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Host range and reservoirs of *Lasiodiplodia theobromae* causing longan fruit rot in Ba Ria–Vung Tau, Viet Nam

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ABSTRACT

Fruit rot caused by Lasiodiplodia theobromae is one of the major diseases of longan. This study investigated the host range and reservoirs of the pathogen in longan farms of Ba Ria–Vung Tau, Viet Nam. A total of 32 fruits were identified as the hosts of L. theobromae. Among those, 20 fruits showed rot symptoms after pathogen inoculation. Besides the longan cultivars Xuong com vang, Long, Hung Yen, Edor and Tieu da bo, An Phuoc plum was highly susceptible to L. theobromae. Avocado, guava, rough orange, mandarin, rambutan, jackfruit, banana and sapodilla were moderately susceptible to the pathogen. Solanaceae and Cucurbitaceae fruits did not show clear symptoms after pathogen inoculation. Mycelia and pycnidia of L. theobromae could survive in inflorescence peduncles, fruit pedicels and dried fruit tissues which help the pathogen persist in longan farms through different cropping seasons. The pycnidia in these reservoirs release L. theobromae conidia, thus fostering continuous infection of fruit rot in longan farms.

1. INTRODUCTION

In recent years, fruit rot has emerged as the most serious disease affecting longan (*Dimocarpus longan*) production in Ba Ria–Vung Tau, which damaged ranging 45.42% to 74.17% of fruit clusters (Kien et al., 2024). The disease is caused by a complex of pathogens, among which *Lasiodiplodia theobromae* has been identified as the primary causal agent of fruit rot on the Xuong com vang cultivar (Kien, 2026). Species of *Lasiodiplodia* infect a wide range of host plants in tropical and subtropical regions, with more than 500 reported hosts (Punithalingam, 1980; Rosado et al., 2016). In Viet Nam, *Lasiodiplodia* spp. have been reported to cause black spot disease on mango (Dung et al., 2020), postharvest fruit rot on rambutan (Yen & Phong, 2016) and dieback and gummosis on cashew (Man & Ha, 2006). However, the hosts of *L. theobromae* associated with longan fruit rot have not yet been comprehensively investigated in Viet Nam. Moreover, *L. theobromae* has been reported to persist latently in 189 fruits belonging to 60 families (Salvatore et al., 2020) and to survive saprophytically on plant debris (Coutinho et al., 2017; Alam et al., 2020), indicating diverse persistence strategies on farms. Therefore, a comprehensive understanding of the host range and reservoirs of *L. theobromae* is essential for developing effective management strategies for longan fruit rot.

2. MATERIALS AND METHOD

2.1. Materials

Major commercial fruits in Viet Nam, particularly those cultivated in longan-producing areas of Ba Ria–Vung Tau, were selected for this experiment, as listed in Table 1.

