Citrus stem rot disease (Lasiodiplodia theobromae (Pat.) Griff. & Maubl) problem and their control strategy in Indonesia

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Abstract. Citrus stem rot disease caused by Lasiodiplodia theobromae (Pat.) Griff. & Maubl is a serious disease in citrus orchards in Indonesia. The geographical distribution of these diseases is very broad, almost found in all citrus centers of more than 22 provinces, districts, and cities. Plants of more than 10 years with less intensive maintenance often seriously affected. Alertness and control must be more intensive in productive plants aged more than 5 years old. The disease epidemic problem occurs because of the presence of sensitive citrus varieties, poorly maintained plants, the source of inoculum throughout the year, wide range of host plants, and contamination of agricultural equipment. Sensitive plants in citrus genus including pummelo, orange, tangerine, lime, and lemon. The control strategy is formulated by considering the effectiveness, logical and environmentally friendly to support the development of citrus areas in 24 main citrus centers in Indonesia. Stem rot control strategies include: (1) monitoring of disease incidents in the field, presence of inoculum sources in citrus plants and other hosts, (2) maintaining the cleanliness of the field, sanitation by pruning the branches, (3) optimum maintenance, (4) disinfecting agricultural tools (pruning shears, grafting knives, machetes, saws) with 70% alcohol, (5) Weaning of stems with sulfur lime 2 times a year, (6) Biological control (Trichoderma harzianum, T. asperellum, T. viride, Bacillus subtilis, Gliocladium sp) and or botanical control using cashew nut shell waste (Anacardium occidentale L.), red ginger extract (Zingiber officinale var. rubrum).

1. Introduction
Citrus is a commodity that is idolized because of its high vitamin C content and a taste that is favored by both young and old. Most of citrus in Indonesia are consumed in the form of fresh fruit. The content of vitamin C in citrus is 53.2 mg per 100 g. In addition, there are antioxidants, flavonoids, beta carotene and hesperidin that function as antibody builders and can increase body immunity. The content of these vitamins and antioxidants is believed to be an antidote to the outbreak of Covid-19 so that the demand for citrus is increasing. The production of Indonesian citrus in the last 5 years has increased from 1.78 million tons in 2014 to 2.40 million tons in 2018 [1]. However, citrus productivity varies between 20 - 80 kg/tree at the age of 3-10 years, depending on the variety and plant conditions [2]. The optimum productivity for mandarin is 40 kg/tree and for tangerine is 100 kg/tree. The low productivity of citrus is caused by several factors, among others, because the cultivation has not been carried out optimally, including pests and diseases control.

Stem rot disease or gummosis known as “blendok” disease in Indonesia is the main cause of death in citrus stems and branches in addition to 2 other diseases, namely stem rot (Phytophthora spp) and Huanglongbing (Liberobacter asiaticum). The average loss of citrus production due to Phytophthora,
Diplodia and Huanglongbing (HLB) in Indonesia in 2001-2003 was 60,960 tons or Rp. 236,926,500,000 [3]. Initially this disease was considered harmless, but now gummosis deserves to be called an emerging disease because without realizing it, this disease continues to develop and threatening. If the handling is wrong, then the Indonesian citrus growers are ready to face gloomy times like the 70s. At that time, 80% of the citrus population died from HLB. Therefore, integrated control is needed to prevent the disaster from recurring. In fact, 35–40% of the citrus plant population is infected with stem rot and is spread across 22 provinces, districts, and centers for citrus in Indonesia [4]. Even in 1996, it caused damage to 85% of the 500 ha of pummelo (Citrus maxima) in Magetan, East Java. This pathogen should not be underestimated because it is very opportunistic, causing various types of plant diseases with a worldwide distribution in tropical and subtropical regions [5]. The host range is very wide, estimated to be more than 280 plant species [6,7]. In tropical areas, Botryodiplodia theobromae is known to cause major losses to mango, cocoa, banana and sweet potato farmers [8–10]. Even this pathogen can also cause disease in the fruit if the attack on the stem is not well controlled and the environmental conditions are favorable for disease growth so that it can be carried over until the fruit is harvested.

This paper reviews information and research results on the problem of stem end rot disease in citrus plants in Indonesia, including geographical distribution and economic impact, transmission, host range, detection and control strategies in Indonesia.

2. Causes and symptoms of citrus stem rot disease in Indonesia

2.1. Causes of disease

The cause of this disease is the fungus Lasiodiplodia theobromae (Pat.) Griff. & Maubl, synonym Botryodiplodia theobromae (Pat.) [4,11,12]. The latest report of Henuk et al., [13], disease with symptoms of wet diplodia is caused by B. theobromae Pat. (Teleomorph Botryosphaeria rhodina (Cooke) Arx.) and P. citrophthora. These results are based on morphological characterization and DNA sequence analysis in samples taken from 10 citrus central production of Indonesia: Brastagi (Karo), Kampar, Muaro Jambi, West Tulang Bawang, Garut, Batu-Malang, Jember, Bangli, South Timor Tengah, Banjarmasin, and Banjarbaru. Of the 10 samples symptomatic, only samples from South Timor Tengah were caused by P. citrophthora. While samples from other locations were caused by B. theobromae.

