
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<tbody>
<tr>
<td><strong>High IC\textsubscript{50}</strong></td>
<td></td>
</tr>
<tr>
<td>Thiram</td>
<td>QoI</td>
</tr>
<tr>
<td>Pyraclostrobin</td>
<td>QoI</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>Multi-site/Nitrile</td>
</tr>
<tr>
<td>Captan</td>
<td>Contact</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>QoI</td>
</tr>
<tr>
<td>Difenconazole</td>
<td>DMI</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>DMI</td>
</tr>
<tr>
<td>Propineb</td>
<td></td>
</tr>
</tbody>
</table>

QoI – Quinone outside Inhibitor inhibit fungal protein-tyrosine phosphatase
DMI – DeMethylation Inhibitors disrupt fungal sterol/ergosterol biosynthesis

Fungal inhibition was based on surface area scanning of 16-well plates (pre-filled with PDA ± fungicides via a multi-plate spectrophotometer).
Pathogenic strains: 5 isolates of *P. theae*, *P. microspora* and *P. vigulata*
Inhibition assay on 8 fungicides x 9 concentrations (0-100 ppm) x 5 replicates
PESTALOTIOPSIS LS DISEASE of OIL PALM in MALAYSIA: ON-GOING RESEARCH

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Effects of Si treatments on Pestalotiopsis leaf spot disease

Plants were treated with Ca/K monosilicic acid ($H_4SiO_4$) via soil drenching 1 month before infection.

Each treatment comprises 4 technical replicates with 8 plants/replicate.
PESTALOTIOPSIS LS DISEASE of OIL PALM in EAST & SOUTHEAST ASIA: ON-GOING RESEARCH

CURATIVE CONTROL MEASURES VIA FUNGICIDES

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**On-going: Screening for tolerant/susceptible planting materials against Pestalotiopsis LSD**

From our earlier trials in screening pathogenicity of fungal pathogens, we observed differences in disease severity amongst AAR Commercial planting materials

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Susceptibility towards Pestalotiopsis LSD (co-inoculated with <em>Phomopsis</em> sp. &amp; <em>Aspergillus</em> sp.)</th>
<th>Post 2 weeks after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DxP Cross 1</td>
<td>High</td>
<td><img src="image1" alt="High Susceptibility" /></td>
</tr>
<tr>
<td>DxP Cross 2</td>
<td>Low</td>
<td><img src="image2" alt="Low Susceptibility" /></td>
</tr>
</tbody>
</table>

At post 2 weeks after infection/inoculation, early symptoms of disease symptoms observed with DxP Cross 1

**Susceptible vs. Tolerant lines amongst AAR Commercial Planting Materials**

Each treatment comprises 6 replicates with 8 plants/replicate.

Currently screening planting materials to generate sufficient phenotypic data to correlate with genotyping data.
# PESTALOTIOPSIS LS DISEASE of OIL PALM: Similarities between Latin America and Southeast/East Asia

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<tr>
<td><strong>VECTOR</strong></td>
<td>Hemiptera (<em>Leptopharsa</em>) and Lepidoptera insects</td>
<td>? No direct correlation between disease incidence and vector outbreaks</td>
</tr>
<tr>
<td><strong>SYMPTOMS</strong></td>
<td>Early symptoms appear similar while advance stage is different</td>
<td></td>
</tr>
<tr>
<td><strong>EPIDEMIOLOGY</strong></td>
<td>Associated with insect vector outbreaks?</td>
<td>Associated with temperature and humidity. Disease incidence appears to increase upon the on-set of canopy closure, influenced by temperature and rainfall.</td>
</tr>
<tr>
<td><strong>DISEASE IMPACT</strong></td>
<td>VGM and FFB reduction</td>
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<td><strong>CURATIVE CONTROL MEASURES</strong></td>
<td>Vector control via pesticide</td>
<td>Pathogen control via fungicides</td>
</tr>
<tr>
<td></td>
<td>Fungicide control?</td>
<td>Pesticide control?</td>
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<tr>
<td><strong>PREVENTIVE CONTROL MEASURES</strong></td>
<td></td>
<td>1. Silica can confer tolerance</td>
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<td></td>
<td>against complex pathogen but</td>
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<td>remains to be validated under</td>
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<td></td>
<td>field conditions.</td>
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<td>2. Selection of tolerant</td>
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<td>planting materials.</td>
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NUTRITION and/or BEST MANAGEMENT PRACTICES ON PESTS AND DISEASES?
INTEGRATED PESTS & DISEASE MANAGEMENT

In many cases, addressing P&D outbreaks are often based on tackling the pests or diseases either through chemical or sanitation measures.

Efforts to control Pests & Diseases should be addressed holistically.

In many cases, P&D outbreaks are associated to Environmental conditions and poor agro-management practices.
CASE STUDY: IMPLEMENTATION OF OIL PALM BEST MANAGEMENT PRACTICES ON FFB PRODUCTIVITY & DISEASE (BUD ROT) SUPPRESSION AT COROZITO ESTATE

Prior to the implementation of BMP, Corozito estate, despite recording a steady increase in FFB yield, the number of PC/bud rot cases often peaked following a periods of high rainfall.

Subsequent to the implementation of best management practices such as site specific fertilizer recommendations, drainage, soil and moisture conservation measures, weed management and P&D management, FFB yields continued to increase along concurrent with balanced oil palm and soil nutritional levels but importantly began recording lower disease incidences of bud rot (PC) cases.

Data courtesy of Corozito & C. Manrique
THANK YOU