Table 1. Commercial fruits were selected for this study

No.	Species	English name	Growth stages	Location
1	*Citrus maxima*	Pomelo	Commercial maturity stage	Ba Ria–Vung Tau
2	*Citrus latifolia* Tanaka	Seedless lime	Commercial maturity stage	Ba Ria–Vung Tau
3	*Averrhoa carambola*	Sweet starfruit	Commercial maturity stage	Ba Ria–Vung Tau
4	*Averrhoa carambola*	Sour starfruit	Commercial maturity stage	Ba Ria–Vung Tau
5	*Solanum lycopersicum*	Tomato	Commercial maturity stage	Ba Ria–Vung Tau
6	*Momordica charantia* cv. Tay	Bitter melon	Commercial maturity stage	Ba Ria–Vung Tau
7	*Solanum melongena*	Eggplant	Commercial maturity stage	Ba Ria–Vung Tau
8	*Solanum macrocarpon*	African eggplant	Commercial maturity stage	Ba Ria–Vung Tau
9	*Solanum melongena* var. *esculentum*	Common eggplant	Commercial maturity stage	Ba Ria–Vung Tau
10	*Pouteria campechiana*	Eggfruit	Commercial maturity stage	Ba Ria–Vung Tau
11	*Cocos nucifera* cv. Xiem	Coconut	Commercial maturity stage	Ba Ria–Vung Tau
12	*Citrus aurantiifolia*	Key lime	Commercial maturity stage	Ba Ria–Vung Tau
13	*Syzygium aqueum* cv. An Phuoc	Wax apple	Commercial maturity stage	Ba Ria–Vung Tau
14	*Dimocarpus longan* cv. Xuong com vang	Longan	Commercial maturity stage	Ba Ria–Vung Tau
15	*Dimocarpus longan* cv. Long	Longan	Commercial maturity stage	Ba Ria–Vung Tau
16	*Dimocarpus longan* cv. Long Hung Yen	Longan	Commercial maturity stage	Hung Yen
17	*Dimocarpus longan* cv. Edor	Longan	Commercial maturity stage	Ba Ria–Vung Tau
18	*Dimocarpus longan* cv. Tieu da bo	Longan	Commercial maturity stage	Ba Ria–Vung Tau
19	*Psidium cattleianum*	Strawberry guava	Commercial maturity stage	Ba Ria–Vung Tau
20	*Citrus reticulata* Blanco	Mandarin orange	Commercial maturity stage	Ba Ria–Vung Tau
21	*Mangifera indica* cv. Cat Chu	Mango	Commercial maturity stage	Ba Ria–Vung Tau
22	*Mangifera indica* cv. Cat Hoa Loc	Mango	Commercial maturity stage	Ba Ria–Vung Tau
23	*Manilkara zapota*	Sapodilla	Commercial maturity stage	Ba Ria–Vung Tau
24	*Citrus deliciosa* Tenore	mandarin	Commercial maturity stage	Dong Thap
25	*Mangifera indica* cv. Keo	Mango	Commercial maturity stage	Ba Ria–Vung Tau
26	*Citrus reticulata* × *maxima*	Tangelo	Commercial maturity stage	Ba Ria–Vung Tau
27	*Nephelium lappaceum* cv. Java	Rambutan	Commercial maturity stage	Ba Ria–Vung Tau
28	*Persea americana* cv. Quoc Minh	Avocado	Commercial maturity stage	Ba Ria–Vung Tau
29	*Persea americana* cv. Sap tron	Avocado	Commercial maturity stage	Ba Ria–Vung Tau
30	*Artocarpus heterophyllus* cv. To nu	Jackfruit	Commercial maturity stage	Ba Ria–Vung Tau
31	*Musa acuminata* cv. Tieu	Banana	Commercial maturity stage	Ba Ria–Vung Tau
32	*Psidium guajava*	Guava	Commercial maturity stage	Ba Ria–Vung Tau

2.2. Methods

2.2.1. Survey of the host range of Lasiodiplodia theobromae

Preparation of conidial suspension: A conidial suspension of *Lasiodiplodia theobromae* X07 (10^4 conidia/mL) was prepared from pycnidia formed on potato dextrose agar (PDA) after 21 days of incubation under laboratory conditions (28 ± 3°C).

Preparation of fruit species: Fruits were surface sterilized following the method described by Pipattanapuckdee et al. (2019) and air-dried in a laminar airflow cabinet until inoculation. Each fruit species was arranged in three plastic boxes, as described by Suwanakood et al. (2007). Small plastic boxes (20 × 15 × 10 cm) contained 10 fruits of comparable size to longan fruits, whereas larger boxes (45 × 20 × 20 cm or 45 × 30 × 20 cm) contained 3–6 larger fruits.

Inoculation method: Inoculation was performed as described by Urbez-Torres et al. (2010). Each fruit was inoculated by pipetting 100 µL of the conidial suspension (10^4 conidia/mL) into the pedicel cavity. The boxes were then tightly sealed and incubated under laboratory conditions (28 ± 3°C).