The growth of L. theobromae colonies in PDA media developed fast, regularly circular, pycnidium formed at the age of 21-30 days in isolates from West Java and East Java [4,14]. The pycnidium is scattered or collected under the epidermis or cortex. Conidiophores are shaped like a slender length with a smaller, needle-like tip. Conidium shape is oblong or oval, 1-celled when it is young and 2-celled with transverse bulkhead, dark or black when ripe, and does not have a mucus layer on the outside [11,15]. On average, isolates have mycelium threads or fine hair, like thin cotton, which grows quite a lot and grows abundantly in petri dishes. Initially mycelium is white, then turns gray and black [4,14,16].

According to research by Salamiah [17], the toxin produced by L. theobromae from Banjar plays a role in penetration into the plant tissue in the leaves of Citrus nobilis var. sian Banjar, C. maxima, C. sinensis, C. aurantifolia, and C. hystrix, but does not play a role in the infection process. The observation by Dwistuti et al. [18] using pathogen isolates from East Java on Citrus maxima, C. nobilis, and C. sinensis resulted in leaf necrosis. It is possible that the secretion of toxins plays a role in softening the surface of the leaves or the walls of the tough citrus stem bark so that it can easily enter the plant tissue but has no effect on the infection process. Findings of this kind of toxin were also found and identified in mango [19], Chinese cinnamon [20], coconut [21], and peach [22] infected with L. theobromae. Toxins from several host plants affected by B. theobromae/L. theobromae are summarized in Table 1.
Table 1. Toxin metabolites on *B. theobromae/L. theobromae* in some host plants

| Pathogen          | Host                          | Toxin                        | Mode of action                                                                                           | References          |
|-------------------|-------------------------------|------------------------------|----------------------------------------------------------------------------------------------------------|---------------------|
| *B. theobromae*   | *Cinnamomum cassia*           | Metabolite toxin             | The electrical conductivity increases and causes wilting of sprouts and necrosis of the leaf discs.       | Wang and Li, 2002 [20] |
| *B. theobromae*   | *Citrus nobilis var Banjar C. maxima, C. sinensis, C. aurantifolia, C. histrix* | Crude toxin                  | It has a role in penetration into plant tissues.                                                          | Salamiah, 2009 [17] |
| *B. theobromae*   | *Citrus maxima, C. nobilis, C. sinensis* | Crude toxin                  | Speeds up the incubation period.                                                                         | Dwiastuti *et al.*, 2017 [18] |
| *L. theobromae*   | Tropical fruit                | α-methylene-γ-butyrolactone derivative | Induces symptoms of necrosis.                                                                           | He *et al.*, 2004 [23] |
| *L. theobromae*   | *Mangifera indica*            | Toxin contains non-protein substances | Causes plants to wilt.                                                                                   | Parthasarathy *et al.*, 2016 [19] |
| *L. theobromae*   | *Cocos nucifera* and human    | 3-indolecarboxylic acid, jasmonic acid, lasiodiplodin, four substituted 2-dihydrofuranones, two melleins, dan cyclo-(Trp-Ala) | Modulation of the pathogenicity of *L. theobromae* against plants and also against mammalian cells.       | Félix *et al.*, 2018 [21] |
| *L. theobromae*   | *Prumus persica*              | Metabolite toxin             | Increases the pathogenicity of gummosis in peach.                                                         | Li *et al.*, 2019 [22] |
| *L. theobromae*   | Tropical plants               | Cyclohexenes and cyclohexenones, indoles, jasmonates, lactones, melleins, phenols, and others | Produces bioactive compounds directly involved in host–fungus interactions.                              | Salvatore *et al.*, 2020 [24] |

2.2. Symptoms of disease

In general, the fungus *Lasiodiplodia* in citrus plants in the world causes symptoms of stem cancer, gummosis on branches and stems, shoot blight, and fruit rot [25], especially in tropical and subtropical areas. Plants infected with this disease will die 1-3 years from the first infection, depending on the part of the plant being attacked. If the main stem is affected, the plant will die a year after infection. If the branches or twigs in the canopy are attacked, then it will die 3 years later. Symptoms of the disease found in Indonesian citrus plants include:
2.2.1 Wet Diplodia. Symptoms of wet Diplodia are indicated by the presence of golden yellow gummosis on the affected branches or twigs, and emitting a sour aroma [26]. At an advanced stage, the skin of the plant is peeled off or can even result in death. In severe attacks, gummosis wounds become circular on the main branches and stems and cause the death of the plant parts above the point of attack, and can even cause the death of the plant. The garden was badly attacked and maintenance was not optimal, causing mass death. In inundated areas or paddy fields such as Jember, Banyuwangi, and South Kalimantan, the symptoms of the disease are accompanied by the discharge of white foam (Figure 1).

2.2.2 Dry Diplodia. Symptoms of dry Diplodia are more difficult to recognize at the beginning of the attack, because there is no gum which is a hypersensitive reaction of infected plants to localize pathogens from developing. In further or severe attacks, the bark or branches peel off, there are small gaps, the inside is rotten and the fungus comes out, then dries up or can even lead to death [27]. Severe attacks to coiling on the main branches and stems cause the death of the plant parts above the point of attack, even causing the death of the plant.

2.2.3 Stem-end rot. In Indonesia, this symptom is relatively rare in the field because usually the fruit is not harvested until it is overripe. The fungus attacks the fruit starting from the base of the fruit stalk. The affected part will experience a brownish discoloration and will rot very quickly if it is not controlled. The initial symptom of the fruit is in the form of a pale brown bruise, then a soft rot spreads like being scalded. If the fruit is attacked by the fungus, when it is split, it will show the middle of the fruit starting from the fruit stalk, appearing black fungus spores and mycelium and a slightly sour smell. Disease attacks can occur during the on-farm period in the field and after harvest, namely during storage or delivery. Attacks in the field usually occur on infected stems and are not controlled until the plant bears fruit. Meanwhile, post-harvest attacks occur because the source of infection has been carried over from the field but has not yet been symptomatic, or during a long degreening process [29].

