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Current and Emerging Challenges in the Diseases of Trees

Edited by Cristiano Bellé

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Meet the editor

Cristiano Bellé is a researcher in the field of plant nematology and biological control at Phytus Institute, Brazil. He is also a researcher at the Universidad Nacional de San Agustín de Arequipa (UNSA), Peru. He obtained a master's degree in Agronomy from the Federal University of Santa Maria, Brazil, in 2014, a doctorate degree in Crop Protection from the Federal University of Pelotas, Brazil, in 2018, and a post-doctorate degree in Soil Biology and Microbiology from the Federal University of Santa Maria in 2019. He has experience in the field of agronomy with an emphasis on plant pathology and plant nematology. His work is related to the etiology and diagnosis of plant diseases caused by nematodes and fungi, pathogenicity and host-parasite relationships of plant-parasitic nematode diseases, the interaction between plant-parasitic nematodes and soilborne fungi in plants, and biological, cultural, and chemical control of plant-parasitic nematodes.

Contents

Preface

This Edited Volume is a collection of reviewed and relevant research chapters, concerning the developments within the Current and Emerging Challenges in the Diseases of Trees field of study. The book includes scholarly contributions by various authors and is edited by a group of experts pertinent to Agricultural and Biological Sciences. Each contribution comes as a separate chapter complete in itself but directly related to the book's topics and objectives.

The Book includes the following chapters dealing with the topics: "Devious Phloem Intruder *Candidatus* Liberibacter Species Causing Huanglongbing: History, Symptoms, Mechanism, and Current Strategies", "Anthracnose Disease of Mango: Epidemiology, Impact and Management Options", "New and Emerging Disease Threats to Forest Plantations in Sarawak Borneo, Malaysia", "Main Pests and Diseases in Tropical Forest Species in Nursery" and "Perspective Chapter: Microorganisms and Their Relationship with Tree Health". The target audience comprises scholars and specialists in the field.

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Chapter 1

Devious Phloem Intruder *Candidatus* Liberibacter Species Causing Huanglongbing: History, Symptoms, Mechanism, and Current Strategies

Palaniyandi Karuppaiya, Junyuan Huang and Muqing Zhang

Abstract

Huanglongbing (HLB) or greening is a devastating phloem-intruding bacterial disease that generates various symptoms in leaves and fruits, threatening the global citrus industry. *Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus, and *Candidatus* Liberibacter americanus are the causative agents of HLB in citrus-producing regions around many countries, and these proteobacteria are being vectorized by *Diaphorina citri* and *Triozaerytreae*. The lack of HLB-resistant citrus cultivars, the rapid spread of disease, and the fastidious nature of HLB-proteobacteria have made it difficult to mitigate HLB in the citrus field. There are numerous reports on the control of HLB disease using thermotherapy, chemotherapy, plant defense activators, brassinosteroids, and nanoemulsions. However, there is no evidence of such applicability of the methods mentioned above to complete the elimination or suppression of the pathogen to control HLB disease. We aim to provide an overall picture of HLB disease, its distribution, causal organism, pathogenic mechanism, and current and future strategies for combat against citrus Huanglongbing disease. This review may prompt the researchers toward an integrated and environmentally sustainable methodology for the mitigation/elimination of HLB pathogens.

Keywords: citrus, Huanglongbing, *Candidatus* Liberibacter, control strategy, psyllid

1. Introduction

Citrus fruits are the most predominantly produced fruits worldwide. The citrus species, Rutaceae family, is one of the major fruit crops in the world, which has provided an immune-enhancing source of vitamin C, nutrients, and medicinal value since ancient times [1]. Citrus crops are cultivated in more than 135 countries worldwide [2]. Worldwide citrus production is estimated at over 124.3 million tons annually [3]. Cultivated commercial citrus plants, consisting of rootstock and scion

varieties, have a significant impact on scion growth, fruit quality, yield, and tolerance to biotic and abiotic stresses [4, 5]. Therefore, the selection of rootstock may make a significant contribution to the success or failure of the planting process [2]. However, various biotic and abiotic stresses impede citrus production worldwide, among which Huanglongbing is one of the significant pernicious diseases devastating the citriculture industry in the last few decades. Citriculture industries in Asia, Africa, and America have suffered massive economic losses due to the devastating Huanglongbing (HLB) malady [6].

Citrus HLB (Yellow dragon disease or citrus greening) is one of the highly ruinous diseases in citrus species caused by proteobacteria *Candidatus* Liberibacter species. The casual organisms of HLB have not been successfully cultured on axenic culture to date, and the prevalence of the HLB pathogen in citrus plants was evaluated using a diagnostic polymerase chain reaction (PCR) technique. *Diaphorina citri Kuwayama* (Asian citrus psyllid (ACP)) and *Triozaerytreae* (African citrus psyllid (AfCP)) transmit HLB disease from one citrus plant to another and also feed on many other species of the *Rutaceae* family [7]. The ACP resides in warm and humid zones and is most prevalent in Asia, the Indian subcontinent, Saudi Arabia, Reunion, and Mauritius. Now, ACP has also spread to South and Central America, such as Brazil, USA, and Mexico [8]. AfCP thrives in cold weather and is sensitive to the sweltering climate. AfCP resides in Africa, Cameroon, South Africa, Yemen, Madagascar, and Madeira Island [9]. HLB was first identified as a significant issue of unknown disease in citrus by farmers in southern China at the end of the nineteenth century [10]. HLB was first known a century ago as Citrus "Dieback" in India and "Yellow Dragon shoot Disease" in China, with a clear impact on citrus production in many countries, followed by South Africa, the Philippines, Indonesia, Thailand, Brazil, and the United States [11].

Citrus are susceptible to HLB, that is, nearly all commercial citrus and some citrus relatives. *Poncirus trifoliate* citrus, some *P. trifoliate* hybrids, and a few lemon varieties are considered more HLB tolerant [12]. The most efficient and sustainable strategy against citrus HLB is breeding resistant citrus cultivars. However, conventional citrus breeding is a long-term process that takes about 20 years to develop a new variety. Further, breeding efficiency is affected by gametophytic cross-incompatibility, heterozygosis, pollen-ovule sterility, apomixis, seedlessness, graft incompatibilities, polyembryony, and unstable characteristics [13]. Genetically engineered resistant citrus varieties are yet to be available for commercial cultivation due to the lack of acceptance of GMOs from farmers and consumers. It will, therefore, take many years to develop a promising resistant cultivar against HLB [14].

Many strategies to combat HLB were initiated, such as thermotherapy, antibiotics, plant defense initiators, pesticide, vector control management, chemotherapy, nanotechnology, and a transgenic approach [15, 16]. Beta-lactams, tetracyclines, and silver nanoparticles have obtained better results against HLB malady [17, 18]; however, the emergence of antibiotic resistance to microorganisms and indirect effects on human health and the environment is a significant and increasing risk that certainly restricts the use of antibiotics at the field level [18]. However, no effective strategies to eliminate or repress the HLB pathogens have been identified. This review attempts to provide an overall picture of HLB disease, distribution, casual organism and its pathogenic mechanism, and vector control management, and post the current and possible strategies to mitigate/combat HLB malady in the field.

2. Incidence, distribution, symptomology, and detection of citrus Huanglongbing

2.1 Incidence of Huanglongbing

HLB (also known as Yellow Dragon/shoot disease) was first identified as an unknown disease in citrus trees by citrus farmers in Guangdong Province, China, at the end of the nineteenth century [19], but studies suggest that HLB most likely originated in Taiwan in 1870 where it was known as Likubin ("Drooping disease") [20, 21]. Later, the HLB spread to other parts of China; by 1935, it had become a severe disease of citrus species [21]. Like HLB, dieback was first described in the central parts of India in the middle of the eighteenth century [22]. At that time, it might have been limited, but HLB was recorded in Assam in the eighteenth century and, by 1912, was a devastating disease in Bombay, India. However, the Citrus tristeza virus might cause this disease. Raychaudhuri [23] exhibited that dieback was the same as HLB. African greening disease was first identified in a sweet orange orchard in parts of South Africa in 1929 [24]. Outside of China, HLB was known as the "Greening" disease in South Africa, where extensive research was conducted in the 1950s. In Indonesia, the HLB disease was first noticed in the 1940s and is called the "citrus phloem degeneration" disease [25]. Reinking, in 1919, first described this disease in English as yellowing and leaf mottle of citrus noticed in China. According to International nomenclature rules, the name "Huanglongbing" was considered the official name by citrus pathologists at the 13th conference of the International Organization of Citrus Virologists in China [26]. "Huanglong" means yellowing of the shoot, as well as the yellow dragon (the symptom appears almost like a yellow dragon over the infected trees) and "bing," which means disease in Chinese [10]. Since the discovery of HLB, it has been named differently worldwide [27]. HLB was known outside under the name "citrus dieback" in India [23], "mottle leaf disease" in the Philippines [28], "vein phloem degeneration disease" in Indonesia [25], "yellow branch," "blotchy mottle," or "greening" disease in South Africa [29].

2.2 Geographical distribution

Globally, HLB has been considered one of the significant threats to citrus commercial and sustainable production. HLB was confirmed in citrus-producing regions of various countries, such as Nepal, Bangladesh, Thailand, Pakistan, Japan, Vietnam, Cambodia, Laos, Malaysia, Central African Republic, Comoros, Ethiopia, Kong Hong, Kenya, Madagascar, Malawi, Mauritius, Saudi Arabia, Reunion, Rwanda, Yemen, Zimbabwe, Somalia, Tanzania, Swaziland, and various region of United States of America including California, Florida [7, 27]. HLB has been reported in 24 countries and territories in East, South, Southwest Asia, East, and South Africa. Since then, it has been widely spread in other Asian, American, and African countries [27].

2.3 Symptomology

HLB symptoms are more evident in cold weather conditions than in hot seasons [30]. It is difficult to specify the period between when the citrus tree is affected by HLB and the onset of disease symptoms. It will exhibit in different parts of the plants

or only in infected sectors when it eventually manifests symptoms. It is, therefore, difficult to diagnose and control at the early stage of HLB disease [31]. The HLB-infected tree exhibits symptoms in various parts of the plant depending on the stage of infection. If infection occurs soon after propagation, the entire tree gets affected and turns yellow all over the canopy, which leads to a decline irrevocably. Both the symptoms and the causative organisms were restricted to the infected sector in the event of later infection [27]. Only the infected sector will exhibit symptoms in the case of citrus trees affected by HLB, while the remaining parts will show normal growth and good-quality fruits. The symptoms observed on the HLB-affected tree include a heavy drop in the leaf and out-of-season flushing and blooming. Chronically, HLB-affected trees displayed stunting growth, twig dieback, sparse yellow foliage, or severe fruit drop [24]. The initial stage of HLB is vein yellowing [32], and the secondary level includes (infected leaves) small, upright with various chlorotic patterns similar to that caused by nutrient deficiency, such as zinc, sulfur, iron, boron, manganese, and calcium [33, 34]. In severe cases, the leaves were utterly void of chlorophyll, except for rounded green spots located on the leaves at random places [24]. The most accurate diagnostic symptom for HLB is that the infected fruits are small, lopsided, and taste bitter and salty. HLB-affected trees with premature shedding of green fruit drops while remaining on the tree, in which fruits with yellow halo-like lesions were staying green on the shaded side, hence the name "greening" [7, 34]. Root systems are developed in severely infected trees that exhibit poorly formed roots with few fibrous roots due to undernourishment [24] and repression of new root growth and rootlets decay [10].

HLB disease is challenging to diagnose based on symptoms, particularly during the early stages of the disease. Numerous symptoms of HLB might occur, and citrus trees are often caused by other diseases or nutrient deficiencies that may lead to similar symptoms [11, 30, 35]. Symptoms could be aggravated by other pathogens being coinfected. Several reports from Asian countries postulated that HLB-affected citrus trees are commonly coinfected with the *Citrus tristeza virus* (CTV) [7]. Interestingly, some CTV isolates protect trees against HLB infection [36]. Blotchy mottle leaf is a principal diagnosis of HLB that could be misinterpreted with other diseases, such as stubborn citrus disease caused by *Spiroplasma citri*, a severe infection of CTV phytophthora root rot, zinc deficiency, and waterlogging. Furthermore, it can also be confused with symptoms of leaf-related stress and mineral deficiency [37]. Early stages of citrus blight are also associated with the symptoms of zinc deficiency [38]. For these confusing symptoms of the disease, an unequivocal diagnosis technique is needed for HLB disease.

2.4 Method of HLB detection

Early identification and isolation of *Canditatus* Liberibacter species-infected trees are effective management approaches used to limit the spread of HLB from invading HLB disease-free citrus orchards in local and international trade [39]. Visual examination is one of the most commonly employed approaches for detecting citrus HLB disease. Traditionally, early detection of HLB disease relied primarily on various symptoms in the field, such as blotchy mottle leaf, yellow shoot, aborted seed, and lopsided fruit with green color remaining at the stylar end [40]. Nevertheless, this approach is highly affected by subjective interpretation, diagnostic errors can be higher than 30%, and other biotic and abiotic stress-related problems may worsen diagnosis. HLB symptoms might be confused with diseases such as *Citrus Tristeza*

Closterovirus, *Phytophthora* infection, citrus blight, and specific nutrient deficiencies [41]. Thus, the availability of advanced technologies that enable early and rapid detection of HLB pathogens is crucial [42]. Currently used methods for the diagnosis and confirmation of HLB disease include serology, enzymatic assay, enzyme-linked immunosorbent assays (ELISA), transmission electron microscopy, DNA probes, conventional polymerase chain reaction (PCR), quantitative PCR (qPCR), Fourier transform infrared spectroscopy (FTIR), and mid-infrared spectroscopy. Pereira et al. [43] developed a method for early diagnosis using X-ray fluorescence. The laser-induced breakdown spectroscopy (LIBS) combined with chemometric strategies is used to predict the condition of orchard plants infected with *Canditatus* Liberibacter species successfully. However, these methods did not provide early diagnosis except for the LIBS method. Recently, Tran et al. [44] reported a sensitive and selective label-free biosensor that combines the physical and chemical advantages of carbon nanomaterials such as single-walled carbon nanotubes (SWNTs) in a field-effect transistor (FET)/chemiresistor architecture with selective antibodies against Sec-delivered effector 1 (SDE1), a secreted protein biomarker, for the detection of HLB. Detailed HLB detection techniques have recently been reviewed [42, 45].

3. Causal agents of citrus Huanglongbing

The bacterium associated with citrus HLB was *Candidatus* Liberibacter species, which belongs to the alpha-proteobacteria determined by the 16 s ribosomal DNA sequences and the operon [9]. *Proteobacteria* associated with HLB disease in citrus are successively referred to as *Candidatus* Liberibacter asiaticus (Las) found in the majority of HLB-affected countries, *Candidatus* Liberibacter africanus (Laf) limited to African countries, and *Candidatus* Liberibacter americanus (Lam) limited to America [15]. How *Candidatus* Liberibacter bacterium established its association with citrus species remains unclear.

Scientific classification of *Candidatus* Liberibacter.
Kingdom: Bacteria.
Phylum: Proteobacteria.
Class: Alphaproteobacteria.
Order: Rhizobiales.
Family: Rhizobiaceae.
Genus: *Candidatus* Liberibacter.

3.1 *In vitro* culture of *Candidatus* Liberibacter species associated with HLB

The isolation of *Candidatus* Liberibacter species, causing HLB in an artificial culture medium, was a primary target for many researchers. Davis et al. [46] attempted to isolated *Candidatus* Liberibacter asiaticus in a culture medium from young angular green shoots from HLB-affected trees. A growth film appeared on the bottom of the tube containing broth AD medium. After single-colony isolation, Las and the actinobacteria closely related to *Propionibacterium acnes* remained together. Thus, Las was not isolated in axenic culture. Moreover, actinobacteria are prevalent residents of citrus and psyllids, whether Las is present. Sechler et al. [47] successfully cultivated a single colony of all three *Candidatus* Liberibacter species from HLB-affected leaf midveins and petiole sap in a new medium designated Liber A. The isolated cells were

ovoid to rod-shaped, 0.3 to 0.4 by 0.5 to 2.0 μm, often with fimbriae-like appendages. They isolated two Las and one Lam strains from non-inoculated tissues of inoculated trees and seedlings 9 and 2 months later.

3.2 The pathogenic mechanism of *Candidatus* Liberibacter

Candidatus Liberibacter species are gram-negative, phloem-restricted bacterium associated with the pernicious disease of citrus HLB. Although *Candidatus* Liberibacters have been cultivated in artificial media, traditional molecular and genetic analyses have been difficult to perform owing to declining viability in culture [46, 48]. This difficulty has significantly limited efforts to comprehend the mechanisms of Liberibacter virulence. To date, most insights into the mechanisms of Liberibacter pathogenesis have been acquired through genomic analyses of Liberibacter sequences, host plant transcriptomic, proteomic, and metabolomic data associated with Liberibacter infection, and studies involving surrogates such as *Sinorhizobium* and *E. coli*, and expression in planta [15]. Evidence suggests that Liberibacters species associated with HLB live solely within the phloem tissues of host citrus plants [15]. Las bacterium resides inside a sieve tube and companion cells [47, 49]. The relatively consistent symptomology among various symptom-expressing hosts is one of the hallmarks of diseases caused by Liberibacter species [15].

3.2.1 Liberibacter secretion system and effector protein

The secretome of a pathogenic bacterium represents an array of molecules that play offensive roles during colonization, among which effectors are an important class of proteins capable of suppressing defense and/or manipulating host physiology [50, 51]. Interestingly, Las contain type I secretion systems (T1SSs) and a complete general secretory pathway (Sec), but lack other secretion systems (T2SS and T3SS) [15, 52], which play a significant role in extracellular pathogenic attacks on plant and animal host [16].

Liberibacter genome analyses found a complete T1SSs system in Las and Laf, but not in Lam [15]. Genes encode for serralysin and hemolysin; a T1SSs effector protein has been identified in Las and Laf genomes [53]. Serralysin is a metalloprotease secreted by gram-negative bacteria to inactivate peptides and antimicrobial proteins produced by the host plant. Las bacterium might use serralysin to degrade antimicrobial proteins in the host as its defense mechanism. This degraded protein is used for growth and metabolism by the Las bacterium as a carbon and nitrogen nutrient [16]. On the other hand, the hemolysin gene has been identified in all sequenced Liberibacters, which play an essential role in bacteria survival in the host plant. Las-produced hemolysin triggers ion leakage and water molecules from the host cell that lead to host cell apoptosis [16, 54].

The Secretary pathway (Sec) or Sec-translocon facilitates these effector proteins' transports outside the cytoplasm membrane vital for bacterial viability. The Sec machinery also secretes essential virulence factors in some plant-pathogenic bacteria [15]. *Candidatus* Liberibacter species have a general secretory pathway, which may lead to the secretion of effector proteins [55]. Since *Candidatus* Liberibacter species are phloem-resided bacteria, there is an inference that the bacteria secrete effector proteins directly into the cytoplasm of the host cells and modulate their physiology [56]. The effector protein CLIBASIA_05315 was located in transgenic citrus chloroplasts, resulting in leaf chlorosis and plant growth retardation [57]. Several research

groups are currently focusing on identifying and characterizing the effector proteins of *Candidatus* Liberibacter species, and it is expected that we will have an improved view of this pathogenic mechanism of bacteria in a few years.

3.2.2 Lipopolysaccharides

Lipopolysaccharides (LPS), also known as endotoxin, are critical components derived from the outer membranes of gram-negative bacteria consisting of lipid A, an oligosaccharide core, and an O-antigen. LPSs are involved in outer membrane functions that are crucial for bacterial growth, survival against antimicrobial chemicals, and virulence, particularly within a host-parasite interaction. Lipid A is highly conserved, then the oligosaccharide core and O-antigen [15, 16]. LPSs are classical activators of defense responses in plants during plant-pathogen interaction [58]. Las bacteria use gene encoding active salicylate hydroxylase (SahA) to degrade salicylic acid (SA) and suppress plant defense mechanism. Intriguingly, the *SahA* gene is highly expressed *in planta*, while it is not expressed in psyllid vectors [56]. Las impedes SA-mediated defense responses in the phloem using its SA hydroxylase and maintains significant bacterial titer in citrus HLB disease progression over several years before the tree irrevocably declines. It is yet to be determined whether LPSs of Liberibacter cause callose accumulation in the phloem.

3.2.3 Flagella

The bacterial flagellum organelle, an intricate multiprotein essential for its rotational propulsion, promotes host colonization through adherence and induces plant immune modulation [15]. Las flagella have been reported to trigger host plant defense *in planta* as a pathogen-associated molecular pattern (PAMP) [59]. Microscopic studies found that flagella have not been observed in the *Candidatus* Liberibacter species that reside in the phloem in HLB-infected samples [11]. Despite the small size of the genome, genes associated with flagella biosynthesis have been identified in the sequenced Liberibacter genome [15, 16, 51, 52]. The genes *fliF*, *flgI*, and *flgD* expressed in flagellar assembly and the *motB* gene associated with the motor function were overexpressed *in planta*.

The *flbT*, an essential flagellin regulatory protein that acts as a regulatory checkpoint for flagellin gene expression, is found in the Las bacterial genome, whereas it is not in the Lam genome. The absence of *flbT* in the Lam genome results in no PAMP activation *in planta* [60]. Conversely, *flgL*, *flgK*, and *fliE* were overexpressed in psyllid [61].

3.2.4 Prophages

Several pathogenic bacteria harbor prophages or phage remnants integrated into their genome, encoding lysogenic genes that are proven or suspected virulence factors [59]. Las- and Lam-sequenced genome contains two potential prophages, Type 1 represents prophage SC1, and Type 2 represents prophage SC2. SC1 involved in the lytic cycle of forming phage particles. SC2 was implicated in the lysogenic conversion of Las pathogenesis [60, 62]. Type 3 prophage (P-JXGC-3) was identified in Las samples collected from Southern China. This prophage carries another bacterial defense system, such as a restriction-modification system (RM system) [63]. This RM system is fortified with endonucleases, which cleaves invading DNA that protects

host DNA by altering specific sequences [64]. Type 1 and Type 2 prophages were not detected in the Las strain from Southern China. It is not clear whether these strains contain prophages or have unknown prophages. There are no comprehensive studies to describe the Las prophage repertoire [65]. Among strains observed in an extensive survey of Las isolates in China, it was typical for Las to have a single prophage, with Guangdong isolates harboring mainly the type 2 prophage, whereas isolates from Yunnan are dominated by the type 1 prophage [65]. The Las strain genome from Japan does not contain prophages [56]. Among the Las whole-genome sequences recently reported from different geographic areas around the globe, eight Las genomes contain extensive prophage sequences [63]. A survey of prophage prevalence in southern China revealed active prophage-phage interactions in the Las bacterial strains [63]. The exact function of the RM system has yet to be experimentally determined in Type 3 prophages. However, the lack of a prophage in many Las strains does not relate to the lack of HLB symptoms because Ishi-1 and the Guangdong isolates, which do not contain any prophages, induce similar HLB symptoms as isolates containing prophages [54, 65]. Overall, this evidence suggests that prophages contribute to bacterial virulence but are not required for Las pathogenicity.