Assessment parameters: The time to disease onset and the time to the appearance of clear symptoms in 100% of samples after pathogen inoculation were recorded (hours).

Reisolation and pathogen verification: The pathogen was reisolated from symptomatic lesions on PDA and identified by morphological characteristics, which were compared with the original isolates. Fruits did not show clear symptoms were also sampled at the inoculation site to verify the presence of *L. theobromae* after inoculation.

2.2.2. Evaluation of reservoirs of Lasiodiplodia theobromae in Xuong com vang longan

a. Reservoirs of Lasiodiplodia theobromae of the previous cropping season

Sampling at the floral induction stage: Dried bark tissues from primary and secondary branches (branch orders 1–2) and dried fruit tissues were collected. For each sample type, 30 samples were randomly collected from three farms (10 samples per farm per type). Samples were placed in ziplock bags and stored under cool, dry laboratory conditions. Bark samples were subsequently examined under a stereomicroscope (Olympus CX41) to screen for pycnidia on the surface.

Isolation and identification of Lasiodiplodia theobromae from mycelium: Samples that did not have pycnidia were washed and cut into small segments (2–3 × 2–3 mm). The segments were surface sterilized in 70% ethanol for 30 seconds and rinsed with sterile distilled water. Four segments were plated in opposite pairs on PDA and incubated under laboratory conditions (28 ± 3°C). Two days after incubation, five fungal colonies morphologically resembling *L. theobromae* were subcultured. Two days after subculturing, flowering peduncles of *Imperata cylindrica* were placed on the medium to induce pycnidia and conidia formation. Identification of *L. theobromae* was based on conidial morphology produced in pycnidia at 21 days after induction, following the taxonomic criteria described by Phillips et al. (2013).

Detection of Lasiodiplodia theobromae pycnidia in collected samples: Samples with pycnidia were immersed in distilled water and examined under a stereomicroscope (Olympus CX41) to collect conidia for identification of the pathogen. The incidence of *L. theobromae* in sampled tissues (%) and the type of pycnidia formed-aggregated (conidiomata) or solitary (pycnidia)-were recorded.

b. Reservoirs of Lasiodiplodia theobromae in reproductive organs of the following cropping season

Sampling at the flowering and fruit set stage (March): Dried and fresh inflorescence peduncles (7–10 cm in length) of the following cropping season were collected from the same trees used in the previous cropping season. For each peduncle type, 10 segments were randomly collected from 10 fruit clusters/farm. At the same time, 30 mature longan leaves showing black spot symptoms were collected. All samples were stored under cool and dry laboratory conditions.

Sample collection at the end of the physiological fruit drop stage (April): Developing fresh fruit pedicels and the proximal portions of pedicels were collected. At the same time, dried pedicels and dried longan fruits persisting on the same clusters were also sampled. For each type, 30 samples were collected and stored as described for inflorescence peduncle samples.

Sample collection at the pre-commercial maturity stage (May): Thirty fresh longan fruit pedicels were collected from previously clusters. Samples were stored as described above.

Isolation of persisting mycelial forms and identification of *L. theobromae*: Procedures were conducted as described for bark samples.

Detection and recording of *Lasiodiplodia theobromae* pycnidia: Detection procedures and recording parameters were conducted as described for bark samples.

3. RESULTS AND DISCUSSION

3.1. Host ranges of Lasiodiplodia theobromae

Among the 32 fruits belonging to 17 genera, 12 families and 12 orders (Figure 1), 12 fruits did not show clear symptoms at 5–7 days after incubation (DAI). Nevertheless, the presence of *Lasiodiplodia theobromae* in these samples was confirmed by reisolation.