3. Disease problem on productivity
3.1. Economic impact and geographical distribution
Actually, citrus stem rot is not a new disease, and has been detected since decades ago, only, it is often ignored and considered less dangerous than HLB. This disease was reported in Magetan, East Java in 1996, attacking 85% of the 500-ha pummelo (C. maxima) orchard with mild to moderate attacks (22-
37%) [30]. In South Kalimantan, there were 825,318 trees or 53.9% of the existing plants dying [17]. In the era of 2009 – 2010, the disease began to get worse, and the attack was widespread. It was reported that in 2011, without realizing it, this disease attacked 63,431 ha of the total area of citrus orchards in Indonesia, initially called the result of an HLB attack, because at first glance it was somewhat similar (yellowing leaves, languishing plants then falling off) and inaccurately identifying them. It turns out that the current facts show that it is not only HLB that attacks citrus this time, but the gummosis disease caused by the fungus \textit{L. theobromae}. Some of the symptoms are wet spots on the trunk, branches, or twigs and HLB does not have symptoms of wounds on the trunk and branches that secrete gum, but there are also those that do not secrete gum. When the twig or stem is attacked, the leaves turn yellow and fall. Plants will die when the attack has surrounded the main stem. Meanwhile, HLB attacks are only 2-3% [31]. In 2014 it was reported that the average loss of production and yield losses due to attacks by HLB, Diplodia, stem rot, and fruit flies was 3,218,000 tons and IDR 59,114,000,000 [4].

**Table 2. Increase in the area of citrus stem rot disease in Indonesia during 2016-2019**

| Island   | Province                  | Increase in the area of citrus stem rot disease in Indonesia (hectare) |
|----------|---------------------------|---------------------------------------------------------------|
|          |                           | 2016  | 2017  | 2018  | 2019  |
| Sumatera | Aceh                      | 2.06  | 78.4  | 95.0  | 0.435 |
|          | North Sumatera            | 7.20  | 0.0   | 49.5  | 33.445 |
|          | West Sumatera             | 1.28  | 0.6   | 4.0   | 0.155 |
|          | Riau                      | 15.38 | 0.0   | 6.5   | 54.193 |
|          | Jambi                     | 4.70  | 0.9   | 0.7   | 0.010 |
|          | South Sumatera            | 4.58  | 13.3  | 3.6   | 4.263 |
|          | Bengkulu                  | 0.02  | 1.0   | 0.0   | 0.000 |
|          | Bangka Belitung           | 0.05  | 0.0   | 0.0   | 0.000 |
| Java     | West Java                 | 0.22  | 0.1   | 3.1   | 25.208 |
|          | Central Java              | 9.95  | 1.7   | 83.0  | 93.053 |
|          | East Java                 | 11.64 | 0.0   | 67.3  | 32.245 |
| Kalimantan| West Kalimantan          | 318.26| 6.0   | 15.8  | 36.853 |
|          | Central Kalimantan        | 0.00  | 0.2   | 0.0   | 0.000 |
|          | South Kalimantan          | 21.23 | 5.4   | 1.8   | 5.374 |
|          | East Kalimantan           | 0.00  | 0.3   | 0.0   | 3.260 |
| Sulawesi | North Sulawesi            | 0.75  | 0.2   | 0.0   | 0.000 |
|          | Central Sulawesi          | 0.00  | 0.0   | 0.5   | 1.392 |
|          | South Sulawesi            | 4.68  | 3.9   | 7.8   | 1.672 |
|          | Southwest Sulawesi        | 0.00  | 2.8   | 217.0 | 24.943 |
|          | East Sulawesi             | 0.00  | 0.5   | 4.0   | 1.043 |
|          | Gorontalo                 | 0.14  | 0.0   | 0.0   | 1.200 |
| Bali     | Bali                      | 4.99  | 0.6   | 6.4   | 2.888 |
|          | West Nusa Tenggara        | 0.00  | 0.0   | 0.0   | 0.610 |
|          | Tenggara                  | 0.44  | 0.0   | 0.0   | 5.833 |
|          | Maluku                    | 2.43  | 0.0   | 48.0  | 22.773 |
|          | North Maluku              | 0.00  | 2.0   | 0.0   | 0.000 |
|          | **Total**                 | 409.98| 117.9 | 1,361.1| 350.844|

Source: Directorate of Horticultural Protection, Indonesian Ministry of Agriculture [32]

The disease is quite widespread and has a serious impact on citrus cultivation both in Indonesia and abroad. The disease can be found in the United States, Cuba, India, Malaysia and Thailand. In Indonesia, this disease is found in all citrus centers on more than 5 islands, namely Java (West Java, Central Java,
East Java), Sumatera (Aceh, North Sumatra, Riau, West Sumatra, Bengkulu, Jambi, South Sumatra, Lampung), Kalimantan (West Kalimantan, South Kalimantan, Central Kalimantan, East Kalimantan), Sulawesi (West Sulawesi, Central Sulawesi, South Sulawesi, Southeast Sulawesi), Bali, West Nusa Tenggara, East Nusa Tenggara, and Maluku. In 2016-2019, it was noted that the spread of disease was more widespread, found in 26 provinces (Table 2) which was wider than the previous report of 22 provinces, so awareness of early symptoms and control must be more intensive in productive age plants (above 5 years) [3].