3.3 Phloem dysfunction of HLB-affected citrus

Las bacteria reside within phloem and colonize sieve tubes [15, 16, 66]. Phloem dysfunction is a primary modification due to hyperactive differentiation of vascular cambium and hypertrophy of parenchyma cells surrounding the necrotic phloem pocket that may determine the development of HLB symptoms [32, 67]. HLB-associated Liberibacter secretes virulence factor and Sec-dependent effectors (SDEs) into phloem that stimulates HLB symptoms by interfering with either phloem or companion cell protein and genes of the host [15]. The secreted SDEs and virulence factors may interact with plastids, mitochondria, vacuole, and endoplasmic reticulum in the host phloem and target host genes and proteins to promote pathogen growth and disease development and suppress host immune responses [15]. Eventually, it leads to phloem malfunction in the host plant due to the Liberibacter virulence factors and SDE effects on sieve tubes and companion cells, which provide protein and transcripts to the sieve elements. Necrotic phloem was found in the HLB-infected plants due to starch (**Figure 1**) and callose deposition [32]. Callose accumulation was observed in sieve plates of Las-infected citrus [67]. Phloem dysfunction is generally associated with phloem sieve elements plugged with extensive deposition of callose and phloem protein 2 [67, 68], followed by phloem cell wall distortion and sieve element collapse [69]. Subsequently, photoassimilate transport was significantly blocked due to necrotic phloem [15, 16, 66, 68], which might result in substantial quantities of starch particles in almost all living cells of aerial parts, including phloem parenchyma and the sieve tube elements [32, 70]. The excessive accumulation of starch and zinc deficiency in chloroplast disrupts the thylakoid resulting in nonuniform loss of chlorophyll that triggers noticeable blotchy mottle appearance in the HLB-infected leaves [40, 70, 71]. The anatomical transverse section of HLB-infected leaf midrib exhibited phloem collapse with cell wall distortion and thickening in Valencia sweet orange and SB siblings [72]. In addition, hyperactive vascular cambium regenerates new phloem in the HLB-infected trees, consisting of assemblies of sieve elements, companion cells, and phloem parenchyma cells, but lacks phloemic fibers [72].

In addition to anatomical changes, several metabolic imbalances and genetic reprogramming are noticed in HLB-affected plants [57, 66]. Salicylic acid and

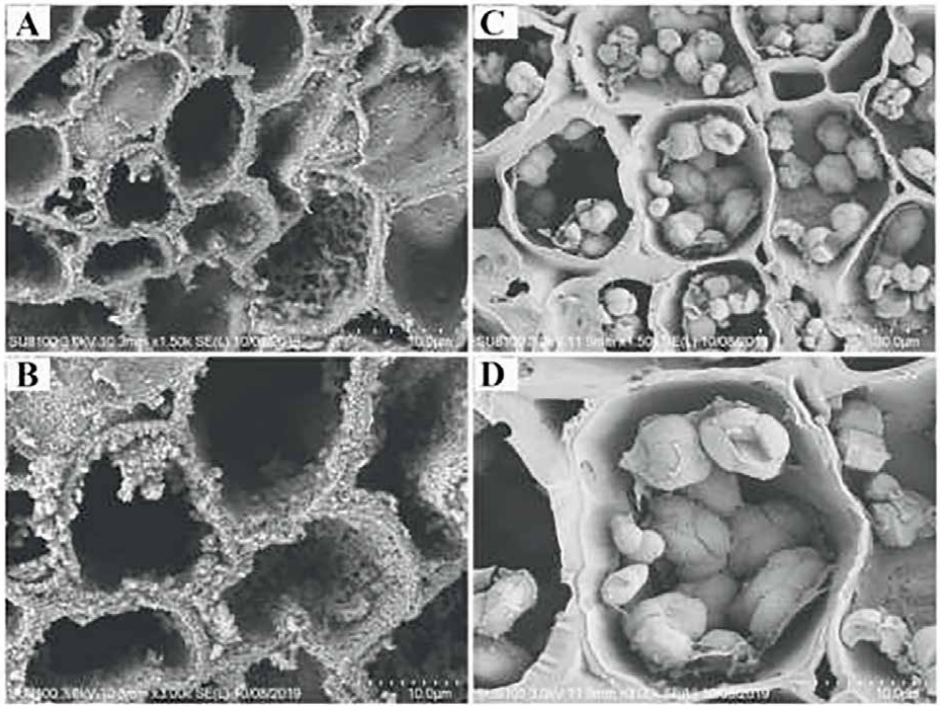

Figure 1.
SEM micrographs of transverse section of healthy and HLB-infected citrus petiole. *A and B. Healthy plant; C and D. HLB-infected plants.*

downstream signaling play a key role in provoking plant defense mechanisms against biotrophic pathogens [73, 74]. Wang and Trivedi postulated that a protein with potential salicylate hydroxylase activity might convert salicylic acid into catechol [75]. Salicylic acid pathway depression was observed in HLB-susceptible citrus plants [76]. Based on the *Candidatus* Liberibacter and plant interactions mechanism literature, we suggest the pathogenic mechanism of *Candidatus* Liberibacter species associated with citrus HLB in the following model (**Figure 2**).

4. Transmission of citrus HLB

The graft transmitted HLB was due to a viral disease [77]. Soon afterward, similar opinions were put forward in South Africa, strengthened by the results of grafting trials showing that greening was inconsistently transmitted to healthy plants. Lin [21] confirmed that HLB was transmitted through grafting in China, thus establishing the causative agent as a pathogen. McClean and Oberholzer [78] confirmed the graft transmissibility of African greening in 1965. The pathogen is not easily transmitted to progeny trees propagated by buds from infected trees, possibly due to sieve tube necrosis and uneven pathogen distribution, but more transmission occurs if stem pieces are used. No infection could be obtained when material from apparently healthy sectors of diseased trees was used. In 1964, natural spread by exposing seedlings to insects in a HLB-affected orchard developed yellowing symptoms similar to greening [79].

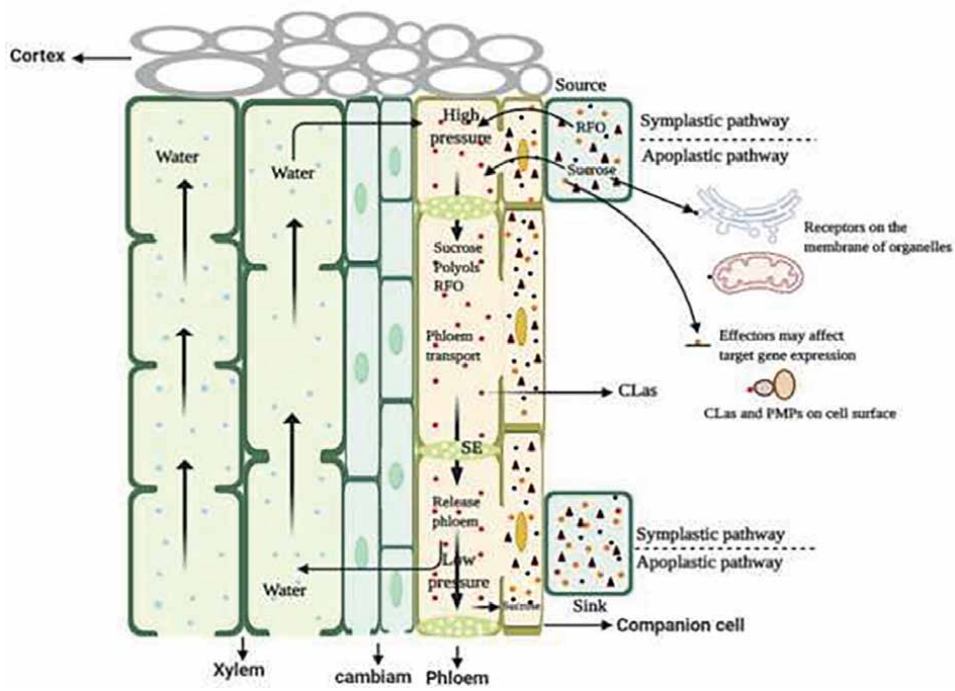

Figure 2.
Illustration of* Candidatus *Liberibacter virulence mechanisms in the plant. Candidatus *Liberibacter species associated with HLB (red circles) live in phloem elements. Phloem is mainly liable for the distribution of the carbohydrate from the source to the sink. Nutrients are transmitted to the phloem either through the apoplastic pathway or the symplastic pathway.* Candidatus *Liberibacter species may secrete effector protein (sec-dependent effectors) and virulence factors (orange and blue circle, red triangles) into phloem sieve elements and companion cells to interfere with host target (genes and protein) that can cause cell necrosis, cell death, and phloem malfunction. Effectors or virulence factors may interfere with phloem organelles, such as mitochondria, plastids, or endoplasmic reticulum, to trigger cellular responses. Some effectors (SDEs) may directly or indirectly affect the expression of target genes. In addition,* Candidatus *Liberibacter species may trigger plant immune responses through pathogen-associated molecular patterns leading to cell death and callose accumulation, resulting in inhibition of phloem transportation. The presence of* Candidatus *Liberibacter species and its metabolic activity may interfere with the function of the phloem by interrupting the osmatic gradients and integrity of phloem transportation. Abbreviations: PMPs—Pathogen-associated molecular patterns; RFO—Raffinose family oligosaccharide.*

Two insect vectors are responsible for the rapid transmission of citrus HLB from Las-infected citrus to healthy citrus species, Asian citrus psyllid *D. citri* in Asia and America, and the African citrus psyllid, *Triozaerytreae* in Africa. The acquisition feeding period is 30 min or longer, and the pathogen remains latent for 3–20 days. The inoculation feeding period is 1 hour or more [80].

Asian citrus psyllid is widespread around the world and found in hot and humid conditions and lower-lying areas in China, India, Myanmar, Taiwan, Philippine Islands, Malaysia, Indonesia, Sri Lanka, Pakistan, Thailand, Nepal, Ryukyu Islands (Japan), Afghanistan, Saudi Arabia, Reunion, and Mauritius [81]. Asian citrus psyllid firstly evolved in India [82], then spread in South America in the 1940s, invading Brazil, Argentina, and Venezuela, and then invaded the West Indies (Guadeloupe), Abaco Island, Grand Bahama Island, Cayman Islands, and the USA in the 1990s. In 2001, ACP was found in the Dominican Republic, Cuba, Puerto Rico, and Texas [83, 84]. Asian citrus psyllid has been reported more recently in many new Americas, including Mexico, Costa Rico, Belize, Honduras, and the states of Alabama, Arizona, California, Georgia, Louisiana, Mississippi, and South Carolina, USA [85].

African citrus psyllid (AfCP) thrives in cool and moist temperatures, at higher areas about 100 to 500 m above sea level. And it is sensitive to excessive heat and exists in Africa from the islands of the Indian Ocean through east and central Africa to South Africa, Saudi Arabia, Yemen, the northwestern region of the Iberian Peninsula, Cameroon, Kenya, Ethiopia, Zimbabwe, Tanzania, Malawi, Galicia, northern Portugal, Swaziland, Madagascar, Rwanda/Burundi, and Reunion [86]. Psyllid populations in Africa, Saudi Arabia, and Yemen might be able to adapt and settle under a wide range of environmental conditions, such as equatorial, arid, and warm temperate climates with varying temperatures and rainfall [86].

5. Current strategies to combat citrus HLB

5.1 Vector control

5.1.1 Biocontrol of vector

Biocontrol uses natural enemies by import, augmentation, and conservation to control the population density of disease pathogens or pests in agriculture [87]. Asian citrus psyllid (*Diaphorinacitri*) was controlled using a practical method of import (from Asia) and free of *Tamarixia radiata* in Florida citrus groves [88]. Natural enemies, *Diaphorencyrtus aligarhensis* and *Tamarixia radiata*, were imported from Taiwan and Vietnam to Florida citrus orchard and released as a biocontrol agent for *D. citri* [89]. *T. radiata* became more widely established parasitoid wasps than its counterpart *D. aligarhensis*. In urban and suburban regions, the release of *T. radiata* could considerably benefit commercial citrus growers by reducing latent psyllid populations and preventing the further spread of HLB disease [87]. *T. radiata* has been firmly established in commercial citrus-producing countries, including Réunion Island [90], the Philippines [91], Indonesia [92], Guadeloupe [93], and the USA [94], where it is spread throughout the state [88]. It appeared inadvertently in Brazil, Venezuela, Mexico [95], Puerto Rico [83], and Texas [31].

In addition to wasps, many insects native to Florida are recognized as *D. citri* predators, including several ladybeetle and spiders [81]. *Cyclonedas anguinea*, *Harmonia axyridis* Pallas (Coccinellid beetles), and *Olla v-nigrum* Mulsant were the leading killer of nymphal psyllids. In addition to *Ceraeochrysa*, *Hibanavelox* (becker), spider, and *Chrysoperla rufilabris* Burmeister (lacewings), *Tamarixia radiata* (parasitoid) also contributed to the additional mortality in Florida citrus groves. Coccinellid beetles are considered one of the most important natural enemies of *D. citri* populations in central Florida. Besides this, intraguild predation (IGP) causes more than 95% of immature *T. radiate* mortality [96]. Van den Berg et al. [97] noted that the spiders are the critical predators of *T. erytreae*, followed by chrysopids, coccinellids, syrphids, hemerobiids, Hemiptera, and predatory mites in citrus groves under the control of an integrated management program. Adults and larvae of *O. v-nigrum* (Mulsant) preying on premature Asian citrus psyllid were noticed in citrus groves throughout Florida [98].

A range of fungi species was reported to infect Asian citrus Psyllid, particularly under humid conditions [81], including entomopathogenic fungi, *Isaria fumosorosea*, *Lecanicillium lecanii*, *Beauveria bassiana*, *Capnodium citri*, *Cladosporium sp. nr. Oxysporum*, *Metarhizium anisopliae*, and *Hirsutella citriformis* that were used against HLB vectors as biopesticides [99–101]. *Isaria fumosorosea* is reported to have great

potential to control different insect pests [102]. *H. citriformis* conidia with *synnemata* produced *in vitro* and *in vivo* were subjected to adult *D. citri* exhibits an increased mortality rate [100, 103]. In a 2-year field investigation in citrus groves in Florida, adult *D. citri* (ACP) was killed by *H. citriformis* following the rainy season [104]. In laboratory conditions, the fungal strains of *Isaria fumosorosea* (ESALQ-1296) and *Beauveria bassiana* (ESALQ-PL63) accounted for 77.8 and 78.4% of adult *D. citri* mortality, respectively, while in semifield conditions, adult *D. citri* mortality rate was as high as 83.5% with ESALQ-PL63 and 80.6% with ESALQ-1296. During 1 year, the monthly use of these two fungal strains in commercial citrus groves exhibited adult *D. citri* mortality ranging from 96.1% in December 2011 to 57.8% in October 2012. In addition, this study found that the mortality rate increased under high humidity conditions [99]. *Isariajavanica* and *Acrostalagmus aphidum* were also identified as biopesticides against *D. citri* in China [105]. The use of fungal species, such as *Metarhizium anisopliae*, *Cordyceps bassiana*, and *Isaria fumosorosea*, was shown to decrease larger populations of nymphs than adults of *D. citri* in the Persian lime groves [106].

5.1.2 RNA interference for vector control

RNA interference, a process in which a double-stranded RNA exerts a silencing effect on the complementary mRNA, has become a powerful tool in entomology. Advantages, such as ease of use, specific targeting, and lack of environmental persistence, make RNAi techniques highly attractive for crop protection against many insect pests [107]. The main challenges in using RNAi-based pest control methods are compelling target gene selection and reliable delivery of dsRNA. The overexpression of dsRNAs in transgenic plants has induced RNAi in targeted insects [108, 109]. The transgenic approach in citrus, however, is slow and difficult. Hajeri et al. [110] targeted *D. citri* endogenous *Awd* (abnormal wing development disc) gene for silencing by using a CTV-RNAi vector, resulting in impaired wings in *D. citri* that would potentially limit the ability to fly and successful transmission of CLas bacteria between citrus trees in the field. In addition, decreased *Awd* gene in nymphs resulted in malformed-wing phenotype in adults and increased adult mortality. Taning et al. [111] postulated that a small dose of dsRNA (dsAK, dsSOD) administered through in Planta system (iPS) bioassay was sufficient to trigger the RNAi mechanism, causing significant suppression of the targeted transcript and increased mortality in ACP.

5.1.3 Horticultural mineral oil for vector control

Petroleum-based horticultural mineral oils (HMO) are a vital constituent of integrated management programs for many pathogens and several phytophagous arthropods pathogens that affect the productivity of fruits, vegetables, and ornamentals in the commercial cultivation field as well as greenhouse conditions [112]. Since the 1980s, HMOs have been employed to control mites and scales in China [113]. HMO controls citrus leaf miner, citrus rust mite, citrus red mite, red scale, chaff scale, spiny whitefly, and Asian citrus Psyllid in citrus [114, 115]. By lowering the number of HMOs used in treatment to 0.25 ± 0.5% and maximizing the number of sprays during each season, a significant level of pest control was achieved without the threat of phytotoxicity. The combined treatment with oils and *Isaria fumosorosea* showed that the survival rate of adult psyllids was lower than that of oils used alone [116]. Kumar et al. [117] postulated that the combined treatment of entomopathogenic *Isaria fumosorosea*

and HMOs dramatically reduced *D. citri* populations, where the maximum mean survival for *D. citri* was 12.5 ± 0.7 days. Similarly, Tansey et al. [118] revealed that mixes of insecticide and HMO application considerably decreased the populations of nymph and adult *D. citri* in Valencia orange groves in Florida. Conversely, Qureshi et al. [119] disclosed that HMO alone did not control *D. Citri* populations because the mean suppression of nymph and adults for more than 3 weeks was only 36 and 50%, respectively.

5.2 Las bacterial control

5.2.1 Antibiotics

Antibiotics are crucial for controlling bacterial diseases in fruit-bearing trees, vegetables, and ornamentals. Although antibiotics can be detected on plant surfaces using delicate analytical chemistry techniques for up to a month after application, their ability to inhibit bacterial growth is lost within a week [120]. In-plant disease control, nearly 40 antibiotics were screened; only streptomycin and tetracycline were used extensively in fruit trees [121]. The only commercially applied treatment for HLB was tetracycline, which is bacteriostatic rather than bactericidal, in Reunion Island's orchards [122, 123]. Tetracycline was the only approved antibiotic injection in trees injected directly into the trunks of HLB-affected citrus trees in China, Indonesia, India, Taiwan, and South Africa during the 1970s [36, 117, 124]. Although the symptoms of HLB were considerably decreased, this antibiotic trunk injection method was not in practice owing to its phytotoxicity and labor costs. The use of penicillin-carbendazim antibiotics in citrus trees showed significant control of HLB disease. The antibiotic disadvantage is a reduction in the fruit size owing to phytotoxicity and the residues of the antibiotics in citrus fruits [125]. The development of therapeutic compounds and bactericidal agents to control devastating HLB could provide an additional solution for an effective integrated disease management program. However, other than selective antibiotics, nonselective bactericide is recommended for general use in most crops, particularly citrus [126]. The combination treatment of streptomycin with penicillin efficiently eliminated or repressed the Las bacterium compared with the separate administration of either antibiotic [126]. The treatment of penicillin combined with streptomycin also significantly reduced the bacterial titer of Las in greenhouse citrus plants. Kasugamycin and Oxytetracycline combination therapy *via* trunk injection significantly reduced HLB bacterial titer in the field. However, the combination of kasugamycin and streptomycin was not effective against the bacterium of Las [127]. Penicillin with oxytetracycline combination therapy has been more effective in controlling citrus pathogens [128] but may require annual treatment [20]. Among the 31 tested antibiotics, some were effective at reducing and eliminating Las bacterial titers in inoculated rootstock and the treated scions of citrus plants, such as ampicillin, carbenicillin, penicillin, cefalexin, rifampicin, and sulfadimethoxine [20]. Oxytetracycline has therefore been suggested to be used more frequently in combination treatment [129, 130] with penicillin or kasugamycin against HLB to control the progression of bacterial resistance and maximize the antibiotic efficacy against HLB pathogenic bacteria [131]. The Environmental Protection Agency (EPA) of the USA allows citrus growers to spray streptomycin and oxytetracycline as routine treatments in the citrus field several times per year [132]. Oxytetracycline (1 g/L) was delivered to leaves of HLB-infected trees through the

foliar application, and oxytetracycline was found in all leaves, although at reduced levels than in the directly applied leaves [132]. However, the phytotoxicity of tetracycline should be considered [20]. Antibiotics tested to combat HLB malady are tabulated in **Table 1**.

S.No	Antibiotics	Working concentration (mg/L)	Effectiveness	Phytotoxicity
1	Actidione	25	High	Highest
2	Validoxylamine A	100	Partly	Less
3	Zhongshengmycin	100	Partly	Less
4	Amikacin sulfate	100	None	NIL
5	Gentamicin sulfate	100	None	NIL
6	Hygromycin B	150	Partly	NIL
7	Kanamycin sulfate	100	Partly	None
8	Kasugamycin hydrochloride	100	None	NIL
9	Neomycin hydrate trisulfate	50	None	NIL
10	Spectinomycin dihydrochloride pentadrate	20	Partly	None
11	Streptomycin sulfate	100	None	NIL
12	Tobramycin	20	None	NIL
13	Ampicillin sodium	100	High	Less
14	Carbenicillin disodium	100	High	Less
15	Penicillin G potassium	100	High	Less
16	Cefalexin	100	High	Less
17	Vancomycin hydrochloride	40	None	NIL
18	Lincomycin hydrocloride	100	None	NIL
19	Cycloserine	50	Partly	NIL
20	Rifamycin sodium	50	Partly	Less
21	Rifampicin	50	High	Less
22	Rifaximin	50	Partly	Less
23	Colistinmethane sulfonate sodium	20	None	NIL
24	Polymixin B sulfate	300	None	NIL
25	Cinoxacin	300	None	NIL
26	Ciprofloxacin hydrochloride	300	Partly	NIL
27	Sulfadimethoxine sodium	100	Partly	Moderate
28	Sulfamethoxazole	100	Partly	Moderate
29	Sulfathiazole sodium	100	Partly	Moderate
30	Chloramphenicol	30	Partly	Less
31	Oxytetracycline hydrochloride	100	High	Highest

Table 1.
Antibiotics effectiveness against CLas bacterium and phytotoxicity.

5.2.2 Thermotherapy

Heat treatment or thermotherapy of planting material is a century-old disease control method that has proven effective against various pathogenic microorganisms. Thermotherapy, simple in principle, can eliminate the conserved pathogens depending on temperature/time regime and can cause mild injuries to the host during the treatment. Heat is mainly generated by water, vapor, or air [133]. The main advantage of thermotherapy treatment is that it is more environmentally friendly than harmful agrochemicals. Thermotherapy has proven to be an effective strategy against HLB that helps to enhance the vigor of citrus trees and promotes new root growth and development. The efficacy of thermotherapy against HLB pathogens depends on the temperature and citrus varieties [134]. Therapy could recuperate HLB-affected citrus plants by eliminating or suppressing Las bacterial titers at temperatures above 40°C [6, 134]. *Candidatus* Liberibacter asiaticus is a heat-tolerant phloem-limited bacteria that can withstand a temperature of about 35°C, while *Candidatus* Liberibacter americanus is heat-sensitive [135]. Thermotherapy could eliminate HLB pathogens from valuable horticultural trees associated with shoot tip grafting [136].

Lin opined on eliminating yellow shoot disease with water-saturated hot air treatment of graft wood 48–58°C with no loss of tissue viability [137]. In India, the thermotherapy of budwood at 47°C for 2 hours of diminished disease incidence, and more prolonged treatment eradicated the pathogen [138]. Heat treatment at temperatures around 38–40°C for 3 or 4 weeks killed HLB pathogens in young infected plants or citrus seedlings grafted with infected tissues [138, 139]. In South Africa, HLB-infected budwoods were treated with hot water baths at 51°C for 1 hour, 49°C for 2 hours, and 47°C for 4 hours, eliminating HLB pathogens with some loss of viability at higher temperatures [140]. In HLB-affected trees topped with polyethylene fiberglass sheets for 2 to 5 months, the number of diseased fruits decreased. However, this technique is not feasible for extensive use in citrus groves [27]. The HLB-affected citrus seedlings were continuously exposed to 40 to 42°C heat therapy for 7 to 10 days, significantly reducing titer or eliminating Las bacteria. This treatment can be helpful to combat HLB-affected plants in greenhouse and nursery settings [134]. Ehsani et al. [141] also postulated a decrease in HLB symptoms in groves of citrus trees after heat treatment. The combined thermo- and chemotherapy of sulfathiazole sodium or sulfadimethoxine sodium was more effective at 45°C than in thermotherapy alone, chemotherapy alone, or a combination of thermotherapy at 40°C and chemotherapy [142]. The temperature treatment at 45°C for 8 h per day for a week and a combination of ampicillin sodium, actidione, and validoxylamine A as a bark paint on grapefruits plant significantly reduced Las titer [143]. Two-year-old graft HLB-affected citrus reticulate treated with thermotherapy at 45°C and 48°C showed diminished HLB symptoms and Las titers 8 weeks after treatment in the greenhouse condition [144]. Commercial and residential citrus trees covered with portable plastic enclosures exposed to elevated temperatures through solarization showed vigorous growth in 3–6 weeks after treatment. Although commercial citrus trees showed Las after heat treatment, many trees generated extensive flushes and grew strongly for 2 to 3 years after therapy [145]. Inner bark heat treatment with 60°C–0.03 MPa-30s in 9-year-old citrus plants exhibited significantly reduced Las bacterial titer with vigorous plant growth from all treated HLB-affected trees [146]. Abdulridha et al. [147] reported that HLB-affected trees with canopy cover were treated with combined hot water and steam therapy at 55°C for 90 seconds. The temperature distribution inside the canopy cover was not uniform; the canopy temperatures were more significant than the trunk

temperatures. The mobile thermotherapy treatment needs to be improved to increase the temperatures around the tree trunk to nearly the same temperature as a canopy. Vincent et al. [132] postulated that heat treatment from 43 to 54°C for no longer than 45 s showed adverse effects on citrus tree growth.