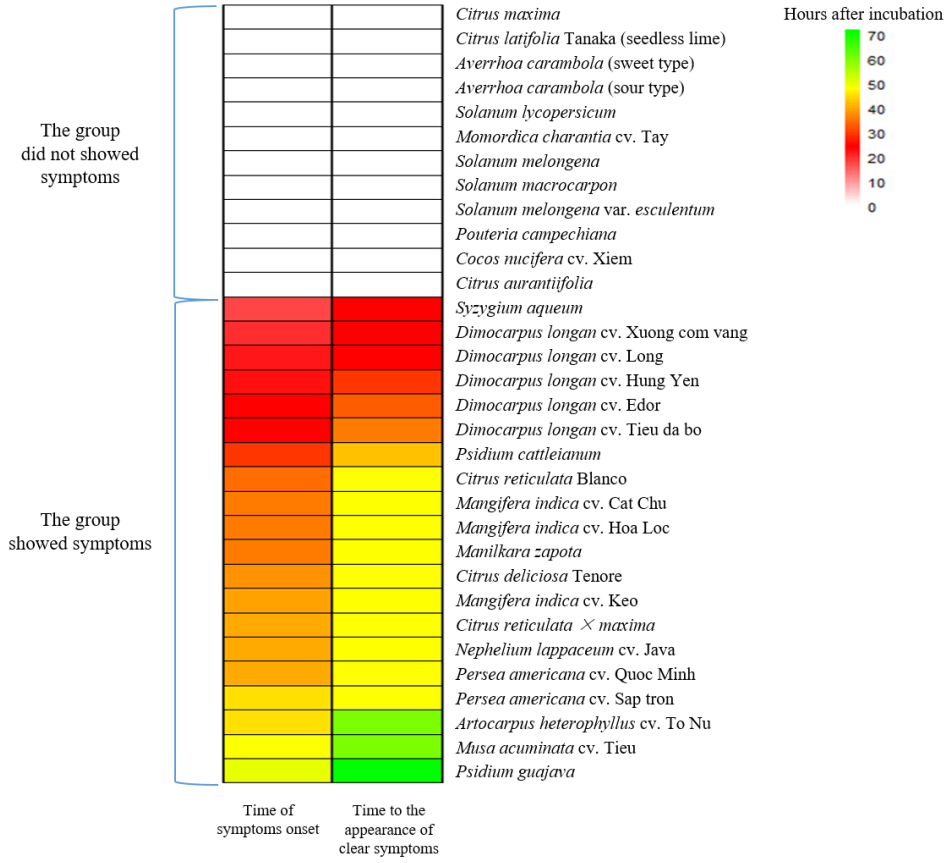

Figure 1. Host range of *Lasiodiplodia theobromae* and the time of appearance of fruit rot symptoms

Note: The time to symptom appearance increases along the color scale from red to green

Among these, Rutaceae fruits accounted for the largest proportion, with three species *Citrus maxima*, *C. latifolia* Tanaka and *C. aurantiifolia*. This was followed by Oxalidaceae, represented by two species: *Averrhoa carambola* L. *(sour and sweet)*. The families Arecaceae and Sapotaceae were each represented by one species (*Cocos nucifera* cv. Xiem) and *Pouteria campechiana*, respectively. The fruit vegetable group comprised six species, including five species of the family Solanaceae-*Solanum lycopersicum*, *S. macrocarpon*, *S. melongena*, *S. melongena* var. *esculentum* and *Momordica charantia* cv. Tay of the family Cucurbitaceae.

Twenty fruits were identified as hosts of *Lasiodiplodia theobromae*. These hosts were predominantly members of the family Sapindaceae, including *Dimocarpus longan* and *Nephelium lappaceum* cv. Java. The family Rutaceae comprised three species (*Citrus reticulata* × *C. maxima*, *C. reticulata* Blanco and *C. deliciosa* Tenore). The family Myrtaceae was represented by three species, including *Syzygium aqueum*, *Psidium cattleianum* and *P. guajava*. The family Anacardiaceae included three mango cultivars, namely 'Cat Chu', 'Hoa Loc' and 'Tuong' (*Mangifera indica*). The family Lauraceae was represented by two avocado cultivars, 'Quoc Minh' and 'Sap Tron' (*Persea americana*). The remaining hosts included *Artocarpus heterophyllus* cv. To Nu of the family Moraceae, *Musa acuminata* cv. Tieu of the family Musaceae and *Manilkara zapota* of the family Sapotaceae.