The fluctuation of attacks from year to year varies, in 2016 it was 409,98 ha, then it decreased in 2017 to 117,9 ha. In 2018 there was a quite high increase of 1.361,1 ha, and decreased again in 2019 to 350,844 ha, possibly influenced by changes in climatic conditions and less optimum crop management methods. The highest fluctuation in increased area attacked in 2016 occurred in West Kalimantan province, in 2017 and 2018 the highest was in Aceh, and in 2019 the highest was in Central Java.

3.2. Transmission
This fungus spreads very quickly, causing the death of plants in Indonesia, both in the nursery and after the productive phase in the field. Infected citrus plants show symptoms of rot at the base of the stem and can be accompanied by the formation of gummosis and produce a sour aroma [26]. Transmission and spread of disease occur through wounds, insect vectors (Nitidulidae, Coleoptera), spraying through the air (droplets), splashing water [11] and agricultural tools contaminated with fungi. The level of disease attack is closely related to the level of orchard management. Usually in orchards that are not maintained, Diplodia attack is very high [33]. High disease severity tends to occur in farmers’ fields that perform mechanical weeding less than six times per year, pruning that is not intensive, use of herbicides, plant population >1000 plants/ha, and use of laying hens’ manure of ≥25 kg/plant/year [34]. According to Salamiah [11], pathogenic inoculums can survive on citrus plants, seeds, fruit peel, plant stumps, and tree branches that are still healthy, but are not found in the soil. The dispersion mechanism and distribution pattern of \textit{L. theobromae} are not well known, but it is suspected that this fungus enters the land through latent propagules on the scion and rootstock [35].

\textit{Lasiodiplodia} is a genus of fungi that has a very wide distribution both geographically and in a range of hosts. Pummelo varieties are very susceptible, almost 90% of pummelo plants in Magetan are infected with Diplodia disease. In favorable conditions, namely at high humidity, lack of plant nutrients including micro intake, high temperatures, and lack of water in the dry season, the pathogens will soon germinate and then penetrate into the plant tissue. The amplitude (difference in temperature day and night) is high, especially the dry season, which is an environment that facilitates the development of this fungus. The critical period for pummelo (\textit{C. maxima}) varieties occurs in the middle of the rainy season with humidity ≥ 80% which fulfills the requirements for fungal growth or in the dry season where plant conditions are less than optimal so that plant defense is lacking [27].

On fruit, fungi sporulating on the dead skin and spores can survive from one season to the next, then during the rainy season, many fungal cones are trapped in the base of the fruit stalk (lobe). The fungus will enter the plant tissue through the wound formed during the formation of the abscission layer. Anything that will lead to the formation of the abscission layer, i.e., injury, over-maturity, ethylene degreening, etc., will contribute to the Diplodia incidence in fruit. The high temperature also supported the development of Diplodia in fruit.

3.3. Host range
The fungus \textit{L. theobromae} belongs to the group of pathogens that are cosmopolitan, polyphagous and opportunistic [36]. The host range is quite extensive in tropical and subtropical regions of the world, consisting of dicot and monocot plants as well as gymnosperms (Table 3). It was reported that more than 280 genera of host plants [37,38] including papaya [39], avocado [39–41], citrus [18,41–43], soursop [40,44], guava, mango [39,44], grapevine [45], nuts, jackfruit [40], banana [9,39], sapote [39,40], mangosteen [46], and apples [4]. Plantation crops that are the host including cocoa [9], eucalyptus [44], coconut [21], jabon [47], rubber tree [48], and pines [36,40]. Salamiah \textit{et al.} [41] found a range of host
pathogens attacking cashew and avocado plants. Sandra [49] reported diversity in citrus, cocoa, rubber, banana, and mangosteen in South Kalimantan. The variability of the symptoms of the disease and the range of hosts that these fungi possess indicate that there may be variations in morphological and molecular characters, it is possible that this species has several strains. From several \textit{L. theobromae} isolates taken from infected citrus stems in East Java, a diversity of fungal isolates was obtained, but the DNA band profiles formed from PCR amplification using ITS 3 and ITS 4 primers were indistinguishable.