HLB is a systemic disease. Efficient elimination of Las bacteria from the entire citrus tree, including roots, is vital to managing the disease. The current thermotherapy challenge is that although adequately elevated temperatures can reach the above-ground areas of the plant, killing temperatures are unlikely to be attained at the roots where the temperature is mitigated by the soil [148]. Therefore, heat treatment is unlikely to reduce the populations of HLB pathogens in the roots, which then acts as a site for canopy reinfection during flushes. The efficacy of heat treatment in eliminating Las bacterial populations in underground roots must be enhanced to become a feasible part of integrated citrus HLB management [15]. To overcome this barrier, Hoffman et al. [134] suggest that heat treatment, coupled with chemotherapy in HLB-affected plants, can lead to a potential future strategy for controlling citrus HLB.

5.2.3 Plant defense activators to combat HLB

Trunk injection is an alternative target-precise technique for efficiently delivering plant protective chemicals in tree fruit crops. It harnesses the rapid transportation ability of the xylem that enables therapeutic compounds' translocation and subsequent distribution into the canopy where plant protection is needed [149]. There has been limited research on the trunk injection of antibiotics and plant defense activators for better disease control. Several recent field studies have demonstrated the utility of trunk injection of bactericides and plant defense activators in disease management [150].

Treatments with β-aminobutyric acid (BABA), 2,1,3-benzothiadiazole (BTH), 2,6-dichloroisonicotinic acid (INA), ascorbic acid (AA), and the nonmetabolizable glucose analog 2-deoxy-D-glucose (2-DDG) plant defense inducers individually or in combination found effective in suppressing Las bacterial population in plants and sustaining fruit production to a certain extent. Treatment with BABA and BTH was the most effective in reducing the Las population in plant tissues compared with other plant defense inducers [151]. Hu and Wang proved that trunk injection of oxytetracycline in HLB-affected trees exhibited long-lasting suppression of Las populations. It also prevented the tree decline by promoting new growth without the disease [152]. Trunk injections of salicylic acid, potassium phosphate, acibenzolar-S-methyl, and oxalic acid in the HLB-affected tree significantly suppressed the Las titer and HLB disease progress [150].

Brassinosteroids (BRs) are a class of steroid hormones that regulate gene expression, growth, and developmental processes in response to biotic and abiotic stress [153]. The plant defense mechanism of brassinosteroids was mediated by leucine-rich repeat receptor kinase (LRR-RK) BAK1, which serves as a coreceptor for both microbe-associated molecular patterns (MAMPs) and steroid hormone [154], which binds to BRs and FLS2 eliciting microbe-induced immunity. BR treatment showed increasing disease resistance against many pathogens [6]. Canales et al. [155] postulated that applying epibrassinolide as a foliar spray in HLB-infected plants improved immunity against *Candidatus* Liberibacter asiaticus in greenhouse and field citrus plants. *Candidatus* Liberibacter asiaticus titer was markedly reduced in epibrassinolide-treated plants due to the enhanced defense gene expression in the citrus leaves. However, the molecular mechanism of BRs in plant responses under normal and environmentally challenging conditions has remained unclear [155].

5.3 Nanoemulsions to deliver chemicals against Las bacteria

HLB is caused by Las proteobacteria that reside in the phloem of infected citrus trees. It is, therefore, challenging to deliver effective compounds into the phloem through a foliar spray. The presence of wax, cutin, and pectin in plant cuticles prevents the effective bactericidal compounds from entering the phloem through a foliar spraying method. The use of chemical adjuvant enhanced the foliar uptake of agrochemicals [156, 157]. However, foliar spray treatment, including the combination of antibiotic PS and adjuvants in dimethyl sulfoxide and Silwet L-77, did not significantly impact the HLB-affected citrus trees [128]. Therefore, there is a need for candidate adjuvants, which can potentially increase the permeability of citrus cuticles to deliver antimicrobial compounds into citrus phloem.

Nanoemulsions or submicron emulsions are colloidal dispersion systems with average droplets size ranging from 50 to 1000 nm that has extensively studied for delivering chemical compounds. Nanoemulsions were pondered as thermodynamically and kinetically stable isotropic dispersions, composed of two immiscible liquids such as water and oil, stabilized by an interfacial film composed of an appropriate surfactant and co-surfactant to form a single-phase [158]. However, the approach efficacy relies on nanoemulsions droplet characteristics, such as low surface tension, tiny size, ample surface area, and low interface tension [159]. Our research group postulated that water in oil nanoemulsions containing ampicillin coupled with adjuvant Brij 35 was used as a foliar spray to enhance the permeability through the citrus cuticle into the phloem and more efficiently eliminated Las bacteria in HLB-affected citrus *in planta* [160]. Ampicillin showed the lowest phytotoxicity to citrus trees infected with Las bacteria [20]. However, the US Environmental Protection Agency (EPA) has not approved the commercial use of ampicillin in crops due to the development of resistant bacterial strains [160]. In another study, oil in water nanoemulsions was formulated using a spontaneous emulsification method, where five different antimicrobial compounds alone combined with Cremophor EL (viscous oil), acetone, and Span 80/Tween 80, which formed tiny droplets, were effectively applied to the bark for efficiently control HLB [161].

Silver nanoparticles (AgNPs) are one of the most investigated and used in agricultural science to enhance the yield and sustainable development of the crop. This has long been reported to have significant antibacterial, antifungal, antiviral, and pesticide effects. AgNPs are used as foliar sprays to prevent the development of rot, mold, fungi, and other plant pathogens [162]. Stephano-Hornedo et al. [18] evaluated the commercially available AgNPs to directly eradicate *Candidatus* Liberibacter asiaticus (CLas), responsible for HLB in the citrus field. The 93 HLB-infected citrus trees administered foliar and trunk injections of silver nanoparticles showed a remarkable reduction of 80–90% in bacterial titer by both methods than control. Compared with other effective treatments involving b-lactam antibiotics, the effectiveness of AgNPs is 3- to 60-fold higher when administered by foliar spray and 75- to 750-fold higher when injected *via* tree trunk. Thus, the silver nanoparticles could be a sustainable method for mitigating citrus HLB. However, AgNPs toxicity to a citrus tree and the environment needs to be warranted before its commercial use.

5.4 Transgenic approach to combat HLB

Globally, insect pests are responsible for significant crop losses through direct harm and transmission of plant diseases [163]. The best long-term alternative strategy

for managing citrus HLB is to develop disease-resistant cultivars in commercial citrus production. Due to the lack of resistant cultivars, developing HLB-resistant plants by conventional citrus breeding is difficult. Resistance occurs in citrus relatives, such as kumquat, where its genetic background influences the quality and yield of the fruit [164]. In addition, conventional citrus breeding is labor- and time-consuming, and very costly as citrus species are polygenic, extremely heterozygous plants with a long juvenile phase. The genetic transformation approach is an essential strategy that would aid in incorporating disease-resistant genes into citrus cultivars to combat the HLB disease. The progression of citrus breeding through genetic transformation is still early, indicating a lack of molecular pathogenesis understanding of innate disease resistance in citrus [165].

Systemic acquired resistance (SAR), a natural plant defense response mechanism, has been well characterized in *Arabidopsis thaliana*. SAR entails signal molecule salicylic acid (SA) to activate defense mechanisms. In response to SA, the non-expression of pathogenesis-related gene 1 (NPR1) is translocated to the nucleus, where it triggers the expression of pathogenic related (PR) genes by interacting with TGA transcription factors, thereby provoking SAR [166, 167]. *Arabidopsis* mutants contain deficiencies in the *NPR1* gene showing decreased PR gene expression induced by SA and SAR, leading to increased susceptibility to pathogens [167, 168]. Conversely, overexpression of the *NPR1* gene in *Arabidopsis* increased the disease resistance to bacteria and oomycete pathogens. Interestingly, the over-expression of *AtNPR1*gene in most plant species does not provoke noticeable adverse effects on plant growth and development [169]. Thus, *NPR1* is a target gene for the genetic transformation of nonspecific resistance in crop plants.

Dutt et al. [170] postulated that the overexpression of the *AtNPR1* gene in Hamlin and Valencia orange cultivars resulted in trees with normal phenotypes, and exhibited increased resistance to HLB. Transgenic trees showed reduced disease severity, and a few lines remained disease-free even after 36 months of planting in a high-disease pressure field. The phloem-expressed *NPR1* gene was equally effective in increasing disease resistance by triggering several indigenous gene expressions involving plant defense mechanisms of signaling pathways. In addition to triggering resistance to HLB, the observed SAR response could protect citrus trees from other major fungal and bacterial diseases, such as black spots and citrus canker [170].

6. Conclusions

HLB is one of the century-old diseases in the history of citrus pathology. The global spread of HLB disease causes economic loss in most citrus-producing countries. The causal agent of HLB, *Candidatus* Liberibacter, impedes understanding its pathogenic mechanism, fastidious nature, and unculturable in artificial conditions. Future research will focus on the isolation and pure culture of these proteobacteria. The rootstock and scion of some tolerant varieties have been noted and used by citrus growers in citriculture. These tolerant varieties have the potential to suppress the progression of HLB in Las-infected trees. The HLB management program recommends the intense psyllid control and removal of Las-infected trees in citrus groves. Citrus farmers focus on maintaining productivity in the HLB-affected trees by using plant defense activators, micronutrients, and fertilizers and paying more attention to water irrigation systems. Besides, thermotherapy is still an efficient methodology for eliminating and suppressing the causal agents in citrus scions and rootstock.

Antibiotics alone or combined with other bactericides have also shown to be effective against citrus HLB. However, antibiotics need to evaluate their phytotoxicity before their commercial use. The combination of thermotherapy and antibiotics, plant defense activators, and thermotherapy provides controlled HLB management efficiency. The formation of nanoemulsion in water in oil (W/O) and/or oil in water (O/W) could offer a practical methodology for the targeted delivery of antimicrobial compounds to the phloem of citrus by foliar spraying method to control citrus HLB. In addition, transgenic orange cultivars over-expressing the *AtNPR1* gene exhibited enhanced resistance to HLB. Transcriptome analysis between susceptible, tolerant, and resistant citrus varieties provides new insights into HLB tolerance by revealing defense-related genes, biological pathway signaling, hormones, transporters, carbohydrate metabolism, phloem-related genes, and secondary metabolism. In addition, some potential targets have also been identified, such as *DMR6-like* and *NPR1-like* genes for future HLB-tolerant citrus breeding [171]. Epibrassinolide as a foliar spray in HLB-affected plants improved the immunity against *Candidatus* Liberibacter asiaticus in the greenhouse and citrus field. However, further studies on the impact of eBL and nanoemulsion loaded with antibiotics in HLB-affected citrus plants are warranted to understand the complexity of citrus pathophysiology and fruit productivity. Researchers have investigated many control strategies to combat *Candidatus* Liberibacter species, but no effective management strategies have been developed. More studies are needed to investigate a sustainable and environmentally friendly strategy to control citrus HLB in the form of an antimicrobial agent in citrus groves. Meanwhile, biotechnological approaches such as transgenic, gene editing, and host-induced gene silencing may provide an unprecedented opportunity for long-term HLB management tools.

Based on the extensive prevention strategy experiments in the citriculture field by Chinese farmers, it has been shown that the control of HLB disease can be carried out in the three-pronged approach.

1. **HLB-free seedlings.** Selection of HLB-free citrus saplings, rootstocks, and scions. Furthermore, infected root stocks/scions might be cured through thermotherapy.

2. **Removal of infected plants.** Identification of HLB-affected plants by utilizing a suitable pathogen detection system and removing infected trees or infected sectors.

3. **Suppress the psyllid.** Control of psyllids to reduce the spread of HLB pathogens in the field and biological control of vectors might be desirable methods to control the vector populations rapidly and cost-effectively.

Nanotechnology-driven farming is still early, but it is an exciting and challenging field of research to be developed in the future, especially if the proper emphasis is placed on understanding the fundamental interactions between nanoscale materials and crop plants [172]. Future nanotechnology will enable the development of biosensors for early diagnosis of disease, new methods for suppression of disease pathogens in field and greenhouse conditions, and new molecular tools for understanding pathogenic mechanisms in pathogens and plants [173]. Nanotechnological investigations in phytopathology have increased dramatically over the last decade. Nanomaterials can be engineered as biosensors to diagnose plant diseases and as a

means of delivery of genetic material, probes, and agrochemicals. Nanotechnology has been incorporated into disease management strategies, diagnostic tools, and molecular tools. Nanotechnologies could provide an alternative treatment to citrus farmers to be integrated into their existing HLB management programs in the citrus groves.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 2

Anthracnose Disease of Mango: Epidemiology, Impact and Management Options

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Abstract

Mango is one of the frequently cultivated seasonal fruit crops in several tropical and subtropical regions. It is consumed as whole fruits apart from serving as raw materials for most industries that are into mineral production. Mango production is, however, constrained by diseases, pests, and poor post-harvest handling of fruits. Anthracnose disease, caused by *Colletotrichum gloeosporioides* Penz and Sacc, is one of the most important yields limiting constraint in mango production across the globe. The disease occurs in both the field and post-harvesting. In the field, it affects aboveground parts, such as the stem, branches, leaves, flowers, and fruits. Anthracnose disease reduces the shelve life and marketability of mango fruit. In Ghana, anthracnose disease is responsible for about 30% yield/fruit loss. Most farmers do not control it, although some have resorted to the application of various fungicides not registered for mango anthracnose disease management in Ghana. This chapter will highlight on the importance of the disease on the mango industry in Ghana, control strategies currently employed thereby reducing the over-reliance on chemical control option and propose ways to minimize the effect of the disease in the country.

Keywords: aetiology, anthracnose, botanicals, *Colletotrichum* spp., epidemiology, mango

1. Introduction

Mango (*Mangifera indica* L) is a major fruit crop with an immense economical relevance in Ghana. Due to the special qualities of its fruits, it is also considered as one of the most frequently considered fruits in various fruit markets across the global. Some of the qualities mango are delicious taste, beautiful colour, excellent carotene content, excellent flavour and attractive flavour [1]. The crop is known to contribute up to a half of the overall tropical fruits that are produced globally [2]. Africa contributes about 10–15% of the total mango produced per annum. In Ghana and other neighbouring countries, the crop serves as one of the most frequent

non-traditional fruits exported to other countries. Mango in Ghana is currently in high demand in the form of both fresh and dry fruits, juice and flavours, and jams. In as much as mango production in Ghana and other production countries has seen some increase in recent times, it is frequently faced with challenges such as pests and diseases attack. One of the most devastating diseases is the anthracnose disease. The mango anthracnose disease caused by *Colletotrichum gloeosporioides* Penz and Sacc is a major fungal disease of economic importance to mango production across the globe [3–6]. To manage this menace, various strategies has been adopted by various stakeholders in the mango production value chain [7]. These strategies ranges from pre-harvest to post-harvest. Both the pre-harvest and post-harvest control measures are successfully implemented through proper field hygiene, after harvest treatments or a combination of the two. However, the management strategy chosen at any point by an individual must be consumer friendly, economical, and environmentally friendly. In composite, these management strategies can further be categorised into cultural, chemical, biological, use of resistant cultivars, and/or their integration [7].

2. Impact of anthracnose on mango production in Ghana

Different kinds of diseases affect productivity of several plants contributing significantly to global food/fruit shortage. Destructions/Catastrophe caused by the outbreak of plant diseases in multiple plants as well as its impact on humans such as starvation, malnutrition and death have been well publicized. In Ghana, the impact of diseases on tree and horticultural crops have been reported and well documented. Mango production in Ghana has gained prominence in recent times with the projection of overtaking cocoa as the most economically valuable crop. The potential gains due the country from mango production is however, threatened by the existence and outbreak of multiple diseases due to the crop's susceptibility to several fungal pathogens. Mango is highly susceptible to diseases due to the high water-nutrient content which serves as a perfect media for fungal pathogen development [8]. Production and export of mango in Ghana keeps dwindling due to the prevalence and severity of diseases such as the anthracnose [9]. Among the many diseases limiting mango production is the Anthracnose disease caused by the fungus *Colletotrichum gloeosporioides* Penz. & Sacc. It is a major limitation to mango production as it has been reported to has led to a yield loss of 30% in the Yilo Kobo District of Ghana [10]. Similarly, about 39% yield loss in mango production in India has been attributed to anthracnose disease [11]. The disease is so devastating in crop production that it has also been reported that 60% of harvested avocado fruits affected by the disease were discarded and rendered unmarketable in Kenya [12].

Although the disease leads to massive losses in the field, the most significant loss occurs during post-harvest conditions. The post-harvest damage due to the disease is of more interest and economically relevant because it reduces the fruits quality as well as shelf life which invariably affects standard for the export market. This pose great challenge to various actors along the value chain especially those involved in international trade as low-quality fruits cannot be marketed on the international market. Although in some instances, producers and traders may sell these low-quality fruits on the local market, differences in revenue from export and local markets leads to serious economic loss to all stakeholders as well as mango exporting countries like Ghana as they are denied of foreign exchanges.

Attempts to mitigate the effects of anthracnose disease on mango production have centred mainly on the employment of conventional pesticides to treat trees and even fruits at harvest and storage. Although effective in minimizing the severity of the disease, this strategy is limited due to the growing negative concerns associated with fungicides on the environment, human health and rapid surge in antifungal resistance by several pathogens. In addition to these, is the high cost of these pesticides leading to increased cost of production to the farmer and reduced income which can further increases poverty in the rural economy.

3. Aetiology and epidemiology of anthracnose disease of mango

3.1 Aetiology of anthracnose disease of mango

Anthracnose disease of mango is one of the serious diseases of mango worldwide as it affects the crop from nursery through growth (pre-harvest) to harvested fruits (post-harvest) [11, 13, 14]. Many reporters have confirmed that two closely related species of Colletotrichum are involve in disease initiation and development. These species are *Colletotrichum gloeosporioides* Penz and Sacc. (Teleomorph: *Glomerella cingulata*) and *Colletotrichum acutatum* (Teleomorph: *Glomerella acutata*) [15–17]. In Ghana, literature has predominantly reported that the *C. gloeosporioides* is the causal agent of the disease [18, 19].

3.2 Epidemiology and disease cycle

The disease initiation, growth and development of the pathogen are influenced greatly by the nutritional and environmental factors of the host plant (Mango). Among the nutritional factors are sources of nitrogen and carbon [20] whereas the environmental factors for disease development wet, humid, hot weather [21] as well as pH and Temperature [20]. The pathogen is also considered generally inactive in dry weather [3].

It is reported that the source of primary inocula of the disease if the abundance of conidia in the tree canopy [3]. The disease attacks every part of the plant i.e. young nursery seedling leave and twigs, young flowers/flower clusters (panicles), young fruits as well as mature fruits. The cycle of the disease begins with the dissemination of the conidia (asexual spore) of the pathogen which are dispersed passively through rain splashes or irrigation water. The plant is inoculated when conidia land on the infection courts or sites (most of which are above ground parts of the plant). Infection and disease development are also achieved on young and immature fruit and tissues, conidia are germinated and penetrated in the cuticles and epidermis to ramify through the tissues. On mature fruits, infections occur immediately when spores penetrate the cuticle. However, they remain at a latent state until climacteric fruit ripening begins. During disease symptoms development, black, sunken, rapidly expanding lesions develop on affected organs. On leaves, symptoms are seen as small, brown-to-black lesions on most affected parts.

On fruits where major destruction occur both in pre and post-harvest, symptoms of black/dark is visibly as well as slightly sunken lesion with irregular shape, then gradually enlarges and subsequently causes fruit rot. The first appearance of symptoms as spots usually coalesces and penetrate deep into the fruit, resulting in extensive fruit rotting. In most green fruits, infections remain latent and largely invisible until

ripening, that fruit that appear healthy at harvest can develop significant symptoms upon ripening. The second symptom type of the fruit is one that commonly refers to as an "alligator skin" in which on the fruits consist of a "tear stain" symptoms, in which appear a linear necrotic region on the fruits that experience superficial cracking of the epidermis. Lesions on stem and fruits may produce visible, pinkish-orange spore masses under wet conditions. During reproduction of the pathogen, sticky masses of conidia and production of fruiting bodies (acevulli) on symptomatic tissues during humid conditions. Repeated cycles of the disease may occur as a result of continuous multiplication of the fungus during the season which can serious epidemics. In the absence of favourable conditions, the pathogen overwinters in plant debris such as defoliated leaves, dead branches and other volunteer tree species.

4. Management strategies of mango anthracnose disease

4.1 Cultural control

One of the main conditions that favours the development of the mango anthracnose is high humidity. It is therefore important to establish mango orchards in areas with a well-defined dry season. This creates an unfavourable condition for the development of the disease [12]. In most tropical regions, flowering in mango plants occurs usually in the dry seasons. Nevertheless, this is dependent on other factors such as photoperiodism, growth stage of the shoot, specie of the mango, ambient temperature, as well as the nutritional state of the soil under cultivation. This brings some level of variations in the flowering period of mango from region to region. It has been noted that a very early flowering, usually before the dry season leads to a huge infection of the disease due to the higher relative humidity at the time of fruit formation and establishment [22, 23]. From another standpoint, a worst scenario of infection is seen when mango trees flower at the latter part of the dry season or when early-stage fruit development coincides with the peak of the raining season [24]. This can lead to a disease incidence of up to 90% in such occasions. For proper anthracnose disease management in the tropics, a plausible approach must be adopted to avoid situations where flowering an early-stage fruit development will coincide with the rainy season. A proper adherence to this approach could cumulatively, leads to a disease incidence and severity near zero present. However, despite the effectiveness of this strategy, it is near inapplicable in the subtropical regions where the main stimulants for flowering in mango is atmospheric temperature rather than lower water deficit. One of the most effective ways of managing the incidence and severity of this disease in these areas is the foliar application of a growth and flowering retardant (e.g. Paclobutrazole) either singly or in combination with potassium nitrate [24, 25]. By that, flowering in mango can be advanced by several days or weeks. Furthermore, traditional cultural practices such as the removal and proper incineration of infected plant parts such as fruits, branches, leaves among others can also be reliable in the cultural management system of mango anthracnose disease. In addition, practices such as removal of dry, infected, or malformed panicles and fruits can serve as one of the effective ways of managing the disease. However, these later practices can be laborious and time consuming on large area farms. Research has also revealed that wrapping of young developing fruits in paper bags as a way of creating barriers between the fruits and any possible inoculum of the disease can immensely reduce the incidence and severity of the disease. However, his method can also reduce the bright red/yellow colour observed in ripped mangoes. This, on a

broader perspective could insinuate that choosing a particular method or practice in managing the disease could highly depend on the objective and the target market of the farmer. For example, most industries that would rather prefer chemical free fruits to bright coloured ones will rather appreciate this approach as compared to the application of various synthetic fungicides other methods such as chemicals.

4.2 Resistant varieties

The use of cultivars that are resistant to disease has always been one of the most effective and economical ways of managing diseases in many crops, including mango. However, in mango, several studies have shown that most cultivars that are commercially available to farmers are susceptible to the disease. Nevertheless, some experiments in controlled environments have reported that there are some few genotypes with some considerable levels of resistance to the mango anthracnose disease (**Table 1**). These traits can be harnessed in future breeding intervention to development some potential cultivars with higher resistance to the disease [26].