Syzygium aqueum cv. An Phuoc showed acute symptoms at 18 hours after incubation (HAI), followed by the five longan cultivars at 20-24 HAI. Other species showed symptoms later, at 29–50 HAI. Similarly, wax apple and longan reached 100% disease incidence earlier (24-36 HAI), whereas the other species required a longer period (42-72 HAI).

Thus, 20 fruits were identified as hosts of *Lasiodiplodia theobromae*. Among these, *Dimocarpus longan* cv. Xuong com vang, cv. Long, cv. Hung Yen, cv. Edor and cv. Tieu da bo, *Syzygium aqueum* cv. An Phuoc was highly susceptible to *L. theobromae*. *Persea americana*, *Psidium guajava*, *Citrus reticulata* Blanco, *Citrus deliciosa* Tenore, *Nephelium lappaceum* cv. Java, *Artocarpus heterophyllus* cv. To nu, *Musa acuminata* cv. Tieu and *Manilkara zapota* were moderately susceptible to the pathogen. Solanaceae and Cucurbitaceae fruits did not show clear symptoms after pathogen inoculation

Previous studies have reported that *L. theobromae* is the most prevalent plant pathogenic species in the genus *Lasiodiplodia*, with a broad host range in tropical and subtropical regions (Rathnayaka et al., 2023). Pedicel and fruit rot symptoms caused by *L. theobromae* have been widely documented (Ismail et al., 2012; Coutinho et al., 2017). In Thailand, *L. theobromae* was identified as the causal agent of fruit rot on longan several decades ago (Suwanakood et al., 2007). On mango, fruit rot caused by this pathogen was first reported in Egypt in 1971 (El-Ganainy et al., 2022). Pedicel rot on avocado, citrus fruits, banana and rambutan caused by *L. theobromae* has also been reported in Indonesia (Karunanayake & Adikaram, 2020). In addition, fruit pedicel rot on sweet orange (*Citrus sinensis*) in Indonesia (Dwiastuti & Aji, 2021), fruit rot on jackfruit (*Artocarpus heterophyllus*) in Taiwan (Ni et al., 2008) and Sri Lanka (Adikaram et al., 2020) and fruit rot on wax apple (*Syzygium samarangense*) in Thailand (Trakunyingcharoen et al., 2015) caused by *L. theobromae* have been confirmed. In Viet Nam, *L. theobromae* has been reported as the causal agent of pedicel rot on pomelo (*Citrus maxima*) in Ben Tre province (Khuong et al., 2023) and black-end fruit rot of avocado (Ngan et al., 2025).

The hosts did not show symptoms after pathogen inoculation, which has also been reported (Salvatore et al., 2020). This pathogen has been detected as latent on bitter melon (*Momordica charantia*) in China (Huang et al., 2012) and on the stems and leaves of *Solanum surattense* in India (Kannan & Muthumary, 2012). *Lasiodiplodia theobromae* has also been reported to cause leaf blight on coconut (*Cocos nucifera*) in Brazil, but did not show symptoms (Santos et al., 2017). Similarly, this pathogen causes gummosis on key lime (*Citrus aurantiifolia*) in Egypt, while symptoms were not observed (Leala et al., 2021). In Indonesia, pomelo (*Citrus maxima*) was reported to be infected by *L. theobromae* but did not show stem canker symptoms (Dwiastuti & Aji, 2021). These observations explain the presence of *L. theobromae* in 12 fruits but did not show clear symptoms.