### Table 3. Host of \textit{B. theobromae}/\textit{L. theobromae} in some countries

| Country | Host range | Resistance varieties | References |
|---------|------------|----------------------|------------|
| India   | \textit{Citrus reticulata} (var. Nagpur and Kinnnow), \textit{Ziziphus mauritiana}, \textit{Eucalyptus grandis}, \textit{Psidium guajava}, \textit{Mangifera indica}, \textit{Citrus limon}, \textit{Annona squamosa}, \textit{Capsicum annuum} | Kinnows grown in arid parts of Punjab (Abohar) India, \textit{Emblica officinalis}, \textit{Achras zapota}, \textit{Jatropha curcas} | Ladaniya, 2008 [42]; Kumar and Patel, 2018 [44] |
| Philippines | \textit{Persea americana}, \textit{Averrhoa carambola}, \textit{Achras zapota}, \textit{Citrus sp.}, \textit{Psidium guajava}, \textit{Mangifera indica}, \textit{Carica papaya}, \textit{Musa sapientum}, \textit{Capsicum annuum}, \textit{Lycopersicum esculentum}, \textit{Luffa 7cutangular}, \textit{Daucus carota} | Not reported | Alzate \textit{et al.}, 1981 [39] |
| Ghana | \textit{Theobroma cacao}, \textit{Mangifera indica}, \textit{Musa sapientum}, \textit{Ipomoea batatas} | Not reported | Twumasi \textit{et al.}, 2014 [9] |
| Brazil | \textit{Pinus spp.} | Not reported | Maciel \textit{et al.}, 2015 [36] |
| Mexico | \textit{Anacardium occidentalis}, \textit{Annona spp.}, \textit{Carica papaya}, \textit{Citrus aurantium}, \textit{Citrus aurantifolia}, \textit{Citrus sinensis}, \textit{Hibiscus sabdariffa}, \textit{Mangifera indica}, \textit{Nephelium lappaceum}, \textit{Persea americana}, \textit{Pinus pseudostrobus}, \textit{Pinus sp.}, \textit{Pouteria sapota}, \textit{Vitis vinifera} | Not reported | Picos-Muñoz, \textit{et al.}, 2015 [40]; Úrbez-Torres \textit{et al.}, 2008 [45]; Pedraza \textit{et al.}, 2013 [12] |
| Indonesia | \textit{Citrus nobilis}, \textit{Citrus maxima}, \textit{Citrus sinensis}, \textit{Interstock Carrizo citrange}, \textit{Interstock Ponnirus trifoliata}, \textit{Anacardium occidentale}, \textit{Persea americana}, \textit{Anthocephalus cadamba} | Interstock \textit{Volcameriana}, \textit{Cocos nucifera}, \textit{Artocarpus heterophyllus}, \textit{Averrhoa carambola}, \textit{Carica papaya}, \textit{Hevea brasiliensis} | Salamiah \textit{et al.}, 2008 [41]; Dwiasuti \textit{et al.}, 2017 [18]; Dwiasuti and Sugiyatno, 2018 [43]; Aisah \textit{et al.}, 2017 [47] |

Pummelo aged 10-15 years are the most sensitive to gummosis disease compared to younger ages or other varieties [27]. This is confirmed by the results of the study by Dwiasuti \textit{et al.} [18] which states that pummelo and sweet orange are more sensitive than tangerines. Meanwhile in South Kalimantan, the attack of Diplodia on citrus plants aged 4-10 years was 245,896 trees, the most compared to plants aged less than 4 years old and more than 10 years old [17].
3.4. Detection of disease
Detection of stem rot disease can be done directly in the field based on the symptoms. However, if there is any doubt, it can be continued by isolating it in artificial media and then observing it under a 400x magnification microscope to see the morphology of the pathogen. Detection can also be done molecularly or with the lock postulate (Table 4). Molecular detection has been observed using various primers successfully amplifying the DNA of *B. theobromae/L. theobromae* fungi from various plants at different locations [49–53]. Dwiastuti [28] also succeeded in detecting the presence of fungi from East Java (Tlekung, Pasuruan, Poncokusumo, Jember, Magetan, and samples from infected fruit) molecularly through PCR amplification using ITS 3 and ITS 4 primers. The success of genomic DNA amplification in molecular techniques is determined by several factors, one of which is the suitability of the primers and the efficiency and optimization of the PCR process.

Table 4. Primer for quick detection of *B. theobromae/L. theobromae* in some host plants

| No | Host                | Origin   | Primer | Product size | References     |
|----|---------------------|----------|--------|--------------|----------------|
| 1  | *Vitis vinifera*    | South Africa | EF622075 | 254 bp       | Alves *et al*., 2008 [50] |
|    |                     |          | EF622055 |              |                |
| 2  | *Eucalyptus* sp.    | China    | HQ332194 | Not reported | Alves *et al*., 2008 [50] |
|    |                     |          | HQ322210 |              |                |
| 3  | *Lysiphyllum cunninghamii* | Australia | GU199367 | Not reported | Alves *et al*., 2008 [50] |
|    |                     |          | GU199393 |              |                |
| 4  | *Mangifera indica*  | China    | ITS5    | 420 bp       | Lin *et al*., 2009 [51] |
|    |                     |          | ITS4    |              |                |
|    |                     |          | Lth1    |              |                |
| 5  | *Vaccinium* spp.    | China    | Lt347-F | 347 bp       | Xu *et al*., 2016 [52] |
|    |                     |          | Lt347-R |              |                |
| 6  | *Citrus sinensis*   | Bangladesh | ITS5F   | 650 bp       | Hasan *et al*., 2020 [53] |
|    |                     |          | ITS4R   |              |                |
| 7  | *Citrus* spp.       | Indonesia | ITS4    | 550 bp       | Henuk *et al*., 2017 [13] |
|    |                     |          | ITS5    |              |                |

4. Environmentally friendly control strategy
Integrated control is considered to be effective and environmentally friendly, namely by combining several appropriate control techniques for the purpose of maintaining ecological balance, developing environmental conditions that can reduce disease progression, and improve health. Holistic management is carried out in an integrated manner by applying good and correct cultivation methods (optimum technical culture); at citrus seed preparation, bud sticks are not near the ground, sanitation, maintaining clean agricultural equipment, biological control and generic fungicides, as well as using fungicides rationally. The critical phases of the pathogen are the time before penetration occurs, in this phase the control is more effective than if the attack is advanced.