4.3 Chemical control

Just like in most scenarios, the application of chemicals seems to be one of the most effective and fastest ways of managing the mango anthracnose disease. Nevertheless,

Name	Designation	Country
'Carrie'	R	Australia
'Caraboa Florigon'	R	Australia
'Tommy Atkins'	R	Australia
'Saigon'	R	Australia
'Kensington Pride'	MR	Australia
'Palmer'	R	Philippines
'Siam'	R	Philippines
'Velei-Colomban'	R	Philippines
'Joe Welch'	R	Philippines
'Fernandin'	MR	Philippines
'Arumanis'	MR	Philippines
'Edward'	MR	Philippines
'Gedong	MR	Philippines
Tjenkir'	MR	Philippines
Paris', 'Fairchild	R	Hawai'i
'Rapoza'	R	Hawai'i
'Haden'	MR	Hawai'i
'Zill'	R	Florida

R = resistant; and MR = moderately resistant.

Table 1.
Mango cultivars resistant to the anthracnose disease [21].

under severe pressures, one has to conduct up to 25 applications in a season to achieve desired results. Also, the choice of fungicide is also dependent on the variation in requirements of proposed destination or purpose of the fruit. For example, Dithiocarbamate (an effective active ingredient for anthracnose disease control) produces ethylene thiourea (ETU) as a bio-product. Due to this, fruits that has a history of the application of ethylene-bisdithiocar- bamates such as mancozeb and maneb are prohibited in the United States. Most frequently, copper fungicides are recommended for the control of most fungal disease, which the mango anthracnose is not an exception. In as much as some success stories has been documented, there are also contrasting studies that has revealed that under higher disease pressures, copper-based fungicides are usually less effective as compared to the carbamates. Not only that, few reports have also reported some phytotoxicity associated with the application of fungicides of various sorts. Post-infection fungicides such as the benzimidazoles has also been used in combination with protectant fungicides such as the imidazole prochloraz to retard the build-up pressure of the mango anthracnose disease [27–29]. This chemical s usually affects the mycelia development of the fungi by supressing the synthesisation of ergosterols, a very important component of the cell membrane of a fungus [30]. One advantage for this combination is that it also retards the build-up of resistance in the population of the pathogen. Other reports have also documented the use of clarified hydrophobic neem oil (70%) to effectively control the mango anthracnose disease.

4.4 Biological control

Biological approach of managing disease is the use of antagonistic organisms in managing diseases. In the cases of mango anthracnose, an array of microorganisms have been postulated to have an antagonistic association on its causative agent (*C. gloeosporioides*) [31]. However, even though this looks very promising, there is no current documentation of commercial implementation of this finding. In expense, keen attention has rather been placed on the postharvest control method. Perhaps, this could be as a result of the ability to manage controlled environment conditions as compare to field situations. Nevertheless, the antagonistic potentials from such biological agents can be harnessed as an intervention to developing a more robust management approach to the disease. An example was cited in a study on the efficacy of essential oils in managing the mango anthracnose disease [32]. As all other disease management approaches, the efficacy of biological control method can be enhanced with an integration of other control measures [33].

4.5 Current research on the management of anthracnose disease in mango with botanicals

Anthracnose an important pre and post-harvest disease of mango infests mango parts such as leaves, twigs, flowers and fruits. The disease can dwindle the productivity of infected mango plants as well as the quality of mango fruits; hence, resulting in economic losses. There is therefore the need to manage mango anthracnose disease in order to enhance the health and productivity of mango plants and also maintain the quality of mango fruits. Several methods such as culture, use of synthetic fungicides, resistant varieties and biological have been employed in the management of mango anthracnose diseases. The use of synthetic fungicides is one of the major methods employed in managing mango anthracnose disease. However, the use of synthetic fungicides is usually accompanied with challenges such as pollution of the

environmental, development of pathogenic resistance, residual toxicity and harmful effects on humans [34, 35]. The excessive and improper use of synthetic fungicides can result in the accumulation of fungicidal residue in plants and plant organs used as food which may pose health risk to consumers. For instance, the residual effects of some synthetic pesticides have been recorded in fruits of melon, guava, orange, peach and mango at levels toxic to human consumption [36]. There is therefore the need to find environmentally friendly alternatives in managing anthracnose disease of mango.

The use of botanical fungicides in plant disease management can help promote sustainable agriculture since botanicals are natural, easily biodegraded into harmless substances; hence do not persist in the environment and plant parts used as food. The antifungal activities of botanicals result from the phytochemical they contain. These phytochemicals which are secondary metabolites produced by plants in nature play an important role in the ability of plant to defend themselves against phytopathogens. Phytochemicals have antimicrobial properties [37]; hence, can prevent or reduce infection when applied on plants or plant parts.

Currently, several studies have documented the use of botanical in the management of anthracnose of mango [38–40]. At the pre-harvest of mango anthracnose disease, studies has showed that aqueous extracts leave of *Eucalyptus camaldulensis* and *Azadirachta indica* inhibited the mycelia growth of *C. gloeosporioides in vitro* and also under field conditions, the foliar application of the extracts reduced the incidence and severity of anthracnose on mango plants [38]. Furthermore, literature has also reported that mango fruits treated by dipping in aqueous extracts of *Ruta chalepensis* at concentration of 50 grams of the powdered plant material in 100 ml of distilled water before storage remarkably reduced the occurrence of anthracnose disease, maintained the quality and marketability of the fruits [41]. Some botanicals in comparison to synthetic fungicides have exhibited an equivalent level of antifungal activity against *Colletotrichum* spp. For instance, studies has showed that essential oil of basil leaves inhibited the mycelia growth of *C. acutatum* that caused anthracnose disease in fruits of mango cat hoc variety, and also significantly reduced the incidence and severity of anthracnose on the mango fruits comparable to those treated with the synthetic fungicide Tolent 50 WP (Prochloraz) [40].

Botanicals can be used for the eco-friendly management of both the pre- and post-harvest anthracnose disease of mango [39]. Their studies reported that the pre-harvest anthracnose disease of mango was effectively controlled by the foliar application of aqueous extracts of *Azadirachta indica, Eucalyptus camaldulensis, Allium sativum, Zingiber ofcinale* and *Calotropis procera,* and at the post-harvest stage, mango fruits sprayed with the botanicals reduced the anthracnose infection of the fruits and improved their quality. Although many botanicals have exhibited some potential to be used in managing anthracnose disease of mango, the main challenge is that most of these findings have not moved beyond the research stage and as such not readily available to mango growers and marketers. To encourage the use of botanical in the management of mango anthracnose disease, there is the need to develop and register botanicals which have exhibited the potential to manage the disease into commercial botanical fungicides products which can easily be assessed by mango growers and marketers.

5. Postharvest treatments

Usually, fruits that are contaminated or infected with disease inoculum shortly before harvesting do not show any symptom of the disease due to latency. This state of

infection at the time of harvest leads to a lethal level of the disease shortly after harvest. This leads to huge quantitative loss of the produce. As much as synthetic fungicide applications still remains as one of the most basic and effective ways of managing the disease [42], their application to the fruit after harvest seams not to be safe for human consumption as it could lead to fatality [43]. This calls for an eco-friendlier approach in managing the disease postharvest, as an effective alternative for chemical control. Various treatments are applied to the fruit to prevent or perhaps retard the development process of the disease [44]. This treatment usually has to do with temperature manipulation [45]. At the onset of ripening of the fruit postharvest, fruits can be refrigerated at 10°C to retard the development of the disease. However, it must be noted that fruits should not be chilled before ripening to reduce or avoid chilling injury and further reduce the quality of the fruit. Fruits may also be dipped in hot water (usually at temperatures 50–60°C) for a duration of up to 15 minutes [46]. The effectiveness of this method has made it to been known as one of the most effective and environmentally friendly postharvest control methods of the mango anthracnose and has been recommended by several disease control departments across the globe. Fruits may also be exposed to vapour heat, forced-air dry heat for about 3–6 hours at appropriate temperatures. However, in all these treatments, the temperature levels and duration of exposure depends greatly on the variety of the subject. Also, various treatment combinations has been found to be effective in the management of the disease [47]. For example, beromyl has been reported to be very effective in the management of quiescent infections of the anthracnose disease in mango when it is coupled with the various hot water treatments. On the other hand, prochloraz has also been found very effective when combined with cold water treatment. However, this was said to be less effective as compared to the beromyl treatment. It is also important to note that the effectiveness of any of these methods has a direct baring with the variety or cultivar. This means that in choosing a particular postharvest management method, one as to put into consideration the variety of the mango. For example, morphological feathers such as skin thickness of mango varies from variety to variety. For optimum effectiveness, treatment should be applied to varieties as may be applicable. The overall effectiveness of postharvest treatment approaches is said to be moderate in the management of the disease. This could perhaps be because treatments are only aimed predominantly at managing the disease and not as a protectant. In summary, the following treatments has been recommended to be effective for postharvest management of mango anthracnose; scrubbing with 1% NaOCl, hot water dip (50-55oC for 3–10 minutes), hot benomyl dip (500–1000 ppm), hot/cold prochloraz dip (400–l000 ppm), hot imazalil (1000 ppm), hot water +20 k RAD irradiation, hot water +75 k RAD irradiation, hot benlatc/iprodionc (1000 ppm) + 75 k RAD irradiation + waxing [48, 49].

6. Colletotrichum: current status and future directions

6.1 Colletotrichum: current status

Colletotrichum species are important cosmopolitan pathogens of many plant species. Globally, *Colletotrichum* can cause anthracnose disease on various types of suitable host plants at the pre- and post-harvest stages resulting in economic losses in crop production.

In the absence of the host plant, the inocula of *Colletotrichum* species can survive unfavourable conditions on plant debris, alternate and collateral hosts, and volunteer

crops on harvested crop fields. There has been continuous first reports of anthracnose disease caused by *Colletotrichum* species on various plants across the world [50–53]. This indicates that the inocula of *Colletotrichum* species are persistent in the environment and expanding their plant host range; hence, remain a major threat to crop cultivation.

Cross infection of anthracnose disease caused by *Colletotrichum* species from one species of plant or its product to another has been reported [54, 55]. A great diversity of *Colletotrichum* species can cause anthracnose disease on a particular host plant and also cross infect another suitable host. *Colletotrichum* species such as *C. asianum, C. cliviicola, C. cordylinicola, C. endophytica, C. fructicola, C. gigasporum, C. gloeosporioides, C. karstii, C. liaoningense, C. musae, C. scovillei*, *C. siamense* and *C. tropicale* were found to cause mango anthracnose disease and also cross infect other crops [56].

Methods such as chemical, culture, biological and use of resistant varieties have been employed in the management of anthracnose disease. A major challenge confronting the management of anthracnose disease caused by *Colletotrichum* is how to properly identify closely related species of the pathogen causing the disease, since some of the *Colletotrichum* species have similar morphological characteristics and show similar symptoms on infected plants [56, 57]. To avoid the issue of improper identification, several studies have advocated the use of molecular methods for accurate identification of *Colletotrichum* species [54, 56, 57]. Accurate identification of Colletotrichum species causing a particular plant disease would aid in the proper management of the disease.

6.2 Colletotrichum: future directions

Anthracnose disease of plants caused by *Colletotrichum* species is real and remain a major threat to crop production now and into the future. Going forward, the general public should be sensitized about plant diseases caused by *Colletotrichum* species. This would make the general public aware of the disease and as such play a key role in managing the disease. For example, people who are conscious of the disease would help minimize the rate at which they aid in spreading the *Colletotrichum* inocula from one anthracnose infected plant or its products to another of the same species or others of different species which may also be suitable host for the pathogen.

Going forward, there is the need to properly identify the particular species of *Colletotrichum* causing a plant disease to allow for the proper management of the disease. Expects in the field of fungi identification should be consulted when identifying *Colletotrichum* species and the identified species confirmed using molecular methods. Anthracnose disease of plants caused by *Colletotrichum* species should be of public concern since it can pose a major threat to food security in the future. More studies should be conducted on the epidemiology, aetiology and sustainable methods of managing diseases caused by *Colletotrichum* species.

7. Conclusions

Mango anthracnose disease is one of the most important economic diseases of mango in many mango production areas across the globe. It affects the almost all above ground parts of the plant and demands both preharvest and postharvest approaches for effective management [17]. The management regimes range from timely application of appropriate fungicides on the field, smart use of traditional cultural practices, the use of resistant cultivars, to postharvest treatments such as dip

treatments and refrigeration. Adequate knowledge of this disease is one of the most essential requisites in choosing the most appropriate control measure for an optimum control.

Conflict of interest

Authors declare no competing interest.

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Chapter 3

New and Emerging Disease Threats to Forest Plantations in Sarawak Borneo, Malaysia

Annya Ambrose, Jack Liam and Razak Terhem

Abstract

The planted forest area in Sarawak is the largest planted forest in Malaysia, which has been developed since 1997 to sustain the decline in the production of natural forests. As of December 2021, the total area of plantation forests reached 551,704 hectares (ha), dominated by fast-growing exotic species mainly Acacia species (55%), Falcataria moluccana (15%) and Eucalyptus (14%). The study showed Acacia was infected with red root rot disease of Ganoderma philippii and brown root rot of Phellinus noxius, Ceratocystis wilt disease caused by Ceratocystis. fimbriata sensu stricto (s.s) complex and pink disease caused by Erythricium salmonicolor, while F. moluccana was infected by gall disease namely Uromycladium falcatarium. Eucalyptus pellita diseases were infected namely by G. philippii red root rot disease, stem canker disease caused by Botryosphaeriaceae pathogen and bacterial wild disease caused by Ralstonia solanacearum. Ceratocystis wilt disease of Acacia mangium shows disease incidence (DI) accounted at 68% (serious) as compared with other diseases observed in this study. This will be the first baseline study that is conducted to observe and assess the diversity of the present, new and emerging pathogens and the damage they cause to exotic planted species of Sarawak.

Keywords: Acacia diseases, baseline study, disease incidence, Eucalyptus diseases, fast-growing exotic species, *Falcatria* disease

1. Introduction

The timber and timber-based products are the fourth largest contributor to Sarawak's export earnings after natural gas, petroleum and palm oil products. The timber industry, in particular, contributed to RM6.31 billion (USD 1.43 billion) in royalty, premium and cess revenue over a ten-year period, from 2010 to 2021. In addition, it also provides employment, creates business opportunities and facilitates road access for the rural communities that subsequently support eradication of poverty and sustainable development of the country.

Log supply from the natural forests peaked in the 1990s at about 20 million m^3 and declined to 4.07 million m^3 in 2019. The concern for the sustainability of natural forests has become a major topic of discussion these days. Sarawak is committed to conserving a healthy forest environment for the long term, including biodiversity conservation and

ecosystem functioning with an aim to strike balance between the development and economic needs of the nation. Sarawak has a total land area of approximately 12.4 million hectares of which 7.7 million hectares or 62 per cent is under forest cover. Under the 'Sarawak Land Use Policy,' 7 million hectares have been allocated for sustainable forestry and conservation. Out of these, 6 million hectares are Permanent Forest Estates (PFEs) and 1 million hectares are Totally Protected Areas (TPAs). The remaining 5.4 million hectares are for agriculture and other development purposes. Under the Sarawak Forest Policy, the key component is sustainable forest management (SFM). SFM is implemented based on the guidelines in the Forest Statement Policy 1954.

Sarawak embarked on large-scale forest plantations in 1996 as a long-term strategy to provide a new source of wood material for the State's wood-based industries. Hitherto, Sarawak is the main player in Malaysia's forest plantation industry in the county where the total area of its plantation forests reached 551,704 hectares with three (3) main species planted, dominated by *Acacia* (55%), *Falcataria moluccana* (19%) and *Eucalyptus* (14%). Sarawak hopes to establish one (1) million hectares of industrial forest by 2025 by planting fast-growing species. It is hoped to produce 15 to 25 million m^3 a year when the target is achieved. In addition, the planted forest will relieve pressure on logging in natural forests in terms of both area size and intensity.

Currently, the forest plantation area could only produce an average production yield per ha of 70–120 m^3 assuming fully stocking at the age of 8 years trees. However, to better secure the future wood supply that Sarawak needs for its domestic and international markets, the production yield per ha should be increased to an average of 120–210 m^3 to be on par with other timber-producing countries such as Vietnam, China and Indonesia. One of the major attributes that had been identified in contributing to the poor growth and performance is the occurrences of disease attacks as well as the unavailability of genetically improved planting materials that are disease-resistant.

The research effort on forest health in Sarawak is still in the infant stage; however, its capacity has increased over the past decade. Surveys and basic quantitative evaluations were conducted during the period 2015–2020 in response to the plantation development observed in Sabah and Indonesia. Surveys and evaluations for forest health are crucial, and they should be conducted immediately and systematically in order to obtain related information such as disease incidence, signs and symptoms, contributing factors, and pathogens as well as silviculture treatments.

This information is expected to be used by stakeholders and policy-makers in developing an appropriate disease management strategy to control the disease infection on their plantations. Hence, this is the first baseline study conducted to identify and evaluate the incidence and the damages of the known major diseases as well as the emerging disease that could pose a threat to the vitality of forest plantation tree species in Sarawak.

The significant information gathered from this study will definitely provide an overview of the risk and threats of the pathogens and the management options that could be undertaken for a countermeasure.

2. Materials and methods

2.1 Forest health surveillance (FSH)

Forest health surveillance (FHS), a formal and regular inspection of the planted forest, was performed in this study through the establishment of permanent sampling

plot (PSPs) surveillance and monitoring plots to assess the disease incidences and occurrences. FSH was undertaken between the period years 2015–2020, in various plantations selected randomly from the four forestry administration regions of Sarawak namely Miri, Bintulu, Sibu and Kuching (**Figure 1**). Field observations were carried out on forests planted with three major exotic tree species (**Table 1**).

We relied on information gathered by local foresters as well as our own observations to select the surveyed plantations in each studied forest region. Basically, the largest operational forest plantations were selected to better represent each forest region.

The plots were established by 30 × 30 m plot (the size of a plot varies in order to obtain a minimum of 100 living trees per plot) * or alternatively by line transect with 10 planting rows and 10 trees per rows approach, which subsequently 100 trees will be assessed, assuming every tree were still alive (full stocking). All the trees assessed were labelled accordingly. GPS coordinates were recorded at every corner of the plot

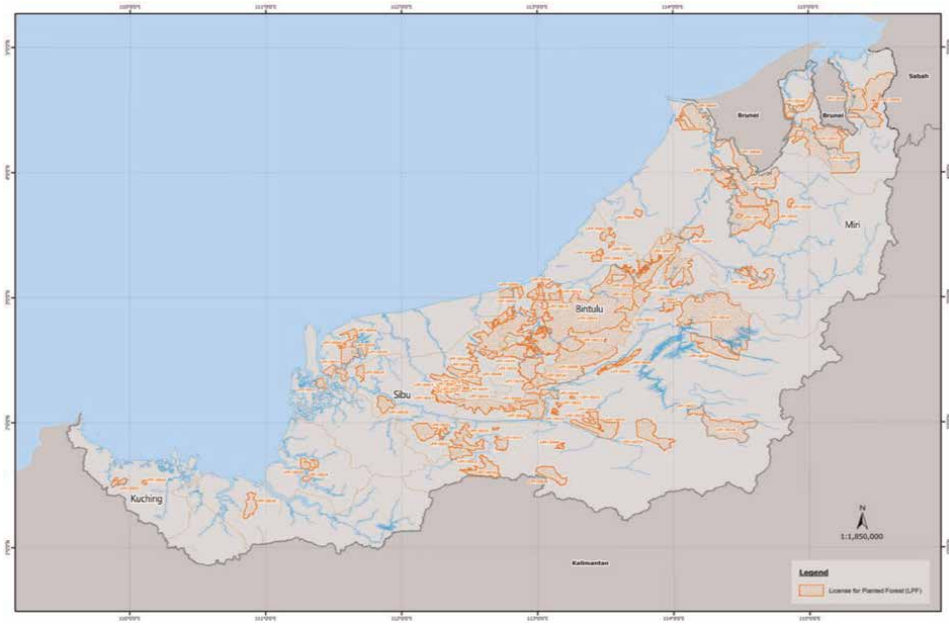

Figure 1.
Map of Sarawak showing the distribution of forest tree plantation which are highlighted areas with red border.

Host	Area in 2021 (ha)	Planting region	Native/ Exotic	Potential end-uses
Acacias	301,653.30	Kuching, Miri, Bintulu, Sibu	Exotic	Veneer, sawn timber, wood chips
Eucalyptus pellita	78,834.51	Kuching, Miri, Bintulu, Sibu	Exotic	Solid timber, sawn timber, timber for furniture
Falcataria molluccana	106,276.20	Kuching, Miri, Bintulu, Sibu	Exotic	Wood chips, veneer

Table 1.
Details of forest plantation species that were surveyed for disease pathogens.

established and marked clearly with a peg. The plots were established at least 20 m from the boundary of plantation, roads or forest gaps. Details of plots are shown in **Table 2**.

2.2 Disease assessment, sampling and disease incidence (DI)

Trees with signs and symptoms such as the presence of any fungal fruiting bodies, swelling and cracking of the stems, branches and canopy appearances (i.e. wilting, yellowing, or defoliations) were observed and thoroughly recorded. Various observations were also taken, including the presence of animal damage, and appearances of insect/pests (i.e. termites' mounds, defoliators, borers or boreholes).

Samples of diseased leaves, branches, stems and roots were taken from diseased trees and collected for laboratory examination. The surface of cankers on stems was examined on the field using hand lenses for dark pycnidia [1] or any signs of fruiting bodies. Trees showing symptoms of branch death, wilting foliage, bark discolouration or sap exudation typical of Ceratocystis as well as showing signed of wood boring insect activities and sweet gaseous smell of pineapple were examined by chopping into the woody xylem at the base of the tree (most infections occur through the root system) or further up the stems for the characteristic xylem discolouration. Longitudinal strips of wood xylem (approximately 0.5 cm thick and around 10 to 15 cm long) were then cut from the discoloured xylem. In some cases, diseased trees were also felled and were cut into several sections measuring between 10 and 20 cm in length. The samples were then placed into paper bags before being transported to the Forest Pathology Laboratory, Industrial Forest Research Centre (IFRC), Kuching. The samples were stored at 4°C prior to fungal isolation.

Below ground variables were recorded for each tree on roots exposed to 20−50 cm from the base of the tree that exhibits symptoms of root rots. Variables recorded included are the presence or absence of any kind of root rot (red, brown, white) as well as the presence or absence of insect pests such as termites' tunnelling activities. Samples of insect pests were also collected for further identification.

The surveys aim to estimate the number of trees with any signs and symptoms of disease infection and thus determine the disease incidences (DI).

The DI were calculated by the following formula:

$$\mathrm{DI} = \left(\frac{\boldsymbol{n}}{\boldsymbol{N}}\right)\boldsymbol{x}\ \mathbf{100}\%. \qquad (1)$$

Where:

n = number of infected standing tree

N = total number of standing trees in one plot.

The disease incidences were then evaluated based on the indicator and rating as guided in **Table 3**. Values DI were reported in the results as ranges and are based on the plot data over the years of plot assessment.