3.2. Reservoirs of *Lasiodiplodia theobromae* in fruit tissues and branches of the previous cropping season

The pathogen showed high prevalence in fallen fruit tissues on farms and dried branch bark prior to the floral induction stage (83.33% and 96.67%, respectively). At this stage, the pathogen persisted in both mycelial and pycnidial forms. Mycelia were detected as latent infections within both fruit tissue and branch bark (Figure 2e). In contrast, conidiomata were predominantly formed in branch bark (Figures 2a and 2b), whereas pycnidia were mainly observed in fruit tissues (Figure 2c). The morphological similarity between conidia produced from mycelial cultures on PDA (Figure 2f) and those obtained from pycnidia in fruit tissues and branch bark (Figure 2d) indicates that the same pathogen persisted on farms.

Saprophytic reservoirs of *Lasiodiplodia theobromae* in the mycelial form on plant debris have been widely reported (Liu et al., 2012; Phillips et al., 2013; Coutinho et al., 2017; Alam et al., 2020). However, reports on the occurrence of *L. theobromae* pycnidia on plant debris remain limited. Nevertheless, the formation of conidiomata in dried citrus branches in the United States (Zhang, 2014), in grapevine trunks and fruits in China (Zhang et al., 2022) and the presence of pycnidia in dried fruits of *Bactris hirta* Mart. (Arecaceae) in Brazil (Vitoria et al., 2012) demonstrate that *L. theobromae* is capable of persisting in both mycelial and pycnidial forms on farms.

The lower frequency of mycelial detection in fruit tissues compared to branch bark is likely attributable to high soil temperatures during the local dry season, which may negatively affect the survival of pathogen mycelia in fallen fruit tissues. In contrast, dried branch bark retained on trees likely maintains higher moisture levels, thereby reducing environmental stress on the pathogen. These observations are consistent with reports from Pakistan, where *L. theobromae* was detected more frequently in dried mango branches retained on trees (91.6%) than in dried fruits (34.3%) on farms 4–5 months after harvest (Alam et al., 2020).

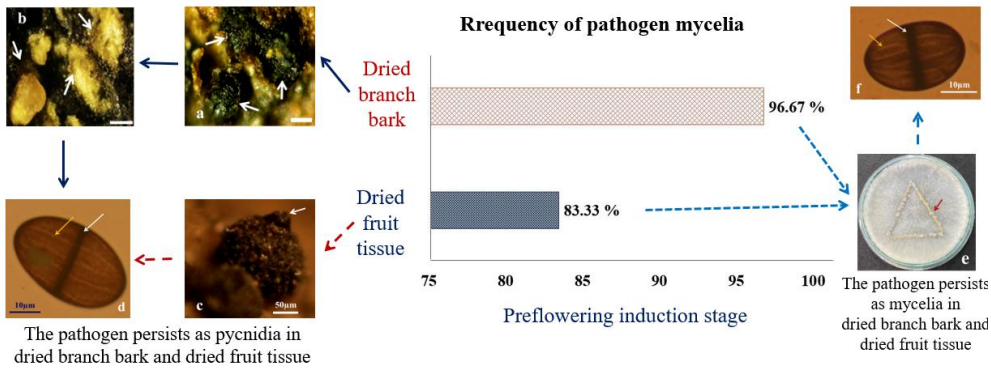

Figure 2. Reservoirs of *Lasiodiplodia theobromae* in dried longan branch bark and fruit tissue prior to preflowering induction stage

Note: (a) conidiomata in branch bark (white arrows); (b) transverse section of conidiomata; (c) pycnidium formed in dried fruit tissues (white arrows indicate ostioles); (d, f) mature conidia (white and yellow arrows indicate septa and longitudinal striations, respectively); (e) mycelium isolated from branch bark and dried fruits (red arrows show pycnidia formed on Imperata cylindrica peduncles). Scale bars: (a–b) 100 µm; (c) 50 µm; (d, f) 10 µm.