4.1. Preventive control during seed preparation
When preparing the seeds, use healthy bud sticks. Budding process are done not too low and not come into direct contact with the soil. It is recommended that the budding height is 20-25 cm above the ground. This is done to avoid transmission through rainwater splashes. After that, spray the plant with fungicide: the slurry “bubur California”, Copper, Benomyl, Carbolineum.

4.2. Sanitation
Sanitation is an important control component. Sanitation is done by pruning dry branches. The results of old research reported that pruning dry twigs regularly could reduce the attack by 18.46 - 90.58%. Pruning dry and diseased branches or twigs is carried out at the limit of 1-2 cm from the healthy part
then smeared with paraffin or fungicide with active sulfur ingredients. If the attack is severe, do heavy pruning. Cut twigs or sick stems should be burned. Sanitation can also be done by improving garden drainage to keep garden humidity from too high and water is not stagnant. In this sanitation, beetle vector control is also included with systemic insecticides or by mechanical means.

4.3. Keeping agricultural tools clean
Always keep pruning scissors, saws, and grafting blades clean and free from fungal contamination. Before use, the agricultural tools are smeared with 70% alcohol/10% chlorox and follow such a protocol every time it is used on a different crop. The results showed that if the control treatment was carried out, it could reduce the intensity of the attack by 55.4% and the area of the attack by 60.8% [27].

4.4. Cultivation practices
It must be ensured that the manure used is ready/ripe. The use of not yet ripe manure can also accelerate transmission. Raw manure is still decomposing so the temperature around the stem increases and stresses the plant. Raw manure also attracts snails that attack the stems until they are injured. It is at this time that an open wound is easy for *Botryodiplodia* to enter.

4.5. Biological control
Several biological and vegetable products have been found including *Trichoderma viride*, *T. harsianum*, and *T. asperellum* as well as neem. Combination application will be very good by applying 10 kg of oilcake or neem powder followed by watering the soil with 0.5% *Trichoderma* spp in the early stages of infection. Combination with copper oxychlorite fungicide 0.2% can also be an option. Currently, the secondary metabolite biofungicide product that has been successfully assembled is called Boser Tridia 1 with the active ingredient *Trichoderma* sp. This product has been tested in South Kalimantan and provides fairly effective results compared to copper fungicides, Difenconazol or “bubur California” [54,55]. It plays a role in increasing chemical compounds in plants that function in plant defense against pest attack so that plants can withstand and avoid important pest attacks. When applied at the beginning of planting, it can overcome environmental stress because it is related to its role as an impact on plant resistance. It is able to reach the presence of pests in plant tissues, and with various mechanisms according to the content in their secondary metabolites, and easy to prepare, apply, and store.

Application of PGPR (plant growth promoting rhizobacteria) liquid at a dose of 5 cc per liter of water is good for plants resistance to fungi. Liquid volume of 2 - 5 liters per plant, applied 2 times a year for prevention and 4 times a year for recovery [31].

4.6. Chemical control
For prevention, it is done by cleaning the stems with a soft brush or coconut husk and then covering the main stems, primary & secondary branches of plants over 3 years with a generic fungicide “bubur California” (mixture of water, lime and sulfur) at the beginning and end of the rainy season [33]. Plants that have been attacked can be overcome by making homemade fungicides, namely “bubur Bordeaux/bubur bordo”. It is a mixture of CuSO₄, lime, and water. Apply the freshly made “bubur bordo” and within 1-2 weeks, the wound usually dries up immediately [31].

4.7. Control on citrus fruit after harvest
The recommended control is to use safe ingredients, because citrus fruit in Indonesia is mostly consumed in fresh form. Fungus can be prevented by picking the fruit before it is ripe, using 2,4-D in the packhouse and by treating the fruit with the fungicide SOPP, TBZ or imazalil. Store the fruit in cold temperatures immediately after picking and packing, to reduce the potential for infection with pathogens. Lower degreening time and use less ethylene and store fruit at 50°F (10°C).
4.8. Resistant plants

It was found that 16 accessions of citrus plants resulting from protoplast fusion (satsuma mandarin and tangerine var. Madu which were 1.5 years old) had different levels of resistance to the two parent plants and there were 11 citrus plants resulting from protoplast fusion that did not differ from the two parent plants. Classification of resistance of citrus plants resulting from protoplast fusion showed that 8 plants were classified as resistant; 17 plants were classified as moderate; and 5 plants classified as vulnerable [56].

Some citrus farmers in Indonesia have implemented stem rot disease control, although the components are not implemented completely and holistically. The Directorate of Horticulture Protection, the Ministry of Agriculture of Indonesia reported that the area of control that had been carried out in infected citrus centers during 2016 to 2019 was 2481.47 ha, 826.9 ha, 105.1 ha, and 466.9 ha, respectively (Figure 2). From Figure 2, it can be seen that citrus plants are controlled in 24 provinces with various control areas. The highest control was carried out by North Sumatra in 2019, followed by North Kalimantan and West Kalimantan. The diversity of disease control carried out in Indonesia is due to several factors, including inadequate disease recognition, especially in terms of critical periods, expensive control labor, insufficient capital, many citrus farmers in Indonesia who rely on government assistance, lack of human resources, so there is less time to control. Almost all citrus farmers in Indonesia still use conventional methods in citrus cultivation. So that the availability of human labor becomes a mainstay for carrying out all agricultural activities.