2.3 Fungal isolation and identification

2.3.1 Isolation of Ceratocystis wilt disease

The carrot baiting method with minor modification was carried out to isolate Ceratocystis [3]. Pieces of the diseased stem were placed between two slices of fresh

No	Plot ID	Species	Region*	Age (years)	Rotation	Spacing (m)	Types of stand**
1	SGAM1	*A. mangium*	BTU	6	1	3 × 3	CS
2	SGAM 2	*A. mangium*	BTU	6	1	3 × 3	CS
3	SGAM 3	*A. mangium*	BTU	1.5	2	3 × 3	CS
4	SGAM 7	*A. mangiun*	BTU	10	1	3 × 3	CS
5	SGAM 9	*A. mangium*	BTU	1	2	3 × 3	CS
6	PDAM01	*A. mangium*	BTU	3	2	3 × 3	CS
7	PDAM02	*A. mangium*	BTU	3	2	3 × 3	CS
8	GPP-SFC01	*A. mangium*	BTU	<1	2	3 × 3	CS
9	GPP-SFC02	*A. mangium*	BTU	<1	2	3 × 3	CS
10	SFC-DK-001	*A. mangium*	BTU	2	2	3 × 3	CS
11	SFC-DK-002	*A. mangium*	BTU	2	2	3 × 3	CS
12	SFC-DK-003	*A. mangium*	BTU	2	2	3 × 3	CS
13	SFC-DK-004	*A. mangium*	BTU	2	2	3 × 3	CS
14	SFC-DK-005	*A. mangium*	BTU	2	2	3 × 3	CS
15	SFC-DK-006	*A. mangium*	BTU	2	2	3 × 3	CS
16	SFC-DK-007	*A. mangium*	BTU	<1	2	3 × 3	CS
17	SFC-DK-008	*A. mangium*	BTU	<1	2	3 × 3	CS
18	SFC-DK-009	*A. mangium*	BTU	<1	2	3 × 3	CS
19	SFC-DK-010	*A. mangium*	BTU	<1	2	3 × 3	CS
20	BT 1–2016	*A. mangium*	BTU	3	2	3 × 3	CS
21	BT2–2016	*A. mangium*	BTU	2	2	3 × 3	CS
22	SBW 1–2016	*A. mangium*	SBW	2	2	3 × 3	CS
23	BT1–2017	*A. mangium*	BTU	2	2	3 × 3	CS
24	Lws 2-RND	*A. mangium*	MY	1	1	3 × 3	CS
25	Lws-3-RND	*A. mangium*	MY	1	1	4 × 3	CS
26	Lws 4-RND	*A. mangium*	MY	1	1	5 × 3	CS
27	Plot 1AH	*A. hybrid*	SBW	2	2	3 × 3	CS
28	Plot 2AH	*A. hybrid*	SBW	2	2	3 × 3	CS
29	SBW 1-RND	*A. mangium*	SBW	2	1	3 × 3	R&D
30	SBW 2-RND	*A. mangium*	SBW	2	1	3 × 3	R&D
31	SBW 3-RND	*A. mangium*	SBW	2	1	3 × 3	R&D
32	AM4	*A. mangium*	KCH	12	1	3 × 3	CS
33	EP1	*E. pellita*	KCH	11	1	3 × 3	CS
34	EP2	*E. pellita*	KCH	11	1	3 × 3	CS
35	EP3	*E. pellita*	KCH	4	1	3 × 3	CS
36	EP6*	*E. pellita*	KCH	4	1	3 × 3	CS
37	EP5	*E. pellita*	KCH	13	1	3 × 3	CS
38	4	*E. pellita*	BTU	3	2	3 × 3	CS

No	Plot ID	Species	Region*	Age (years)	Rotation	Spacing (m)	Types of stand**
39	5	*E. pellita*	BTU	3	2	3 × 3	CS
40	8	*E. pellita*	BTU	8	2	3 × 3	CS
41	10	*E. pellita*	BTU	4	1	3 × 3	CS
42	11	*E. pellita*	BTU	2	2	3 × 3	CS
43	PDEP01	*E. pellita*	BTU	2	2	3 × 3	CS
44	Plot 5 Bt	*E. molluccana*	SBW	2	2	3 × 3	CS
45	Plot 5 Bt	*F. molluccana*	SBW	2	2	3 × 3	CS
46	SFC-WTK003	*F. molluccana*	SBW	<1	2	3 × 3	CS
47	PD-AF-01	*F. molluccana*	BTU	3	2	3 × 3	CS
48	PD-AF-02	*F. molluccana*	BTU	3	2	3 × 3	CS
49	GPP-SFC03	*F. molluccana*	BTU	<1	2	3 × 3	CS
50	GPP-SFC04	*F. molluccana*	BTU	<1	2	3 × 3	CS
51	SFC -Bt-0018	*F. molluccana*	BTU	4	2	3 × 4	R&D
52	SFC-SY-001	*F. molluccana*	MY	2	2	3 × 3	CS
53	SFC-SY-002	*F. molluccana*	MY	2	2	3 × 3	CS
54	SFC-SY-003	*F. molluccana*	MY	2	2	3 × 3	CS
55	SFC-SY-004	*F. molluccana*	MY	2	2	3 × 3	CS
56	SFC-LimbaJ	*F. molluccana*	MY	2	2	3 × 3	R&D

Forestry Region (Figure 1**): BTU, Bintulu; SBW, Sibu; KCH, Kuching; MY, Miri; R&D.*
***Types of stand: R&D, Research and Development; CS, Commercial Stands.*

Table 2.
Details of permanent sampling plots established and evaluated.

Indicator	No	Light	Medium	Serious
Diseases incidence	Affect less than 10% of standing trees	Affect 10–29% of standing trees	Affect 30–59% of standing trees	Affect more than 60% standing trees

Table 3.
Indicator and ratings for evaluating the impact of forest health status in Sarawak [2].

carrot and incubated in plastic containers that served as moisture chambers for 4–6 days. After 5–8 days, a pale orange ascospore mass appeared on the tip of the perithecia was then picked with a sterile syringe needle and transferred into a Petri dish plated with Potato Dextrose Agar medium and incubated at 26°C. All the isolates were identified based on the analyses of morphological characteristics [3–5]. The characteristics and structures of the culture, such as perithecium, ostiolar hyphae, ascospore and conidia (barrel-shaped and cylindrical-shaped) (μm) of all the fungal isolates, were measured and described. Morphological identification for this study was conducted by using a Leica compound microscope ICC50HD with a laptop workstation for data & image acquisition and a stereo microscope of Olympus SZ61 (SZ61TR-2X-DI-TP-118000A-SET) at Forest Pathology Laboratory, IFRC, Kuching.

2.3.2 Isolation of root rot disease

Direct isolation was carried out to isolate the pathogen of root rot disease. Root tissues bearing disease symptoms were cut into several pieces approximately 3 mm × 3 mm in size and were placed on malt extract agar (MEA) and incubated at room temperature for the fungus to grow. The cultures were then transferred to MEA for morphological studies.

2.3.3 Isolation canker disease

Segments of symptomatic plant parts were incubated in moist chambers for 2–3 days to induce the development of fruiting structures. These were then transferred to MEA and incubated at 25°C. Isolation from symptomatic tissue was also made directly onto MEA. Isolations were made onto MEA from fruiting structures occurring on cankered stems [6, 7].

3. Results

3.1 Disease incidence (DI)

In this study, a total of 56 PSPs comprising 32 of *Acacia*, 17 of *Eucalyptus pellita* and 14 of *F. mollucana* have been established and assessed with approximately 5600 trees of the three major species planted have been evaluated, data recorded and analysed. The study showed that DI was varied between tree species.

3.1.1 Acacia

Approximately 3200 *Acacia* stand aged between less than 1-year-old and 12-year-old were assessed in this study with three prominent diseases observed; Ceratocystis wilt disease, root rot disease and pink disease. Based on **Table 4**, Ceratocystis wilt disease was the most devasted and important disease observed. DI was recorded the highest with mortality recorded at 68% in the Bintulu region based on disease indicators rating as depicted in **Table 2**, and the DI rating is categorised at a serious level. The disease was observed in stands less than 1-year-old also in the Bintulu region with DI recorded at 1.9%. The DI is predicted to increase over time with rotation length. The disease was not observed in Miri and Sibu region; however, DI of 3% of the disease was documented in Kuching region in 12 -year-old *A. mangium*.

The disease incidence of root rot was recorded between 0 and 37% in trees aged less than 1 year old to 10 year old. 10 years of plantation of *Acacia mangium* in the Bintulu region recorded the highest incidence of the disease, and no incidences were recorded in the young stand. The disease rating based on the highest DI is light. Root rot disease seems to be the most encountered disease in most of the plots, with 17 out of 32 PSPs observed with the disease.

The pink disease was only observed in Sibu region and had affected 2-year-old taxa seed source trial plot established by Forest Department Sarawak. DI of pink disease was recorded at 5%.

No	Plot ID	Species	Region*	Disease Incidence (DI)			Age (years)	Rotation	Spacing (m)	Types of stand**
				Root rot	Ceratocystis wilt	Pink disease				
1	SGAM1	*A. mangium*	BTU	22	0	0	6	1	3 × 3	CS
2	SGAM 2	*A. mangium*	BTU	22	0	0	6	1	3 × 3	CS
3	SGAM 3	*A. mangium*	BTU	29	0	0	1.5	2	3 × 3	CS
4	SGAM 7	*A. mangiun*	BTU	37	0	0	10	1	3 × 3	CS
5	SGAM 9	*A. mangium*	BTU	6	0	0	1	2	3 × 3	CS
6	PDAM01	*A. mangium*	BTU	17	13	0	3	2	3 × 3	CS
7	PDAM02	*A. mangium*	BTU	0	68	0	3	2	3 × 3	CS
8	GPP-SFC01	*A. mangium*	BTU	0	0	0	<1	2	3 × 3	CS
9	GPP-SFC02	*A. mangium*	BTU	0	0	0	<1	2	3 × 3	CS
10	SFC-DK-001	*A. mangium*	BTU	9.5	0	0	2	2	3 × 3	CS
11	SFC-DK-002	*A. mangium*	BTU	3.6	7.1	0	2	2	3 × 3	CS
12	SFC-DK-003	*A. mangium*	BTU	0	2	0	2	2	3 × 3	CS
13	SFC-DK-004	*A. mangium*	BTU	0	0	0	2	2	3 × 3	CS
14	SFC-DK-005	*A. mangium*	BTU	3.8	10	0	2	2	3 × 3	CS
15	SFC-DK-006	*A. mangium*	BTU	1.4	0	0	2	2	3 × 3	CS
16	SFC-DK-007	*A. mangium*	BTU	0	4	0	<1	2	3 × 3	CS
17	SFC-DK-008	*A. mangium*	BTU	0	15.5	0	<1	2	3 × 3	CS
18	SFC-DK-009	*A. mangium*	BTU	0	12.2	0	<1	2	3 × 3	CS
19	SFC-DK-010	*A. mangium*	BTU	0	1.9	0	<1	2	3 × 3	CS
20	BT 1–2016	*A. mangium*	BTU	8.3	35.2	0	3	2	3 × 3	CS
21	BT2–2016	*A. mangium*	BTU	3	3.5	0	2	2	3 × 3	CS
22	SBW 1–2016	*A. mangium*	SBW	1.2	0	0	2	2	3 × 3	CS

No	Plot ID	Species	Region*	Disease Incidence (DI)			Age (years)	Rotation	Spacing (m)	Types of stand**
				Root rot	Ceratocystis wilt	Pink disease				
23	BT1–2017	*A. mangium*	BTU	7.2	41.2	0	2	2	3 × 3	CS
24	Lws 2-RND	*A. mangium*	MY	0	0	0	1	1	3 × 3	CS
25	Lws-3-RND	*A. mangium*	MY	0	0	0	1	1	4 × 3	CS
26	Lws 4-RND	*A. mangium*	MY	0	0	0	1	1	5 × 3	CS
27	Plot 1AH	*A. hybrid*	SBW	1.2	0	0	2	2	3 × 3	CS
28	Plot 2AH	*A. hybrid*	SBW	1.2	0	0	2	2	3 × 3	CS
29	SBW 1-RND	*A. mangium*	SBW	0	0	5	2	1	3 × 3	R&D
30	SBW 2-RND	*A. mangium*	SBW	0	0	3	2	1	3 × 3	R&D
31	SBW 3-RND	*A. mangium*	SBW	0	0	2	2	1	3 × 3	R&D
32	AM4	*A. mangium*	KCH	3	3	0	12	1	3 × 3	CS

Forestry Region (see **Figure 1): BTU, Bintulu; SBW, Sibu; KCH, Kuching; MY, Miri; R&D.*
***Types of stand: R&D, Research and Development; CS, Commercial Stands.*

Table 4.
Details on DI (DI) of observed diseases recorded in Acacia.

No.	Plot ID	Species	Region*	Disease Incidence (DI)			Age (years)	Rotation	Spacing (m)	Types of stand**
				Root rot	Canker	Bacterial Wilt				
1	EP1	*E. pellita*	KCH		4		11	1	3 × 3	CS
2	EP2	*E. pellita*	KCH		3		11	1	3 × 3	CS
3	EP3	*E. pellita*	KCH		2		4	1	3 × 3	CS
4	EP6*	*E. pellita*	KCH				4	1	3 × 3	CS
5	EP5	*E. pellita*	KCH		2		13	1	3 × 3	CS
6	4	*E. pellita*	BTU				3	2	3 × 3	CS
7	5	*E. pellita*	BTU				3	2	3 × 3	CS
8	8	*E. pellita*	BTU	6			8	2	3 × 3	CS
9	10	*E. pellita*	BTU	6			4	1	3 × 3	CS
10	11	*E. pellita*	BTU		7		2	2	3 × 3	CS
11	PDEP01	*E. pellita*	BTU				2	2	3 × 3	CS
12	PDEP02	*E. pellita*	BTU				2	2	3 × 3	CS
13	Plot 3 EP	*E. pellita*	SBW		1.2		2	2	3 × 3	CS
14	Plot 4 EP	*E. pellita*	SBW				2	2	3 × 3	CS
15	SFC-WTK1	*E. pellita*	SBW				<1	2	3 × 3	CS
16	SFC-WTK2	*E. pellita*	SBW				<1	2	3 × 3	CS
17	SFC-BT	*E. pellita*	BTU			3	2	2	3 × 3	R&D

Forestry Region (see **Figure 1): BTU, Bintulu; SBW, Sibu; KCH, Kuching; MY, Miri; R&D.*
***Types of stand: R&D, Research and Development; CS, Commercial Stands.*

Table 5.
Details on disease incidence (DI) of observed diseases recorded in E.pellita.

3.1.2 E. pellita

Approximately 1700 *E. pellita* stands aged between less than 1-year-old and 13-year-old were assessed in this study with the establishment of 17 PSPs as detailed in **Table 5**. Canker disease was found in 6 PSPs with the highest DI of 7% recorded in 2-year-old stand in Bintulu region. Out of the 6 PSPs, mostly canker incidences were found in Kuching with 11% DI accumulatively. Root rot was observed in the Bintulu region with accumulative DI of 12% in 2 PSPs located within the same area. Bacterial wilt disease with a DI of 3% was encountered in one PSP established within R&D tree breeding plot at Bintulu. No other PSPs recorded such disease.

3.1.3 F. molluccana

A total of 14 PSPs with comprises of approximately 1400 of *F. molluccana* stands of less than 1-year-old and 2- and 3-year-old were established and assessed for DI as

No.	Plot ID	Species	Region*	Disease Incidence (DI) %		Age (years)	Rotation	Spacing (m)	Types of stand*
				Gall Rust	Canker				
1	Plot 5 Bt	*F. mollucana*	SBW			2	2	3 × 3	CS
2	Plot 5 Bt	*F .mollucana*	SBW			2	2	3 × 3	CS
3	SFC-WTK003	*F. mollucana*	SBW			<1	2	3 × 3	CS
4	PD-AF-01	*F. mollucana*	BTU			3	2	3 × 3	CS
6	PD-AF-02	*F. mollucana*	BTU			3	2	3 × 3	CS
7	GPP-SFC03	*F. mollucana*	BTU		1	<1	2	3 × 3	CS
8	GPP-SFC04	*F. mollucana*	BTU			<1	2	3 × 3	CS
9	SFC -Bt-0018	*F. mollucana*	BTU	69.7		4	2	3 × 4	R&D
10	SFC-SY-001	*F. mollucana*	MY			2	2	3 × 3	CS
11	SFC-SY-002	*F .mollucana*	MY			2	2	3 × 3	CS
12	SFC-SY-003	*F. mollucana*	MY			2	2	3 × 3	CS
13	SFC-SY-004	*F. mollucana*	MY			2	2	3 × 3	CS
14	SFC-LimbaJ	*F. mollucana*	MY	1.9		2	2	3 × 3	R&D

Forestry Region (see **Figure 1): BTU, Bintulu; SBW, Sibu; KCH, Kuching; MY, Miri; R&D. Types of stand: R&D, Research and Development; CS, Commercial Stands.*

Table 6.
Details on disease incidence (DI) of observed diseases recorded in F. molluccana.

detailed in **Table 6**. Based on the survey, gall rust disease was the most important prominent disease observed with DI recorded at an alarming 69.7% in an R&D (taxa seed source trials) established at Bintulu. Miri region also recorded gall rust DI of 1.9% in stands less than 1-year-old. Another disease observed affecting *F. molluccana* was canker disease and attacked stands less than 1-year-old also in the Bintulu region with DI recorded at 1.9%. No diseases were observed in the Sibu region.

3.2 Symptoms and signs

3.2.1 Diseases of Acacia

3.2.1.1 Ceratocystis wilt disease of Acacia

Ceratocystis infections observed typically start on the lower stem and move up, causing dark staining of the woody xylem. However, we did observe some symptomatic trees with initial infection starting at the upper stem with dead foliage hanging throughout the tree or on some major branches. The infected blackish gummosis stem upon debarking showed dark brown to black discolouration in the woody xylem, radial pattern in pattern, which is a unique symptom of Ceratocystis wilt disease. Within every plot observed with Ceratocystis, all trees showed these wilt symptoms were noted for Ceratocystis wilt (**Figure 2a–d**). But it was further confirmed upon root excavation to differentiate root rot disease as the latter also exhibits symptoms such as wilting and severe defoliation.

Figure 2.
Ceratocystis wilt disease implicates damages to Acacia mangium *plantation in Sarawak (a–i); (a) blackish lower stem with foamy exudate at the upper stem and wilting and dying 2-year-old tree; (b) blackish streak-like inner bark due to Ceratocystis infection; (c) foamy exudates and slime typical of Ceratocystis infection on stem of the tree; (d) initial infection starting at the upper stem with dead foliage hanging throughout the tree; (e) dead tree with severe bark damage caused by squirrels with wood discolouration; (f) severe bark damage but tree observed free from Ceratocystis infection (g) traces of wood-boring insect holes on the stem;(h) Ambrosia beetle found under bark and the wood appeared with blackish lesion; (i) side view image of wood-boring insect identified as* Immanus desectus *(eggers) collected from the infested tree.*

Some symptomatic and dead trees as young as 6-month-old that were adjacent to the conservation or buffer area were observed with wound damage on the stem of juvenile trees inflicted by squirrels grazing (**Figure 2e**). These wounds would act as the point of entry for Ceratocystis pathogen to infect the trees. However, we did observe that some trees inflicted with these wounds but did not show any Ceratocystis symptoms (**Figure 2f**).

Wood-boring insects were also found present in infected trees, with wood infested exhibited blackish lesions typical of *Ceratocystis* pathogen (**Figure 2g–i**). The wood-boring insects collected from the tree were identified as those of ambrosia beetle species.

Culture characteristics and morphology of Ceratocystis isolates from *A. mangium* were observed and analysed based on morphological characteristics, such as colony form, mycelium colour and reverse media colour [3–5], were further identified as *C. fimbriata sensu stricto (s.s) complex*, having the characteristic of olive-green colonies with the underside of the cultures was light grey at the margin and became darker towards the centre and typical pineapple-fruit odour. The isolates of *C. fimbriata* showed superficial or submerged in the substrate perithecia, with colours ranging from brown to black both at the base and neck of the perithecium (**Figure 3c**). They had globose to sub-globose ascomata with long necks and typical divergent ostiolar hyphae at their tips (**Figure 2**). Teleomorph and anamorph structures were produced within 2 weeks on MEA cultures (**Figure 3d**).

3.2.1.2 Root rot disease of Acacia

The trees showed symptoms typical of red root rot including dying trees at the periphery of rot centres that had wilting leaves. Most of the affected trees were dead stands that exhibited chlorotic/yellowing foliage and loss of foliage.

The diseased trees were observed to be clustered in patches that were roughly circular. The trees with symptoms of root rots were adjacent to one another. Fruit bodies were only observed on the stem of some of the trees. Types of root disease were distinguished by the colour of infected roots, red root rot, brown root rot and white root rot.

Underground symptoms observed include the presence of red-coloured rhizomorphs with blackish exudates (**Figure 4c**) on the surface of the roots and white mycelium (denote as *My* in **Figure 4d**)**.** Yellow mottles of the foliage with patches in the majority of the infected *A. mangium* trees were easy to recognise as typical of red root rot and further identified as *G. philippii*. Trees discovered to be infected with brown root rot were observed with soft, fibrous and totally rotten roots and sandy particles adhering to the rhizomorph (**Figure 4e**). The causal pathogens identified were *G. philippii* and *Phellinus noxius.*

3.2.1.3 Pink disease of Acacia

Pink disease in *A. mangium* was observed both in the base of the main stem and branches and twigs. Trees observed with pink disease (**Figure 5**) initially exhibited silky-like whitish mycelium on the surface of the bark, known as the 'cobweb' stage, infecting the branch and stem. Pinkish to salmon cobweb-like structures were observed soon after known as the pustule stage, and subsidiaries symptoms of wilting of the branch's foliage and subsequently turned brown and branches that extending to all parts of the plant until dieback observed by death in the canopy. Infected branches and stems showed sign of bark crack and scaled bark. Where infection was observed centralised in certain areas of the stem or bark, cankers and wounds developed. In this

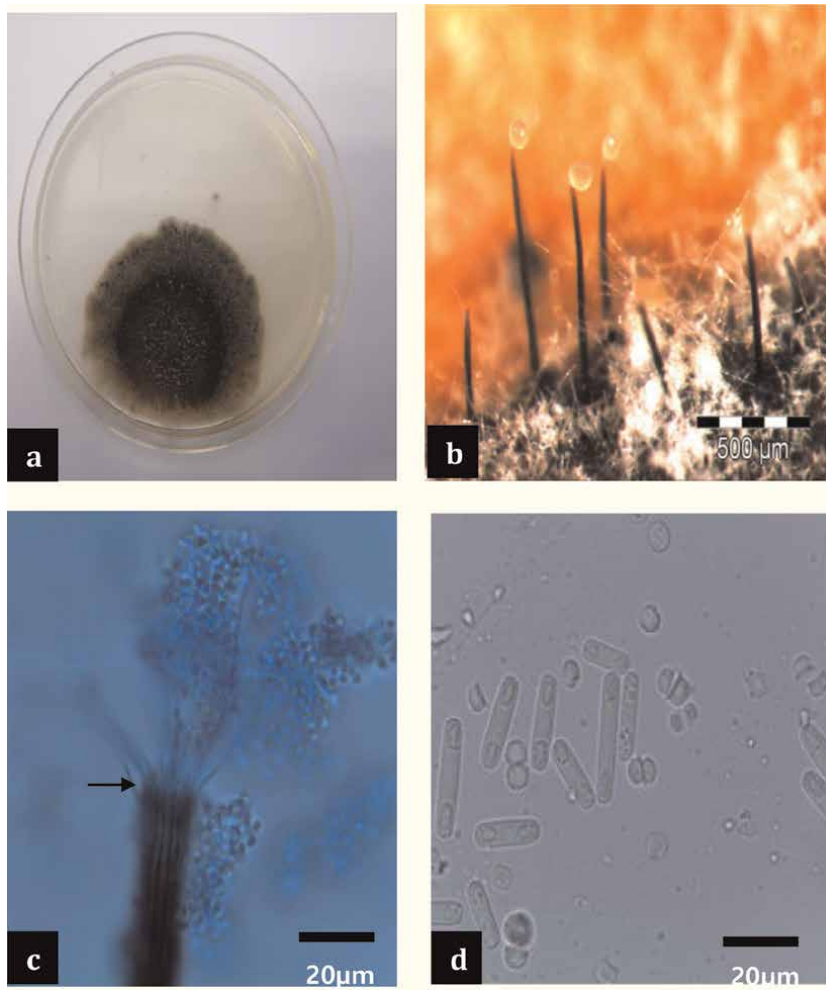

Figure 3.
Microscopic structures of identified Ceratocystis fimbriata *obtained from* Acacia mangium *plantations in Sarawak* ***(a–d);*** *(a) culture of* C. fimbriata *grown on MEA appeared cotton-like, sparse, initially white, later turning to olive grey with white to grey at the margin after 5–7 days with banana-like aroma and salmon-like shiny globose spores; (b) Salmon-like colour ascospores mass extruded at the tips of the long-necked black fruiting bodies (perithecia); (c) long, divergent ostiolar hyphae (arrow); (d) spores of* C. fimbriata., Thelaviopsis *sp.-elongated, cylindrical condia of* Thelaviopsis *sp. (imperfect state) and hat-shaped ascoscopres of* C. fimbriata *(teleomorph or perfect state).*

survey, we observed severe infection in one PSP in Sibu region and inflicted mortality of the tree stands. The symptoms described above showed similarity with the symptoms described [8] and thus, the pathogen of pink disease was identified as *Erythricium salmonicolor*.