3.3. Reservoirs of *Lasiodiplodia theobromae* in longan farms of the previous cropping season

Latent infection of *Lasiodiplodia theobromae* in dried and fresh inflorescence peduncles at the flowering and fruit set stages was highly prevalent, with frequencies of 85.0% and 73.3%, respectively. These results indicate that the pathogen survived in inflorescence peduncles at an early developmental stage. During flowering, fresh peduncles were colonized by the pathogen but did not show symptoms, confirming the latent nature of *L. theobromae* at this stage. In contrast, some dried inflorescence peduncles showed symptoms, suggesting that the pathogen acted as an opportunistic agent when abiotic or biotic stresses predisposed the hosts to develop symptoms (Figure 3). Consequently, the frequency of *L. theobromae* was higher in dried inflorescence peduncles than in fresh ones. The presence of *L. theobromae* in dried mango inflorescences has previously been reported in Pakistan (Alam et al., 2020). This pathogen has also been identified as the causal agent of inflorescence dieback on longan, rambutan and mango in Puerto Rico (Serrato-Diaz et al., 2020).

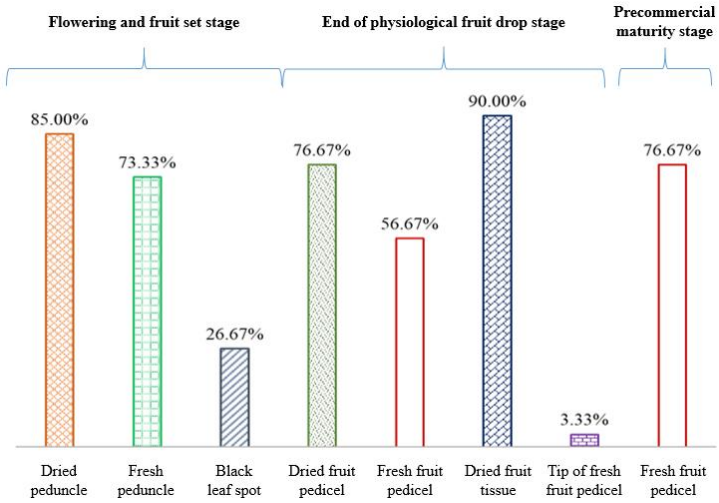

Figure 3. Occurrence frequency of the mycelial form of *Lasiodiplodia theobromae* from flowering to the pre-commercial maturity stage

Note: Inflorescence peduncles and fruit pedicels were sampled at the inflorescence–fruit node. Dried tissues were sampled at the pedicel end of physiologically dried fruits persisting on the fruit clusters. Fresh fruit pedicel ends are the tissue surrounding the pedicel cavity.

The presence of *Lasiodiplodia theobromae* on longan leaves was detected as an opportunistic pathogen in association with *Pestalotiopsis* sp., the causal agent of black leaf spot disease. The detection frequency of *L. theobromae* in leaf samples was 26.67% (Figure 3). Previous studies reported a comparable detection frequency of *L. theobromae* in dried mango leaves (27.6%), where *Colletotrichum gloeosporioides* and *Alternaria alternata* were also recorded at frequencies of 32.2% and 32.3%, respectively (Alam et al., 2020). These results indicate that *L. theobromae* functions as a secondary pathogen in longan leaf spot disease.

The end of the fruit drop stage corresponds to a period of physiologically active fruit development, particularly in seed growth. At this stage, *L. theobromae* was detected latently in 56.67% of healthy fruit pedicels. This frequency was lower than that observed at the pedicel fruit junction (76.67%) and in the pedicel end tissues of physiologically dried fruits (90.00%). In contrast, the presence of *L. theobromae* in the pedicel end tissue of fresh fruits was very low (3.33%) (Figure 3). Overall, the pathogen was detected more frequently in pedicels and dried fruits than in living tissues of the same organs, demonstrating its capacity to persist saprophytically.