![Figure 2. Control area of stem rot disease in Indonesia during 2016 – 2019](Source: Directorate of Horticultural Protection, Indonesian Ministry of Agriculture [32])

5. Future directions in research of citrus stem rot disease

Future research that needs to be further developed and strengthened are: 1) Studying the nature and diversity of pathogens in citrus and planning a national program to get new varieties that are resistant through a selection breeding program, 2) Studying the disease epidemic in detail, 3) Studying plant resistance mechanisms and vaccinating plants from seeding using biological microbes that are compatible with plant growth, 4) Development of the production process and mass production of effective biological control agents and MS products, 5) Regional collaboration through the establishment
of a Phytophthora working group for Southeast Asian countries who share the problem of L. theobromae, 6) Initiation of modern agricultural application research using mechanical means to replace labor, 7) Evaluation of the effectiveness of a modified postharvest degreening treatment (i.e., using a lower temperature) in reducing the incidence and severity of Diplodia stem rot, 8) Testing new citrus postharvest fungicides (i.e., Natamycin and Propiconazole), essential oils, and chlorine dioxide (ClO2) on in vitro postharvest growth and development of L. theobromae and on inoculated fruit, 9) Development of delivery methods for the promising ingredients identified in (2) above (e.g. wet water, incorporation into wax, controlled release formulations contained in sachets for volatile production, etc.) to control inoculated Diplodia SER on grapefruit or fruit with natural infectious ingredients.

6. Conclusion
L. theobromae is a dangerous pathogen and can cause death for citrus plants other than HLB. Infection can also affect the fruit that has been harvested and stored. The problems of various cultivation methods, underestimating the effects of this disease, and the large number of host plants and the large potential for transmission, make it difficult to implement disease control in the field. Environmentally friendly control recommendations are available, in the future it needs to be tested more thoroughly for application in the field. The future research approach is directed at obtaining new, resistant varieties through a breeding selection program, studying the opportunities for plant vaccination, mass production of secondary metabolite products from the control agent consortium, evaluating the effectiveness of post-harvest treatments in reducing the incidence and severity of the Diplodia stem rot disease.