3.2.2 Diseases of E. pellita

3.2.2.1 Root rot disease of E. pellita

Root rot disease infecting *E. pellita* in this study was observed to be very detrimental and trees dying in patches. The first signs typical of root rot disease attacked

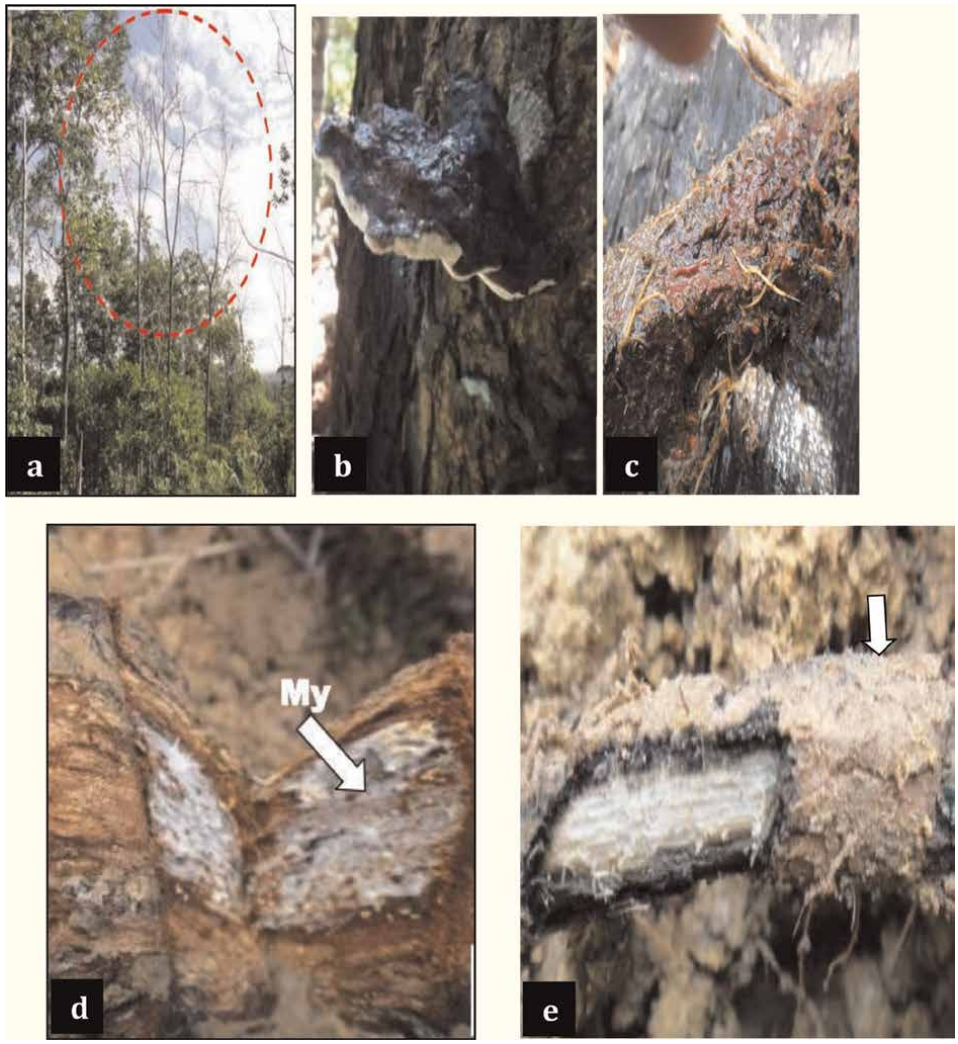

Figure 4.
Root rot disease of Acacia mangium*; (a) 10-year-old trees with severe defoliation and open gaps are the most advanced symptoms of the disease; (b) Ganoderma fruiting body on infected trees; (c) red-coloured leathery rhizomorphs with blackish exudates on infected roots; (d)whitish mycelium on the under bark; (e) Brown root rot caused by the pathogen* P. noxious *with soft roots, fibrous and totally rotten and sand like soil (arrow adhering to the rhizomorphs).*

were yellowing of the foliar, and falling with twigs and branches die. The whole crown seems to be burnt-like (**Figure 6a**) and subsequently, the tree died with the tree became bare. The base of the stem and roots appeared rotten, and discolouration of bark stem was observed with fine white threads typical mottled pattern of mycelia growth below the bark (**Figure 6b**). Fruit body body's shape is like a fan, semicircular, hard and woody and the bottom surface is white; the upper middle is dark brown and measured around 50 cm was seen growing on the stem's base. The roots were then uprooted revealing red leathery rhizomorph covering the root system. Following the symptoms observed especially the characteristic of the fruiting found on the tree, the pathogen was identified as *G. philippii* [9].

Figure 5.
Erythricium salmonicolor *pink disease in* a.mangium. *(a)* E. salmonicolor *'cobweb' stage of infection.; (b)* E. salmonicolor *'pustule stage; (c) incrustation pink cobweb-like colonies on upper stems; (d) patches of tree death caused by the pink disease pathogen* E. salmonicolor.

3.2.2.2 Canker disease of E. pellita

Symptoms of canker disease of *E. pellita* varied in each region where it was detected. Canker found in 13- to 11-year-olds (**Figure 7a–e**) in Kuching was found some at the base of the stem and others slightly higher up the stem (**Figure 7b**). The cankered stem characteristics are swollen and misshapen and the bark fissured with traces of drying blackish gummosis, but all trees were still alive without any sign of dying. The canker found in 2-year-old PSP trees stand in Bintulu showed characteristic of the whole bark cracking with stems blackened and leaves yellowing and wilting with mortality recorded. Based on the different symptoms observed in PSPs assessed, there were possibilities that more than 1 pathogen caused this disease. A small portion of the bark of one tree in 2-year-old stand in Bintulu region was collected for

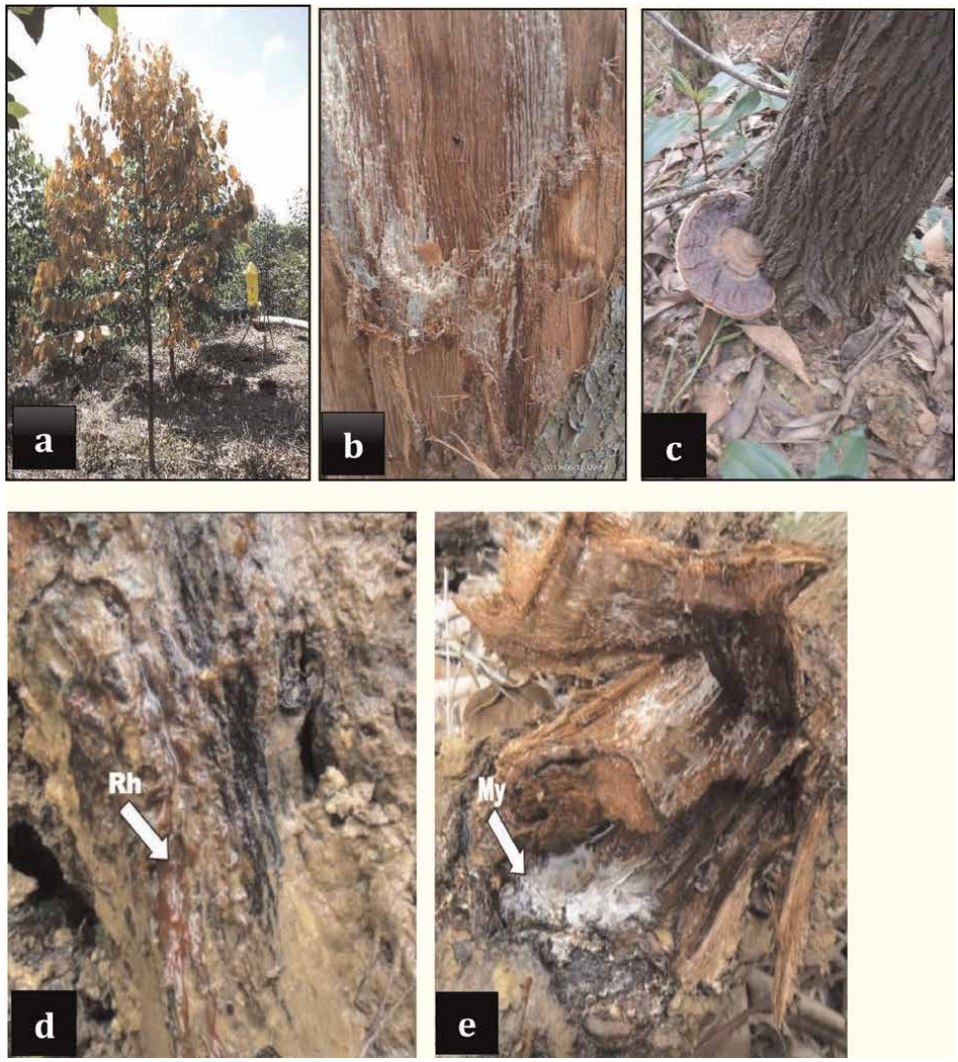

Figure 6.
Root rot disease of Eucalyptus pellita*; (a) 6-month-old tree with burned like foliar and blackish stem; (b) mycelium on under the bark of the basal stem; (c) Ganoderma root rot fruiting body; (d) red-coloured leathery rhizomorphs with blackish exudates on* E. pellita *root; (e) rotting and fibrous root system.*

examination in the laboratory. We managed to identify the pathogen as Botryosphaeriaceae based on the conidia and the culture characteristic grown on MEA as guided (**Figure 8**) [10, 11].

Based on silviculture regimes were not recorded by the management of the plantation. It was observed weeding was not incorporated into the silviculture regime.

3.2.2.3 Ralstonia solanacearum—*bacterial wilt of Eucalyptus*

The symptoms started with foliar exhibiting chlorotic, yellowish to reddish and gradually spreading to the branches started to wilt and trees will succumb to mortality. The inner stem exhibits vascular discolouration of blackish to brownish and bacterial ooze from the wood when observed using a hand lens (**Figure 9**). The

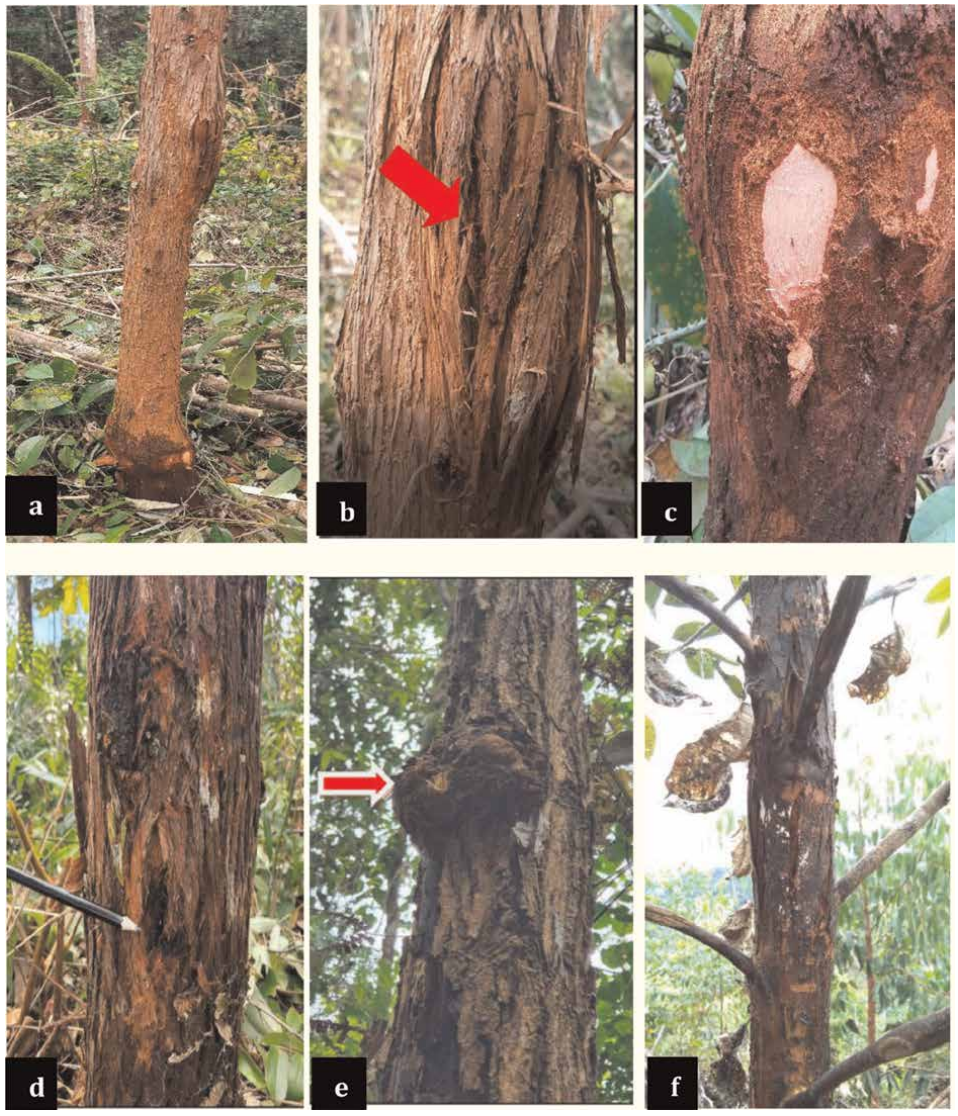

Figure 7.
Canker disease symptoms of Eucalyptus pellita*; **(a–e)** stem cankers on 11-year-old* E. pellita *(a) double' canker at basal and middle stem on 11-year-old; (b) close-up showed cracked and fissured bark on 11-year-old; (c) wood under bark seems to be healthy on 11-year-old; (d) canker developed from injury because of self-pruning and on 11-year-old (e) upper stem canker with swollen bark on 11-year-old; (f) huge canker swelling on 2-year-old* E. pellita *at Bintulu region.*

infected trees were chopped and burned. The possible pathogen would be *R. solanacearum* Smith based on disease symptom described [12, 13].

3.2.3 Disease of Falcataria molluccana

3.2.3.1 Uromycladium falcatarium*—gall rust of* F. molluccana

The disease was observed to cause severe damages and mortality to young trees of *F. mollucana,* especially in the Miri region. The disease causes severe damage to all

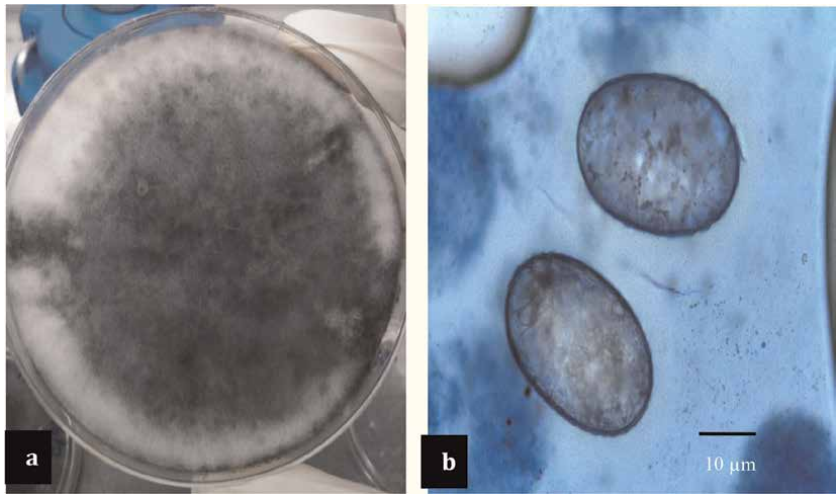

Figure 8.
Botryosphaeriaceae pathogen of canker disease of Eucalyptus pellita*. (a) Culture morphology on MEA; (b) conidia of* Botryosphaeria.

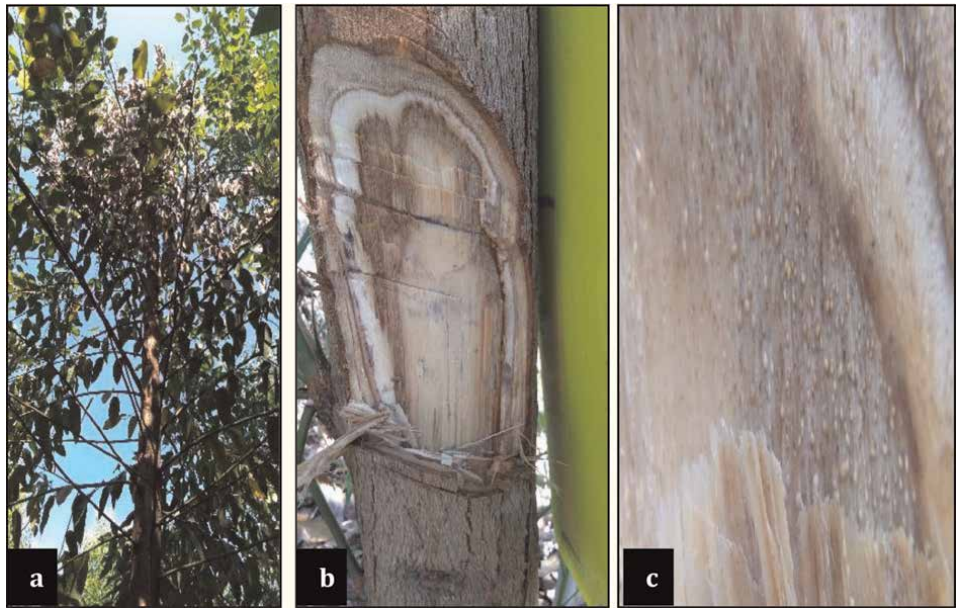

Figure 9.
Bacterial wilt sign and symptoms in Eucalyptus pellita*. (a) Wilting and drying branches symptom on 1-year-old eucalyptus; (b) colour changes turning blackish-brown on eucalyptus 1-year -old stem further symptoms (c) bacterial exudates (ooze) on wood.*

developmental stages of the plant, from seedlings in the nursery to mature trees in the field. Early symptom of infected trees observed was rigid, crooked bending stems, branches or shoot and greenish to reddish necrotic lesion (**Figure 10b**). The symptoms then developed to the development of large chocolate-brown, irregularly shaped, broccoli-like or whip-like galls on the stem and branches (**Figure 10c**).

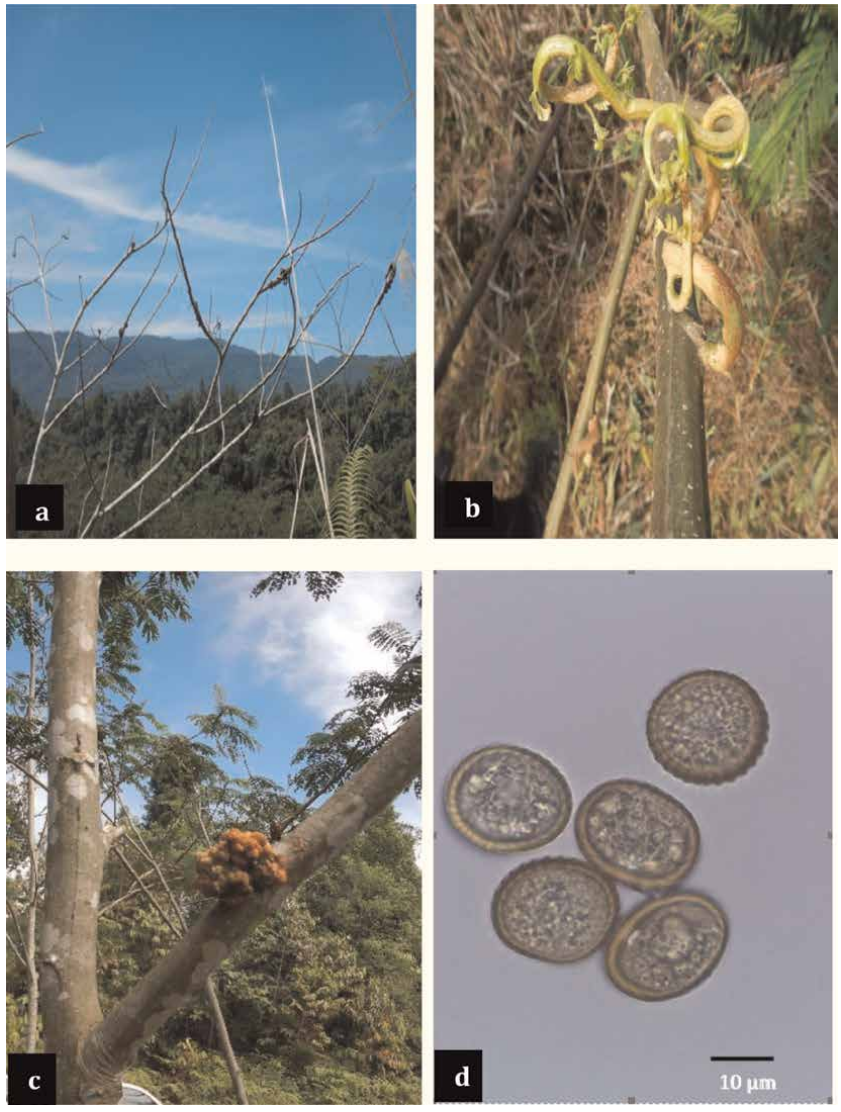

Figure 10.
Gall rust of F. mollucana*. (a) Whole crown dieback and tree died in 2-year -old tree stands at Miri region; (b; c) the shape of gall in the twig; (d) spores with ridged longitudinal striations typical of* Uromycladium falcatarium *pathogen.*

The disease pathogen was identified as *U. falcatarium* based on the symptoms observed in this study as well as referred [14].

4. Discussion

This is the first baseline data of disease incidence (DI) of the current as well as emerging pathogen threats to plantation forests in Sarawak. It comprised about 5 years of field surveys as well as monitoring the health on a yearly basis. PSPs were

established randomly across all the four regions of Sarawak and were representative of the 521702.27 ha of exotic tree species. Overall, seven species of plant pathogens were recognised as either current or emerging threats to the forestry sector. *Ceratocystis fimbriata* is of the greatest concern due to its significant increase in geographical range, the successive rotation and fear of spread or 'jumping host' from Acacia to other species such as *Eucalyptus*.

4.1 Diseases of Acacia

4.1.1 Root rot disease

This disease is considered one of the most important diseases of *A. mangium* plantations in Indonesia and Malaysia [15–17]. The pathogen builds up in every successive rotation and caused tree mortality in trees as young as 3-month-old in particular in the 2nd and 3rd rotation trees based on observation. Tree death can exceed 50% in some areas within less than 20 years of establishing the first rotation [18]. The trees with symptoms of root rots were adjacent to one another. It is a known fact that the root systems of trees are entangled with one another, and thus, one infected root will have higher possibility of infecting the roots of the tree next adjacent. As such, the trees infected with root rot appeared to be centralised and clustered in patches that were roughly circular. The nearer the planting distance, the probability spread of infection will increase exponentially.

Currently, the spread of root rot diseases is control by excavating and destroying the infecting trees as well as construction of drainage trenches filled then with fungicides, which will probably minimise the roots contact of infected trees to healthy trees adjacent. However, mostly all plantation owners commented that such practices is not economically viable and opted to just leaving the infected trees as it are. From general overview, root rot disease will always be a menace as the pathogen fungi, which are present in native forest left in infected stumps and roots when native forest is cleared for plantations, and these then act as inoculum sources and it will definitely increase with successive rotation [16]. Land preparation and silviculture management are the only way to minimise the inoculum build up as the wood debris left after planting will act as the food source of the fungi.