During this stage, the pathogen was rarely detected in the pedicel end tissue of fresh fruits, as the fruit pedicel inhibited the development of *Lasiodiplodia theobromae*. This may be attributed to the abundant sap in young pedicels, which can suppress pathogen infection, a phenomenon similarly observed in mango. The fruit pedicel, therefore, plays a crucial role in preventing *L. theobromae* invasion (Johnson et al., 1992; Alam et al., 2020). Retention of fruit sap and a portion of the pedicel (2–3 cm) has been reported to suppress *L. theobromae* infection (Hassan, 2006). Studies on the role of the pedicel in longan fruit rot are limited; however, longan pedicels are shorter and contain less sap, which may result in weaker defense mechanisms. Consequently, fruit rot symptoms appeared earlier in longan than in mango.

Pycnidia of *L. theobromae* were also observed on some physiologically dried fruit pedicels at this stage, suggesting that the pathogen is able to shorten its latent phase to complete its life cycle once latent mycelial colonization from the pedicel into the fruit tissue has occurred (Figure 4). Similar observations have been reported in citrus, where *L. theobromae* formed pycnidia on dead branches retained on trees in Florida, USA (Zhang, 2014).

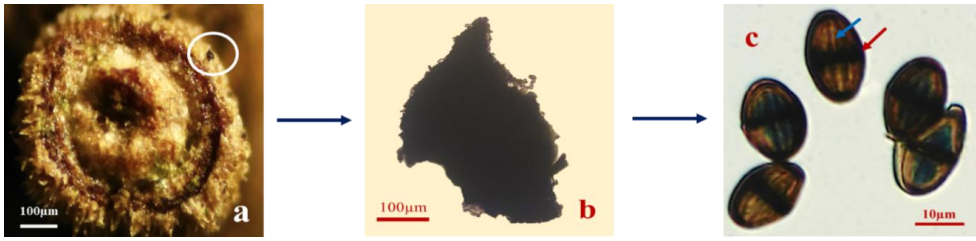

Figure 4. Presence of pycnidia of *Lasiodiplodia theobromae* in physiologically dried fruit pedicels persisting on Xuong com vang longan fruit clusters

Note: (a) peduncle of a physiologically dried fruit in the cluster (white circles indicate the locations of pycnidia); (b) pycnidia in fruit pedicel; (c) mature conidia of L. theobromae formed within the pycnidium (red and blue arrows indicate septa and longitudinal striations, respectively). Scale bars: (a–b) 100 µm; (c) 10 µm.

During the period from fruit development to the precommercial maturity stage, the presence of *Lasiodiplodia theobromae* in fresh fruit pedicels was high (76.67%). This increase is likely attributable to the continuous release of spores from pycnidia formed during the physiological fruit drop stage, which subsequently infected fruit pedicels. In mango, infection by *L. theobromae* during the flowering stage often results in fruit drop; therefore, dissemination and infection leading to mango fruit rot predominantly occur after the physiological fruit drop stage (Alam et al., 2020). These observations suggest that successful dispersal and infection of *L. theobromae* into fruit pedicels after the physiological fruit drop stage are key factors contributing to the increased presence of the pathogen in pedicels. Peterson (1978) also reported a rapid increase in *L. theobromae* associated with stem-end rot of avocado during fruit maturation.

Overall, *L. theobromae* persisted in longan farms from the previous cropping season in both mycelial and pycnidial forms. In the subsequent cropping season, mycelia of the pathogen could survive in inflorescence peduncles, fruit pedicels and dried

fruit tissues until the disease showed symptoms toward the end of the cropping season. In addition, pycnidia of *L. theobromae* could survive on dead inflorescence peduncles, fruit pedicels and dried fruits after the physiological fruit drop stage.

4. CONCLUSION

Twenty fruits were identified as hosts of *Lasiodiplodia theobromae* causing longan fruit rot in Ba Ria–Vung Tau, while twelve fruits were identified as latent hosts.

Mycelia and pycnidia of *Lasiodiplodia theobromae* help the pathogen persist in longan farms through different cropping seasons.

The pycnidia in these reservoirs release *L. theobromae* conidia, thus fostering continuous infection of fruit rot in longan farms

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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