References
[1] Center for Agricultural Data and Information Systems 2019 Agricultural Statistics 2019 ed A A Susanti and T Heni (Jakarta: Center for Agricultural Data and Information Systems Ministry of Agriculture)
[2] Supriyanto A and Zamzami L 2014 Strengthening the Competitiveness of Citrus in the Domestic and Global Markets Strengthening the Competitiveness of Agricultural Products ed Haryono, E Pasandaran, K Suradisastra, M Ariani, N Suratno, S Prabawati, M P Yufdy and A Hendradi (Jakarta: Indonesian Agency for Agricultural Research and Development) pp 195–204
[3] Directorate of Horticultural Crop Protection 2008 Data on the number of attacks, distribution and control of citrus pest and diseases in Indonesia 2005-2010
[4] Dwiastuti M E, Agustina D and Triasih U 2016 Biodiversity of citrus stem rot disease (Botryodiplodia theobromae Pat.) in East Java Prosiding Seminar Nasional II Tahun 2016, Kerjasama Prodi Pendidikan Biologi FKIP dengan Pusat Studi Lingkungan dan Kependudukan (PSLK) (Malang: Universitas Muhammadiyah Malang) pp 94–109
[5] Faber B, Bean T, Daugovish O, Soto J D and Howell A 2016 C Citrus Res. Technol. 37 94–7
[6] Domsch K H, Gams W and Anderson T 1981 Compendium of Soil Fungi Advances in Microbial Physiology vol 1 (Academic Press)
[7] Khanzada M A, Lodhi A M and Shahzad S 2004 Mango dieback and gummosis in Sindh, Pakistan caused by Lasiodiplodia theobromae Plant Healc. Prog.
[8] Amusa N A, Adegbite A A, Muhammed S and Baiyewu R A 2003 J. Biotechnol. 2 497–502
[9] Twumasi P, Ohene-Mensah G and Moses E 2014 African J. Agric. Res. 9 613–9
[10] Meah M B, Plumbley R A and Jeger M J 1991 Mycol. Res. 95 405–8
[11] Salamiah 2008 Agrin 12 86–99
[12] Pedraza J M T, Aguiler A J A, Diaz C N, Oriz D T, Monter Á V and Mir S G L 2013 Rev. Fitotecnia Mex. 36 233–8
[13] Henuk J B D, Sinaga M S and Hidayat S H 2017 Biodiversitas 18 1100–8
[14] Retnosari E, Henuk J and Sinaga M 2014 J. Fitopatol. Indones. 10 93–7
[15] Wardlaw C W 1932 Ann. Bot. 46 229–38
[16] Henuk J 2010 Identification and pathogenicity test of the causes of stem rot in citrus (Citrus spp.) from several citrus production centers in Indonesia (Institut Pertanian Bogor)
[17] Salamiah 2009 J. HPT Trop. 9 158–67
[18] Dwiastuti M E, Ketut Budiarta G N and Soesanto L 2017 J. Hortik. 27 231–40
[19] Parthasarathy S, Thiribhuvanamala G, Faisal P M and Prabakar K 2016 J. Appl. Nat. Sci. 8 559–64
[20] Wang J and Li R Y 2002 For. Res. 15 387–93
[21] Félix C, Salvatore M M, DellaGreca M, Meneses R, Duarte A S, Salvatore F, Naviglio D, Gallo M, Jorrit-Novo J V, Alves A, Andolfi A and Esteves A C 2018 Mycologia 110 642–53
[22] Li Z, Zhang H and Li G 2019 Fungal Biol. 123 51–8
[23] He G, Matsuura H and Yoshihara T 2004 Phytochemistry 65 2803–7
[24] Salvatore M M, Alves A and Andolfi A 2020 Toxins (Basel). 12 457
[25] Slippers B and Wingfield M J 2007 Fungal Biol. Rev. 21 90–106
[26] Vernière C, Cohen S, Raffanel B, Dubois A, Venard P and Panabière F 2004 J. Phytopathol. 152 476–83
[27] Triwiratno A 2002 Gummosis disease control (Botryodiplodia theobromae Pat.) Socialization of Horticultural Pest Explosion Control (Directorate of Horticultural Protection)
[28] Dwiastuti M E 2015 Technology assembly of microbial antagonistic secondary metabolite to control citrus stem rot and fruit rot (Botryodiplodia theobromae).
[29] Zhang J 2014 Lasiodiplodia theobromae in Citrus Fruit (Diplodia Stem-End Rot) Postharvest Decay: Control Strategies (Elsevier) pp 309–35
[30] Supriyanto A, Dwiastuti M E, Hardiyanto and Riati R 2003 Diplodia fungal disease (Botryodiplodia theobromae Pat.) in citrus Lokakarya Penelitian Tanaman Jeruk dan Hortikultura Subtropik pp 143–7
[31] Wiyono S 2011 The citrus killer called gummosis (Pembunuh jeruk bernama blendok)
[32] Directorate of Horticultural Crop Protection 2020 Data on the number of attacks, distribution and control of citrus pest and diseases in Indonesia 2016-2019
[33] Triwiratno A and Supriyanto A 1999 Study of the application of the gummosis disease control technology package (Botryodiplodia theobromae Pat.) in pummelo in Magetan Regency Prosiding Seminar Nasional Hortikultura (Yogyakarta: Fakultas Pertanian UPN “Veteran”) pp 196–205
[34] Widyasiti I G A 2017 Epidemic factors of Lasiodiplodia theobromae stem rot disease in citrus orchard in Bangli Regency. Bali (Institut Pertanian Bogor)
[35] Cysne A Q, Cardoso J E, Maia A de H N and Farias F C 2010 J. Phytopathol. 158 676–82
[36] Maciel C G, Muniz M F B, Mezzomo R and Reiniger L R S 2015 Lasiodiplodia theobromae associated with seeds of Pinus spp. originated from the northwest of Rio Grande do Sul, Brazil Sci. For. 43 639–46
[37] Nunes C A 2012 J. Plant Pathol. 133 181–96
[38] Farr D F and Rossman A Y 2019 Fungal databases, systematic, mycology and microbiology laboratory, ARS, USDA USDA
[39] Alzate W T and Tandingan I C 1981 USM Res. J. 3 25–38
[40] Picos-Muñoz P A, García-Estrada R S, León-Félix J, Sañudo-Barajas A and Allende-Molar R 2015 Rev. Mex. Fitopatol. 33 54–74
[41] Salamiah, Badruzsaufari and Arsyad M 2008 J. HPT Trop. 8 123–31
[42] Ladaniya M S 2008 Postharvest Diseases and Their Management Citrus Fruit: Biology, Technology and Evaluation (Elsevier) pp 417–49
[43] Dwiastuti M E and Sugiyatno A 2018 RJOAS 6 476–87
[44] Kumar H and Patel D S 2018 Int. J. Pure Appl. Biosci. 6 845–7
[45] Úrbez-Torres J R, Leavitt G M, Guerrero J C, Guevara J and Gubler W D 2008 Plant Dis. 92 519–29
[46] Alam M S, Begum M F, Sarkar M A, Islam M R and Alam M S 2001 J. Biol. Sci. 4 1224–7
[47] Aisah A R, Soekarno B and Achmad 2017 P. J. Penelit. Hutan Tanam. 14 85–101
[48] Febbiyanti T R, Wiyono S, Yahya S and Widodo 2019 J. Agron. 18 41–8
[49] Sandra F K 2011 *The diversity of Botryodiplodia theobromae fungi from various host plants based on morphology and RAPD patterns* (Institut Pertanian Bogor)

[50] Alves A, Crous P W, Correia A and Phillips A J L 2008 *Fungal Divers.* 28 1–13

[51] Lin Y, Wang Y, Yang H and Wang P 2009 *Plant Pathol. Bull.* 18 225–35

[52] Xu C-N, Zhang H-J, Chi F-M, Ji Z-R, Dong Q-L, Cao K-Q and Zhou Z-S 2016 *J. Integr. Agric.* 15 573–9

[53] Hasan M F, Islam M A and Sikdar B 2020 *J. Adv. Microbiol.* 20 77–90

[54] Ratule M T, Wuryantini S, Sutopo and Dwistutti M E 2017 *Assistance in the Application of Integrated Management Technology for Healthy Citrus Plantation Based on Tidal and Dry Land*

[55] Wuryantini S, Taufik M, Agustina D and Erti M 2018 *Trichoderma harzianum as Diplodia disease control for citrus in swamp area Proceeding the International Seminar on Tropical Horticulture* (Bogor) pp 71–5

[56] Putra D P R, Sulistiyowati L, Cholil A and Martasari C 2013 *J. HPT* 1 16–26