4.1.2 C. fimbriata complex-*Ceratocystis wilt disease*

The pathogen emerged as a force to be reckoned with, as with the concerns about the vitality and survival of Acacia plantations in Indonesia, Vietnam and Sabah since 2010. It had created havoc on the tree plantation industries in Indonesia and resulted in the replacement of approximately 600, 000 ha of *A. mangium* plantation with *E. pellita* and its hybrid [3, 19, 20]. The disease was first observed in Sarawak in early 2010 in the Bintulu region, infected not more than 10 trees adjacent to the roadsides and trees in the vicinity of the conservation area and buffer zone. Over the years, based on observation and feedback from plantation company personnel who worked on the ground, the number of infected trees is increasing and spreading to the region of Sibu, Miri and Kuching as well. Compared to root rot pathogen modes of spread through root contact, the spread of Ceratocystis pathogens through air and water could be considered rapid especially when the trees were inflicted with wounds made by pruning, animal damages such as squirrel observed in the Bintulu region. However, one most important discovery in some of the surveyed plots in Bintulu region was

some infected trees showing sign of recovery from the infection with new sprouts growing out from the trees.

This could indicate resistance and/or tolerance of some of the individual trees. It was later found out that the plantation company has planted improved clones in their area. This justifies that breeding of disease should be actively pursued as a tool to help with the growing problem of invasive pests and pathogens that threaten our forests. However, resistance breeding is sometimes viewed as being too long term (5 years) and too expensive to be practical as lack of scientific understanding between planters and scientists in the matter concerned [21].

There are some assumptions that wood-boring insects could have the possibilities of harbouring the pathogen in their gut and acting as a vector of the disease; however, no concrete studies have been able to prove this theory. The attempted isolation of Ceratocystis from the carcass of the ambrosia beetle collected during the survey using the carrot bait method but none bore any traces of the pathogens, thus concluding that it did not harbour the fungus and thus not the vector in this specific study. Therefore, we presume the wood-boring beetle found is most probably due to secondary infestation caused by abiotic tree stress.

4.1.3 Pink disease

Currently, the pink disease caused by the pathogen *U. salmonicolor* was never been considered a major threat to Acacia plantations as compared to root rot and *Ceratocystis* wilt [22] disease as the reports of the pink disease were very few and it assumed that Acacia species particularly *A. crassicarpa* seems to be resistant to the fungal pathogen of *U. salmonicolor* [8, 23]. In Sarawak, the earliest occurrence of pink disease on *A. mangium* was recorded in 1979 and it reached epidemic proportion in 1987 [24]. The mortality inflicted by this pathogen observed in 2-year-old *A. mangium* indicated that this disease should be taken seriously. As the spores could travel by wind and water, it will be a threat to nurseries and early establishment of seedlings planted.

4.2 Disease of Eucalyptus

4.2.1 Canker disease

Several fungi are known to cause stem cankers in eucalyptus, among them *Botryosphaeria* spp., *Chrysoporthe deuterocubensis* and *Teratospaeria zuluense* (syn. *Coniothyrium zuluense*) [25]. *Chrysoporthe deuterocubensis* is a widespread and important pathogen of plantation eucalypts in the tropics. Basal cankers caused by *C. deuterocubensis* can extend several meters up the stem and have the ability to kill young trees. Where stems have been girdled, young trees may wilt and die suddenly during hot and dry weather [26–28].

Older trees of 11 to 12 years in the Bintulu region at the first rotation that exhibit symptoms of canker in this assessment are growing well and without any concern for mortality. The wood under bark seems to be healthy without any signs of lesion or discolouration although gummosis is present on the canker stem. This might be that trees normally could have survived the initial infection and only will develop basal swellings and severe bark cracking over brown necrotic sapwood [29]. However, younger trees of 2-year-old in this study recorded mortality. The trees are currently in 2nd rotation planting; thus, it could be concluded that DI and severity, as well as tree

mortality in especially young *E. pellita* plantations, can thus only be expected to increase after the species has been grown for several rotations on the same site [16].

4.2.2 R. solanacearum—*bacterial wilt of Eucalyptus*

Bacterial wilt disease of Eucalyptus will pose a difficulty to contain as *R. solanacearum* sensu lato is known to be a destructive bacterial phytopathogen that is able to cause bacterial wilt in over 50 plant families growing in tropical, subtropical and some temperate areas globally [30]. Bacterial wilt arises from the blockage of the xylem tissue by bacterial growth, thereby resulting in wilt symptoms in the aerial parts of infected plants that ultimately die. The symptoms are very much similar to Ceratocystis wilt disease with the inner stem exhibiting blackish-brown. However, to distinguish between the latter and bacterial wilt disease, examination of the plant stem using a hand lens will be required to observe bacterial oozing (**Figure 7**). As with many pests and diseases, the technology of breeding and selection of disease and insect cultivars should be the key to minimising damage and ensuring the productivity of tree plantation industry [30, 31]. However, the process requires scientist to weigh many factors that include pathogen diversity, prevailing environmental conditions and the availability of material for breeding as well to make the management level understand that process is lengthy that requires at least 1 cycle rotation (8−9 years) planting and costly to begin with.

4.3 Disease of *F. mollucana*

Gall rust was found as the predominant disease of *F. mollucana* in part of Miri and Bintulu regions. It was first reported by some plantation owners back in 2012; however, the disease only affected the seedlings in the nursery. The source of infection was deduced from the seeds imported from the island of Jawa Indonesia. This could be related that Indonesia had experienced the major outbreak of the disease, which affected much of *F. molluccana* plantation estate in Java in 2010 [32].

The plantations affected were those of higher elevation of more than 152 m asl with spacing of 3 m^2 that could be considered to be a close gap between trees. The pathogens thrive in an environment with elevations ranging from 152 m asl to 975 m asl, in trees ages 1-year-old to 9-year-old, and spacing between 6 m^2 to 16 m^2 [33] and require fog or mist as well as high relative humidity to ensure infection [34] (m).

4.4 General overview

The loss of yield due implicated by insect pests and pathogens attacks could hamper the overall maximum production yield of the plantation forest. Sarawak, in recent years, has observed the dramatic losses of *A. mangium* plantation in its neighbouring country Sabah due to the incidence of a serious canker and wilt disease caused by *Ceratocystis acaciivora* [19], the conversion of 1 million hectares of the *A. mangium* plantation estate in Indonesia to *E. pellita* Muell. and related hybrids over the past 5 to 10 years as detailed by [20]. These critical developments have since propelled the forest plantation industry players in Sarawak to be more cautious and attentive to any signs of threats especially caused by fungal pathogens.

Insect pests have not been associated with widespread mortality or failure of any acacia plantations in Sarawak so far. However, an investigation survey conducted in early 2022 to determine the cause of mortality in one of *A. mangium* superbulk

plantations in the Bintulu region revealed that termite's infestation further identified as *Coptotermes travians* appeared to be the most prevalent pest that had inflicted tree mortality in the area, which accounted for 24.1% in mortality of trees.

The tree plantations in Sarawak are currently in the second rotation planting but will soon enter the third rotation of planting, and the concerns of increasing pests and diseases attack will definitely be inevitable [35, 36]. There is growing concern that *A. mangium* may no longer be capable of producing commercial yields after three rotations [37]. Significant reductions in productivity have been reported with each successive rotation and reductions have frequently been associated with mortality caused by fungal pathogens [1].

Thus, Sarawak is looking for other alternative tree species to compliment or maybe even replace *A. mangium*. *E. pellita* and its hybrid are seen to be the next species of choice as Eucalyptus species are easy to hybridise as compared to Acacia species, which is due to the long time and the low productivity in multiplication due to the ageing effects [38]. By theory, hybrid clones combine fast growth, increased tolerance to pests and diseases, excellent rooting ability, as well as wood quality suitable for different uses [39].

Other tree species such as *F. mollucana* seem to be at a lower risk of pathogen attack; however, through observation and communication with planters, insect pests such as defoliators moth and borers will contribute to lower quality of the woods produced although mortality caused by these pests are expected of less concern. New exotic species of that Paulownia are slowly making a wave in Malaysia, and some planters in Sarawak are experimenting in planting this tree on a small scale.

The diagnosis and determination of the cause of any damage or disease on a tree can only be made when the trees are still alive. Once the tree is dead, it is very difficult if not impossible to determine the cause of the problem. Therefore, it is very important that tree health inspections and pest and disease surveys are carried out at regular intervals to enable recognition of early signs and/or symptoms of pests and disease infection [40, 41].

Once recognised, the initial stages and the development of symptoms can be followed until the death of the tree. This would go a long way towards better identifying the causes of tree mortality in the plantations. By so doing, instead of just encountering a dead tree during a random inspection, one would have detailed information about when the symptoms first appeared, their pattern of development and spread, mode and rate of spread, and thus a better idea of the agent(s) involved. It is, therefore, very encouraging that the management has decided to set up pest and disease monitoring transects in the young plantations.

As such, different strategies should be employed in better managing the threats of pests and disease, such as good silviculture, increasing the knowledge of proper identification of pathogens and pests threats among the planters, sharing of knowledge and more transparent in R&D data and information between different companies in Sarawak particularly, management consideration in acknowledging that R&D especially tree pest and disease surveillance, as basis it seems but the information gain is invaluable to predict the next outbreaks and to better contain the damages.

5. Conclusion

In view of the survey done in the period of 5 years through FHS, Ceratocystis wilt disease of *A. mangium* emerged as a major disease in hampering the progress of the

Sarawak forest plantation industry. Research effort must be intensified in making sure *A. mangium* could make it to the subsequent rotation. Although *E. pellita* and its hybrid are making a hype to replace *A. mangium* as the main species planted, diseases such as bacterial wilt and canker disease will be a major problem to planters later on if no countermeasure is readily in place.

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Chapter 4

Main Pests and Diseases in Tropical Forest Species in Nursery

Ángel Sol Sánchez, Gloria Isela Hernández Melchor and Facundo Sánchez Gutiérrez

Abstract

This paper presents the monitoring of pests and diseases in nurseries of 10 tropical forest species used in the reforestation of disturbed areas. The work was carried out in a rustic nursery established in Cardenas, Tabasco, under cocoa shade. The objective was to evaluate the presence of pests or diseases in the nursery under natural conditions. Pests and diseases appeared from the seedling stage in germination beds to the adult stage. The fungus *Fusarium* was the most aggressive causal agent that caused the death of seedling in the germination beds, as well as *Curvularia lanata* that massively affected *Tabebuia rosea* plants. Likewise, *T. rosea* is one of the species with the most reported pests, as well as *Lantana camara*. On the contrary, *Hamelia patens* Jacq (Coralillo) did not registered important pests during the monitoring in the nursery.

Keywords: tropical foest species, pests, diseases, nursery, plants

1. Introduction

In the tropics, the diversity of plant species is very high, although the species richness is not so great because they share the habitat with the other biological forms of that environment, such as shrubs, vines, lianas, parasitic epiphytes and palms.

In Mexico, for a long time precious woods such as cedar (*Cedrela odorala* L.), mahogany (*Swietenia macrophylla* King.), spring (*Cybistab Donnell-Smith*) and red (*Haematoxylum campechianum* L.) were exploited to take them to other continents for various cabinetry work, to the extent that the ecosystems of the Mexican tropics were impoverished by an extraction of eugenic type, that is, taking advantage of the best quality trees with straight stems and larger diameters.

This extraction in some cases has caused the disappearance of habitats due to extractive recurrence, thus favoring that the trees do not reach their physiological maturity, although their reproductive maturity does.

On the other hand, the establishment of exotic forest plantations such as teak (*Tectona grandis* L.f.), rubber (*Hevea brasiliensis* [Willd. ex A. Juss.] Müll. Arg.), melina (*Gmelina arborea* Roxb), pine (*Pinus caribaea* Var. Hondurensis [Sénécl] W.H.G.) or eucalyptus (*Eucalyptus* sp), Oil palm (*Elaeis guineensis* Jacq.) by large corporations, has caused the reduction of the surface of the original vegetation,

because when establishing these plantations all the vegetation present is eliminate, and far from supporting the local population, it exterminates the environments of the area, leaving only pockets of impoverished land to restore part of the lost biological diversity.

In this sense, forest nurseries of local species are created in order to provide seedlings to recover the strongholds of impoverished areas, potential environments or even include other environments for conservation.

For this purpose, the availability of germplasm is an activity of transcendental importance in which the possible progenitors are located, the period of flowering and fruiting is expected and later the seed is collected. This seed is taken to the nursery, processed, sown and the emergence of the seedlings is expected. At this stage, the management and care of the seedlings is of utmost importance because they are more susceptible to attack by pests and/or disease of any pest or illness.

Under this concept, in order to generate information about the main pests of some forest species used in reforestation in a mixed way, ten germination and growth beds of various local forest species were established. It is possible that due to the difference in habitat conditions between the site where they grow naturally and the nursery conditions where they were produced, some pests and diseases were present both in germination beds and growth beds and that are reported in this work.

Currently, it is a common practice to produce forest plants in a nursery and when the seed does not come from certified seed banks, these generally already bring the pest from the harvest field in a certain percentage, which are not suitable for cultivation.

When the percentage of impurities has been eliminated, the resulting seed is sown in bags, tubes, trays or germination beds depending on the size of the seed and wait for them to germinate. During the process of germination and growth of seedlings some diseases occur.

1.1 Damping off *Rizotocnia*

This is a type of opportunistic fungus that occurs when excess moisture in the site is above what the plant can dispose of. This excess moisture ends up rotting the stem of the seedling. They generally call it a dryer and it is common in various species of plants in the region such as cocoite, cedar, bojon, guanacaste, to name a few species and generally what is presented is the mycelium since they rarely form structures of sexual reproduction.

This fungus is very damaging as it forms resistant structures called interconnected Sclerotia that infect the roots of newly emerging plants and can rapidly spread over much of the germination bed.

Its presence is greater in alkaline soils with poor drainage, so generally the substrate must be fumigated with methyl bromide before sowing, covered with plastic and uncovered after two days so that there is aeration, and the seed is sown after 72 hours of aeration.

Another very common fungus is Fusarium sp., and it occurs when the seed generally does not go through an antifungal treatment before sowing, so the fungus Fusarium oxysporum attacks the external and even internal structures of the seeds. The newly emerged seedlings are covered with a white mycelium that affects the new organs of the plant, which is controlled using thiabenzadol at a dose of 1 g per l of water.

2. Germoplasm collection

During the year 2020, germplasm of various forest species or species of interest for biological diversity was collected, this in order to produce plants in germination beds, these species were: *Cedrela odorata* L., *Bursera simaruba* (L.) Sarg, *Cordia megalantha* S. F. Blake, *Hamelia patens* Jacq., *Pimenta dioica* L. (Merr), *Tabebuia rosea* (Bertol) Dc, *Lantana camara* L., *Sterculia apetala* (Jacq.) H. Karsten, *Roystonea regia* (Kunth.) O. F. Cook, and *Chrysobalanus icaco* (L.) L.

The collection of fruits was carried out by various methods such as collection from the ground, collection with a pole and hand scissors, manual selection of the fruits in the branches, specifically for the fruits of the royal palm, it was necessary to climb the palm using a team of ropes to electricians. These fruits were transported in jute sacks to the nursery and the benefit was made there.

Since the palm fruits have a very hard test, they were kept in the sack for a week, then they were washed and then physical scarification was carried out to accelerate the germination.

The seeds of all species were scarified to ensure germination. Specifically, for *H. patens* Jacq (Coralillo), because seeds were collected twice and germination did not occur, cuttings of approximately 20 cm in length were collected. These were placed in a bucket with a prepared solution of rootstock in a proportion of 15 g per 5 l of water. Here the basal part of the cutting was submerged for 5 minutes and then it was planted in the bags of soil.

The substrate used for the germination beds was grit enriched with filter cake, which is a residue obtained in the cane juice clarification process, which includes earthy materials and organic impurities [1].

2.1 *Cedrela odorata* L., (red cedar)

The planted cedar germplasm was quality seed, selected and of regular size, and reached its total germination at 18 days, this presented some problems in the germination beds, mainly due to excess water and lack of drainage, which generated problems of germination and presence of *Fusarium* and *Pythium.*

In the already bagged plants, the Meliaceae borer *Hypsiphylla* grandella was present, and caused problems in 6% of the plants, which were lost due to the bored stem [2]. To avoid greater severity of the damage, sprays were applied both to the plants in bags and to the adjacent beds that were in the process of growth.

Worldwide the main pests of cedar are insects of the genera *Anacrusis* sp. (Tortricidae), *Antaeotricha ribbel* (Stenomidae), *Hypsyphylla grandella*, as well as ants of the *Atta* genus. For Costa Rica, the presence of 15 pests in cedar and two diseases caused by Fusarium Sp and *Phyllachora balansae*, in addition to the species *Orthogeomys heterodus* was reported [3].

The presence of a cedar leaf-roller worm was also recorded, the presence of this pest was recurrent at least four times in six months while the plants were in the nursery. In the substrate, a large number of blind chicken caterpillars (*Phyllopaga* sp), which are beetle larvae, were recorded, but they did not cause any damage to the cedar plants.

In some plants the mealybug (*Mastigimas* sp) appeared, covering the entire plant, which caused its weakening and death due to the overpopulation of the mealybug, these plants were eliminated. For Mexico also was reported nine species of pests for cedar [4]. The insects reported for Costa Rica and México are listed in **Tables 1** and **2** [3, 4].

Insect	Family	Affected part
Anacrusis sp	Tortricidae	Foliage
Antaeotricha ribbel	Stenomidae	Foliage
Apatelodes sp	Apatelodidae	Foliage
Atta sp	Formicidae	Foliage
Hypsypylla grandella	Pyralidae	Shoots, seeds
Mastigimas sp	Psillidae	Foliage
Natad sp	Limacodide	Foliage
Phyllocnistes meliacella	Gracilariidae	Foliage
Sematoneura atrovenosella	Piralidae	Seeds
Sematoneura grijmani	Piralidae	Seeds
Taeniopoda sp	Romaleidae	Foliage
Thecla cupentus	Lycaenidae	Foliage

Table 1.
Insects reported for red cedar in Costa Rica [3].

Specie	Common name	Distribution in Mexico
Cicadellidae	sharpshooter	Wide distribution
	mealy louse	Campeche, Jalisco, Oaxaca, Puebla Quintana Roo, Tabasco, Tamaulipas, Veracruz and Yucatán
Atta cephalotes	leafcutter ant	Campeche, Chiapas, Oaxaca, Quintana Roo, Tabasco and Veracruz
Atta mexicana	leafcutter ant	Guerrero, Jalisco, Nayarit Tamaulipas and Veracruz
Hypsypylla grandella	borer	Wide distribution
Chrysobothris	borer	Wide distribution
Nasutitemis	termites	Wide distribution
Phyllophaga	blind chicken	Wide distribution
Tetranychis	red spider	Hidalgo, Oaxaca, Puebla and Veracruz

Table 2.
Cedar pest insects reported for México [4].

2.2 *T. rosea Bertol* dc. (Macuilis)

Despite the fact that macuilis is one of the hardest species in the state and, in turn, one of the hardest woods, it presents a great diversity of pests and diseases in the nursery. One of the main ones that appeared was the Curvularia lanata fungus, which quickly withered a large number of plants, for which it was necessary to remove the burned leaves, apply fungicide and promote ventilation to the growth beds.

Likewise, the so-called spring rust of *Prospodium perornatum* appeared sporadically on some of the approximately five-month-old *Tabebuia rosea* Plants.

The affected part showed its spermogonia as reddish dots on deep green swollen areas; especially in the upper part of the pedicel of the leaf forming an arch. This situation caused the infected leaves to break, for this reason the plant was eliminated.

A recurring pest was the *Eulepte gastralis* worm, which was present at different stages and which caused damage to the leaves causing their skeletonization, whose protection is a mesh of silk and excrement. A large population of blind hen (*Phyllophaga* sp) was recorded in the substrate, however, since its presence was in the rainy season, it did not cause damage to the macuilis plants.

For macuilis, it has been reported that it is susceptible to attack by nematodes of the genus Meloidogyne incognita, which directly attack the root part [5]. *Eulepe Gastralis* (Lepidoptera: Pyralidade) in the nursery presents a relationship between density and stratum so that the plant is affected. Other authors report a wide diversity of pests for Mexico and the Roca coast **Table 3** [3, 4].

Pest	Family	Stage	Type of affectation
Modoryx oielus Clarke	Lepidoptera: Sphingidae	cobra worm a	eats leaves
Megalostis anacoreta L.	Coleoptera. Chysomelidae	greater hog	gnaws buds
Megistops sp	Coleoptera. Chysomelidae	Oak manakin	gnaws buds
Oiketicus kirbyi (Lands-Guilding)	Lepidoptera: Psychidae	básquet worm	eats leaves
Oncideres teddellata (Thoms)	Coleoptera:cerambycidae	spotted harlequin	pierces stems
Phobetron hipparchia Cramer	Lepidoptera Limacodidae	spider worm	eats leaves
Prosarthia teretrirostris Brunn	Ortopthera: proscopidae	flat shackle	eats leaves
Protaleura tabebuiae Dozier	Homoptera cicadellidae	leafhoppers	sucks sap
Rabdotalebra sp	Homoptera cicadellidae	leafhoppers	Sucks sap
Steirastoma hishistrionicum thams	Coleoptera:cerambycidae	harlequin engrabado	Pierce stems
Tenuipalpus sp	Acari: Tenuipalpidae	Flat mite	Sucks sap
Urodera sp	Coleoptera. Chysomelidae	humpbacked beetle	Gnaws leaves
Zygogramma cognata Stal	Coleoptera. Chysomelidae	Engraved morrocoyita	Punches sheets
Amphicerus cornutos (Pallas)	Coleoptera: Bostrichidae	Capuccino	Pierce stems
Archegozetes sp.	Oribatida: epilohmanidae	painted mite	Terminals
Atta sp	Hymenoptera:Formycidae	leafcutter ant	cut leaves
Automeris junonia	Lepidoptera. Saturniidae	spiny worm	eat leaves
Automeris sp	Lepidoptera. Saturniidae	spiny worm	eat leaves
Compsus sp	Coleoptera: Curculionidae	Little	eat leaves
Cryptotermes brevis (W.)	Isoptera: Kalotermitidae	termite	Pierce the base of the stem
Diabrotica sp	Coleoptera:Chrysomelydae	Morrocoyita	Log leavess
Dikraneura sp	Homoptera:Cicadelidae	leafhoppers	Sucks sap, hojas
Edessa leucogramma Perty	Hemiptera:Pentatomidae	Guayacan jackdaw	Sucks sap

Pest	Family	Stage	Type of affectation
Eriophyes sp.	Acarina:Eriophydae	roller	gills leaves
Eulepte gastralis guanee	Lepidotera:Pyralidae	sheet gluer	eat leaves
Gastrotrips sp.	Thysanoptera:Phlaeotripidae	black trips	Micetophage
Halisidota sp	Lepidoptera:Arctiidae	silly chapola	eats leaves
Hemeroplanes Parce F.	Lepidoptera:Sphingidae	cobra worm	eats leaves
Hyphotenemus sp	Coleptera: Solytinae	scolithid	Pierce stems
Lagochirurs araneiformis LInn	Coleoptera Cerambicidae	painted Brown harlequin	Pierce stems and brances ramas
Lepydomys sp	Lepidoptera: Pyralidae	oak borer	pierces terminals

Table 3.
Pests reported for Tabebuia rosea [3, 4].

2.3 *R. regia* (Kunth) O.F. Cook (Royal Palm)

This palm was very susceptible to the Fusarium fungus during the nursery stage, mainly in the pinnae of the fronds. This fungus was present after the rainy season. Causing necrosis on the sides of the pinnae and in the terminal part of the frond, which caused dieback of an entire batch of these palms in the nursery. The fungicide Captan was applied repeatedly with few positive results, although depending on the severity of the damage, the application response could be any other fungicide [6].

2.4 *L. camara* L.

Being a fast-growing species, it is offered as an attraction for various nursery pests. During the evaluation period in the nursery, this plant grew and flourished and in turn attracted pests such as *Tetranychus urticae* (red spider), some type of aphid and some unidentified caterpillar. Disorganized plant growth provided safe haven for these pests, however, they were not considered severe pests as they did not cause problems to the plants.

This species as an aggressive weed for livestock capable of invading agricultural areas if it is not managed properly [7]. In this regard, were reported the presence of 118 pest species, some in all stages of development [8]. In the state of Tabasco, this species is found in abandoned agricultural areas or is part of the ruderal areas, but because the agricultural surface per producer is not very extensive, every two years the land for crops is cleared, for this reason it does not extend aggressively. Some authors delimit a wide diversity of 29 species of common pests for this species, some of the most frequent are *Tetranychus urticae* spider or red spider: this spider weaves its web on the underside of the leaves, so it cannot be seen with the naked eye and feeds on the sap of the leaves [9]. This species of pest was very common in the six months of the nursery and its presence was frequent, its elimination was with chemical products.

Cochineal: The presence of this pest in Lantana plants is common [10], however; in the nursery during the evaluation period it did not appear because the cochineal occurs mostly in sunny areas and the nursery maintains an average of 80% shade.

Bemicia tabaci (whitefly) appeared as a recurring pest in the nursery, its presence was manifested since the seedlings were small; This species lays its eggs on the

underside of the leaves, so the larvae feed on the sap when they emerge. The occasions that arose caused the deaths of secondary branches of the lantana plants, most of the time the plants were pruned.

Hemiptera (aphids) were present during the entire evaluation period that they were monitored. This pest is detected in the mornings on sunny days. These feed on the plants by sucking the sap, the highest concentration of aphids were located in the tender shoots or apical buds. Among the main symptoms are very sticky leaves due to the molasses excreted by these insects. His treatment was with chemicals.

Moths, a type of grayish brown moth called *Neogalea sunia* was present, it also lays its eggs on the plant and when they hatch and grow, they feed on the foliage of the plant and then become its pollinator. It is recognized because the larvae when feeding leave evidence of eating in the form of circles, their eradication is at that stage, as they are very voracious due to their color, they are easily confused with the stem or leaves of the plant and are easily lost.

Some of the diseases reported for Lantana are *Alternaria*, which manifests itself with brown spots on the leaves, in addition to Fusarium, which was very aggressive in the nursery, since it caused the decay of the plant, since it was necessary to apply antifungals, avoid irrigation and encourage the entry of sunlight.

2.5 *B. simaruba* (L.) Sarg

This species, despite being a rough species and well adapted to tropical conditions, presented some species of pest insects; however, the damages were not severe and did not represent damage per se to the plants in the nursery.

It was detected that the insects do not use the mulatto plants as a habitat, rather as occasional visitors, that when the plant is monitored, they quickly disappear by flying.

Some especies of pests for palo mulato, such as the beetles *Xyleborus* spp. and *Platypus* spp., as pests of green wood, *Lagochirus araneiformis* L. and other insects bore into the bark and wood, they also feed on wood and living seedling trees and Lyctus spp. attacks dry wood like termites [11].

On the other hand, despite the toughness of the plant, the *Fusarium* fungus caused serious problems in entire batches of plants because the substrate used retained a lot of moisture, also when the plant was transplanted from the germination bed to plastic containers., there was high mortality caused by Fusarium. This species tolerates abundant irrigation well, but with good filtration and 60% on shade.

2.6 *Cordia megalantha* S. F. Blake (Candelestick)

This species, at the level of plants in germination beds and bagged, did not present serious problems, occasionally some circular insect bites were observed in the dry season of the year.

However, in sowing the germination bed in the bag, mortality was very high due to the presence of Fusarium, which also occurred in royal palm and other species, due to stem, rot.

It was observed that its growth is limited when shade exceeds 65% and in turn favors the arrival of occasional insects that feed on new shoots. Likewise, when the incidence of sunlight exceeds 60%, the leaves turn yellowish and slow down their growth.

2.7 *S. apetala* (Jacq.) H. Karsten

In the nursery stage, important pests were observed, except that the lack of irrigation caused the plants to decay and lose their turgor. The larval phase of Micromartinia mnemusalis has been reported as a pest of S. apetala for Costa Rica [10].

The adult stage corresponds to various moths considered as pests for various species of commercial interest, however, during the monitoring phase in the nursery it was not present. This plant species was one of the least affected by pests or diseases.

2.8 *Pimienta dioica* L.

Pepper is a species of great interest in food and for the extraction of various oils. In some states of the republic it is not cultivated in plantations, but as shade species of other crops, such is the case of cocoa in Tabasco, or borders in pastures. Although it does not receive management, its production is considered good.

Pepper seedling age, it generally does not present pests or diseases, but it is very demanding in relation to well-drained soils and sufficient humidity, it grows well in any type of soil, from very fertile to very poor such as arenosols and savannah soils.

In the nursery and in newly established plantations, this fungus has the greatest impact. Infections present as isolated necrotic lesions that vary in size from light brown to almost black in color and may cause plant death. When the damage is severe, a yellow powder is generated on the underside of the leaf and when the inflorescences are affected, they turn black and finally die. Control could be carried out by spraying an antifungal [12].

2.9 *H. patens* Jacq (Coralillo)

It is a bushy species, abundant in the region, with a wide distribution from Florida to Argentina, and it is difficult to propagate by seeds. In the nursery, the seeds take up to eight months to germinate and their initial growth is too slow, so it was decided to reproduce them by cuttings. The most appropriate thickness for reproduction was one cm in diameter on average.

The cut to chop the stakes must be clean and each stake must not exceed 20 cm in length. When the cut is not clean, the bark is mistreated and that is where the rot caused by *Fusarium* begins.

This species is not of commercial importance, but it is of local use. In cultivated areas, its growth is allowed, as it attracts pests in agricultural crops. It is attracting pollinating bees, hummingbirds and butterflies. There were no pests in the nursery during the evaluation period.

2.10 *C. icaco* (L.) L. (Icaco)

For the establishment of the germination beds of Icaco, the seeds were first collected from the ground and later the fruits of the tree were collected.

The seeds collected from the ground were perforated by some unidentified insect, therefore, there was no germination.

Subsequently, ripe fruits were collected and put to ferment for three days in a plastic bag with the mouth closed, then the pulp was removed, the seed was washed and a physical scarification was performed, under this method germination was obtained at 28 days.

During the germination phase, there were problems with fungi that caused the death of some plants. After transplanting to the bags, the *Fusarium* fungus was also present, causing the death of some plants, and the *Curvularia* sp. fungus was frequent, causing the death of some of the terminal leaves of the plants. For this species it was reported the presence of the diptera *Anastrepha* (flies), as well as *Ceratitis capitata* and *thephytophagous* mite and *Oligonychus bagdasariani* [13].

Other authors report the presence of the Red Spider Tetranychus urticae, which causes yellowing of the leaves at the site of the bites and when it comes to attacking fruits, they acquire a dirty color due to the dark spots of the bites.

3. Conclusion

The presence of pests and diseases in the seedling stage was common. In the germination beds, Fusarium behaved recurrently in most of the germination beds, mainly in *C. odorata* and *R. regia*. The most specific pests *were Hypsiphylla grandella* for *cedrela odorata*, the fungus *Curvularia lanata* for T. rosea, as well as *Prospodium perornatum* for the same species.

Excess shade, continuous irrigation, residual moisture and evapotranspiration were key factors of importance for the development of fungi almost during the six months of monitoring of the plants in the nursery, although shade was removed from the nursery trees twice this year. it reappeared in a short time.

The pests entered the nursery in the area where the nursery adjoins herbaceous and shrubby vegetation, that is, the natural habitat of these organisms is that type of environment. It was recorded that the more the plants grow, the greater the possibility of shelter for pests.

The reason for including shrubby species such as Icaco, or herbaceous species such as lantana and Hamelia, is that in disturbed areas, herbaceous plants are the pioneers of secondary succession, and when reforestation or restoration areas are established, herbaceous species are very important for maintaining soil cover and protection. In addition, they are very important in attracting beneficial insects for Pollination.

Due to the abuse of agrochemicals for various uses, biological diversity (Flora and fauna) has been greatly reduced, so it is necessary to restore ecological conditions and habitats to generate a status of ecological balance at various scales in time. Hence, it is very important to know the pests or diseases that could affect the species used in habitat restoration.

In any environment that has been altered, pests settle in the islands of vegetation present, so their eradication is almost impossible due to their qualities of adaptation to the changing environment.

In this way, the species that are planted will have different functions over time in which the ecosystem normalizes and the insect pests adapt to that environment.

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Chapter 5

Perspective Chapter: Microorganisms and Their Relationship with Tree Health

Rodrigo F. Ramos, Lisiane Sobucki, Estéfany Pawlowski, Janaina S. Sarzi, Jessica E. Rabuske, Lucas G. Savian, Tiago E. Kaspary and Cristiano Bellé

Abstract

The health of plants depends on numerous environmental factors. All plants, including trees, live in close relationship with microorganisms. Plants harbor microbial communities in above- and below-ground tissues, where plant-associated microbial communities are influenced by environmental conditions and host genotype. The microbiome of trees is composed of mutualistic, commensal, and pathogenic microorganisms. Mutualistic microorganisms can help trees obtain nutrients (e.g., phosphorus and nitrogen) and defend against plant pathogens. Ecological interactions between different microbial groups directly influence host health, and endophytic microorganisms can inhibit pathogen growth or induce the expression of genes related to tree defense against these adverse organisms. Hence, understanding host-microbiome-environment interactions are crucial for modulating tree health.

Keywords: plant holobiont, microbial ecology, bacteria, ectomycorrhizas, arbuscular mycorrhizae

1. Introduction

Trees, including those outside of forests (i.e., orchards, gardens, trees in urban environments), are terrestrial plants that provide several ecosystem services essential to life and the economy. Trees contribute to carbon sequestration, animal and insect biodiversity maintenance, lignocellulosic biomass production for the industry, food supply (i.e., fruits and seeds), tourism (i.e., forest nature trails), air filtration in urban areas, and wood production for construction. Given their importance and the benefits trees provide, understanding the factors related to their health is pivotal for proper human intervention in forests, woodlands, and orchards.

Tree health depends on numerous factors, and it is now understood that their health, as well as other multicellular organisms, is closely related to the structure of the microbial community of the holobiont (the host and its associated microorganisms) [1]. The theory of plant holobiont seeks to shed more light on the plant as a meta-organism,

in which plant growth, health, and productivity are closely related to the composition and functions of the microbiota inhabiting different niches in the plant [2]. Within this perspective, trees are not autonomous entities, and their health can be considered a function of the plant microbiota (set of all associated microorganisms) or the plant microbiome (set of all associated microbial genomes) [3–4].

Trees establish different symbiotic relationships with a plethora of microorganisms. Therefore, the plant microbiome, also known as a phytobiome, is composed of commensal, mutualistic, and pathogenic microorganisms [5]. The diverse microbial composition contributes to host-microbial homeostasis, meaning the microbial community structure can modulate and maintain tree health over time. It is now known that the colonization of plant tissues by a pathogenic microorganism and disease development may be related to an imbalance of host-microbial homeostasis. In some cases, this imbalance results from atypical environmental conditions or the loss of partner microbes or ancestor microorganisms that are key to tree health.

The microorganisms that are part of the phytobiome include a wide diversity of prokaryotes distributed in different phyla (Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, etc.), in addition to fungi (including mycorrhizae), protozoa, nematodes, and viruses. In general, the rhizospheric soil and endosphere are the environments most densely populated by microorganisms; nevertheless, the phylloplane is also an important environment for tree-associated microbial communities. Partner microorganisms can contribute directly and indirectly to tree health through nutrient provision (i.e., phosphorus solubilization and nitrogen fixation), plant hormone production (i.e., gibberellins, auxins), pathogen inhibition via competition, and the inhibition of systemic host resistance.

2. The ecological community of microorganisms

Symbiotic relationships are common to all organisms. Trees establish three main symbiotic relationships with microorganisms: commensalism, mutualism, and parasitism (pathogenic microorganisms). Commensals represent a wide diversity of microorganisms that internally and externally inhabit plant tissues; they benefit from the resources made available by the host tree (contact surface, space, moisture, mucilage, cellular debris, etc.) and do not cause any harm to the host. In contrast, the benefits of commensal microorganisms are considered "neutral" to their host [6]. Although the classical definition of commensalism defines the absence of benefits to the host, this may not be truly applicable to the networks of microbial interactions that develop in plants. The development of a community of commensal microorganisms results in the occupation of a niche that, in their absence, would be held by other groups of microorganisms, including pathogens. Hence, these microorganisms' survival strategies, including cooperation with other commensal microorganisms, pose an obstacle to the pathogen colonization and development in these niches. From this perspective, the benefit of a single commensal species may be neutral, although the gains from the presence of a commensal microbial community are positive to the host, despite not being straightforward to establish the actual gains from the commensal relationship between microorganisms and plants.

While the community of commensal microorganisms can act as an obstacle to establishing primary pathogens, this community can harbor opportunistic pathogens. Factors related to this phenomenon include unusual environmental conditions (i.e., long drought periods or excess water), physical damage to plant tissues (i.e.,

mechanical damage to the roots, stems, leaves, and fruits), extreme temperatures, and other factors that can affect key components of plants' "innate immunity" [7]. For instance, acute oak decline (AOD) has been a recurring problem in European forests and is associated with opportunistic pathogens, as in the case of the fungus *Armillaria gallica* Marxm. & Romagn (Agaricales: Physalacriaceae). This fungus is an important saprophytic species in wood decay in forests, and it can develop a wide network of hyphae in the subterranean soil and is unable to colonize vigorously growing hosts [8]. Nevertheless, *A. gallica* invades oak trees weakened by insect defoliation or drought, colonizing the root system and causing root rot [9]. The transition from saprophytic to pathogenic lifestyle indicates that a microorganism can alter its relationship with the host depending on environmental conditions and the plant's immune status [9].

The symbiotic mutualistic relationship is a key survival strategy between plants and microorganisms. It is now recognized that mycorrhizal fungi were undoubtedly the most important microorganisms for the successful terrestrial colonization by plants. The evolution of symbiosis with mycorrhizal fungi occurred simultaneously with the establishment of plants on land 450 million years ago [10]. Arbuscular mycorrhizal fungi (AMF—phylum Glomeromycota) were the first fungi to establish a mutualistic symbiosis with plants, and, currently, this group is associated with the roots of over 85% of all plant species [11]. However, the establishment of plants on land also occurred concomitantly with the diversification of other mutualistic symbioses. In forest environments, symbiosis with ectomycorrhizae (ECM—phyla Basidiomycota and Ascomycota) is found in various gymnosperm and angiosperm lineages [12]. Both mycorrhizae groups contribute to the nutrition of their hosts by making scarce nutrients available (i.e., phosphorus and nitrogen), as well as increasing the tolerance of their hosts to abiotic (salinity and water stress) and biotic (pathogen attack) stress. The biogeographical distribution of these symbionts is influenced by climatic factors, such as temperature and rainfall regime. In tropical forests, mutualistic symbiotic association with AMF is more common, whereas ECM fungi are more diverse in temperate and boreal ecosystems [13, 14].

Another important association that may have contributed to the plants' successful terrestrial colonization is symbiosis with nitrogen-fixing bacteria. Association with nitrogen-fixing, nodule-forming bacteria, termed root nodule symbiosis (RNS), is restricted to four angiosperm orders: Fabales, Fagales, Cucurbitales, and Rosales [15]. Most leguminous tree species in tropical forests are capable of forming root nodules and fixing atmospheric nitrogen [16]. In some situations, trees can establish a mutualistic symbiotic association with rhizobia in conjunction with mycorrhizal fungi. The synergistic interaction between these two types of mutualism can improve plant performance, especially in soils nutritionally poor in phosphorus and nitrogen or saline soils. For instance, co-inoculation of the AMF *Rhizophagus fasciculatus* (Thaxt.) Gerd. & Trappe (Glomerales: Glomeraceae) and the actinobacterium Frankia (root nodule symbiont) improved the tolerance to salt stress of the tree species *Casuarina equisetifolia* and *C. obesai* (Fagales) [17].

Unlike RNS, nitrogen fixation through plant-cyanobacteria association is widely distributed in terrestrial plants [18]. Association with nitrogen-fixing cyanobacteria can occur in below-ground (rhizosphere) and above-ground (phyllosphere) environments. These microorganisms can live with mosses growing on the soil, be associated with bryophytes and arboreal epiphytes, or grow in the leaf cavities of plants [18–20]. In fact, evidence has shown that nitrogen fixation by cyanobacteria is associated with "feather moss" (i.e., *Hylocomium splendens*, *Ptilium crista-castrensis*, *Pleurozium*

schreberi, and *Sphagnum caprifolium*), an important source of nitrogen input into boreal forest ecosystems [21–22].

The phytobiome also harbors pathogenic microorganisms (symbiotic parasitism relationship). Forest pathogens include fungi, oomycetes, bacteria, phytoplasmas, parasitic higher plants, viruses, and nematodes [23]. Despite a plethora of causative agents, the diseases of forest trees are primarily caused by fungal and oomycete pathogens [24]. The development of some tree diseases has been correlated with changes in the phytobiome. Thus, it is believed that the population growth of a particular pathogen may be related to the decline in the population of beneficial microorganisms. For example, one study reported that pine plants with pine wilt disease (PWD) caused by the nematode *Bursaphelenchus xylophilus* had a lower diversity of beneficial fungi and bacteria in the rhizosphere compared to the rhizosphere of healthy pines [25]. Recent studies have focused on analyzing the microbiome of diseased trees in an attempt to map unknown pathogen groups. Research on the microbiome of oak trees with AOD has reported that this concerning disease is caused by a polymicrobial complex, that is, the onset of AOD is related to the interaction of different bacterial species (*Brenneria goodwinii*, *Gibbsiella quercinecans*, and *Rahnella victoriana*) that work synergistically to develop the disease [26–28]. Some studies suggest that the physiological state of the host can influence the abundance and diversity of pathogenic microorganisms. For example, a recent study evaluated the diversity of foliar endophytes in *Platycladus orientalis* and *Styphnolobium japonicum* trees with different ages (individuals ranging from 10 to 5000 years old for *P. orientalis* and 10 to 1700 years old for *S. japonicum*) [29]. The authors demonstrated that the abundance of latent pathogens (fungi) increased as the trees aged. Thus, the abundance of pathogens *Collectotrichum gloeosporioides* and *Botryosphaeria dothidea* in *S. japonicum* and *Pestalotiopsis funerea* and *Amyloporia subxantha* in *P. orientalis* increased linearly with tree age, indicating that tree age is also an important structuring factor for host communities [29].

Although forest pathology is a recent science, it has been growing rapidly in recent decades mainly due to recent tree death events in forest ecosystems throughout Europe and North America. Forest decline diseases have concerned scientists and government bodies and are becoming increasingly problematic for tree health worldwide [28, 30]. Hence, shedding more light on the role of the tree microbiome will be crucial for properly managing forest environments.

3. Plants as microbial habitats

The plant microbiome is highly dynamic and diverse. Plant-associated microbial communities are deeply influenced by environmental conditions (pH, moisture, temperature, and nutrient availability) and host genotypes. Plants harbor microbial communities in above- and below-ground tissues (**Figure 1**). Below ground, the rhizosphere (soil region in intimate contact with roots) is the environment most densely populated by microorganisms [31]. The root endosphere is another important belowground region that hosts a vast diversity of microbial communities. The endosphere is the region encompassing the apoplastic spaces in the root cortex (inside the roots). The host genotype strongly influences the microbial communities of the rhizosphere and endosphere, and this can be considered an "extended root phenotype" (i.e., a manifestation of the effects of plant genes on their environment inside and/or outside the organism) [32].

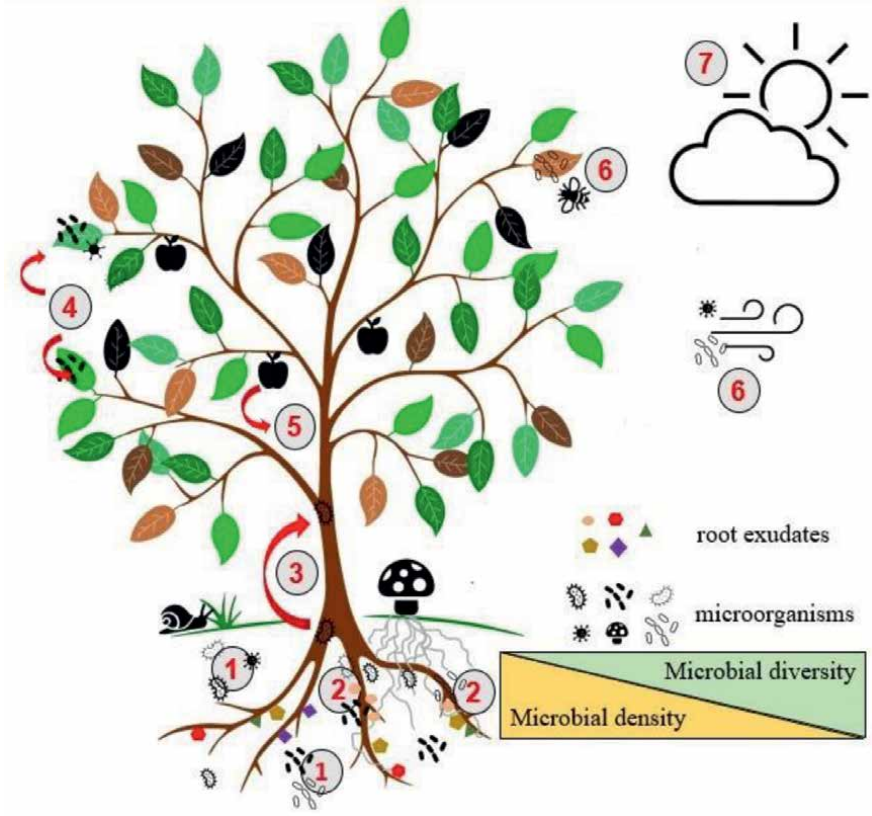

Figure 1.
Interactions between microorganisms and trees. Microorganisms present in the bulk soil that roots can recruit via organic molecule exudation (1). Microorganisms in the rhizosphere and endosphere. The presence of root exudates (sugars, lipids, proteins, and secondary metabolites) stimulates the recruitment of specific microbial taxa. The rhizosphere microbiota is strongly influenced by the physicochemical profile of the exudates of this niche (2). Endophytic microorganisms can inhabit internal regions of the plant without causing disease. Bacteria that inhabit the root endosphere can be translocated via the xylem to other regions of the plant above ground (3). The phyllosphere is the compartment that houses associated microorganisms above ground, and this niche is mainly represented by the leaves (4). Other above-ground compartments may harbor associated microorganisms, such as the outer surface of fruits (carposphere), flowers (anthosphere), and the stem (caulosphere) (5). Microorganisms can be dispersed from one plant to another or from the soil to the phyllosphere by wind, rain, insects, and animals (6). The composition of the microbial community of the phyllosphere is significantly influenced by environmental factors, including solar radiation, temperature, and humidity (7).

Between the volume of soil not occupied by roots and the endosphere region, there is a selection degree of microorganisms. Various studies have demonstrated that microbial species richness is the highest in the bulk soil and that it decreases in the rhizosphere and endosphere (**Figure 1**). In contrast, the population density of specific microorganisms increases from the soil toward the root surface, indicating favorable conditions for the selected microbial species [5]. This phenomenon is called the rhizospheric effect, that is, the composition of root exudates (sugars, oligosaccharides, vitamins, nucleotides, flavones, auxins, and secondary metabolites) modulates the physicochemical conditions of the rhizosphere region and, thus, the plant can recruit and select groups of microorganisms that can proliferate in the specific physicochemical conditions of the root zone [33]. Estimates have indicated that up to 40% of the carbon reserves fixed by photosynthesis are provided in

the rhizosphere, indicating the active role of plants in recruiting microbial communities. Although the root zone harbors a wealth of biodiversity, the rhizosphere and endosphere microbial communities are dominated by four bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria [5, 34]. In the bulk forest soil, the dominant fungal group is Basidiomycota, while Ascomycota is the most prevalent group in plant tissues [9].

Evidence suggests that plants adapt to biotic stress by altering their root exudate chemistry to assemble health-promoting microbiomes [35]. This phenomenon is termed the "cry-for-help" hypothesis, and it posits that plants modulate the chemistry of root exudates to recruit partner microorganisms capable of increasing the plants tolerance to a given stress condition, such as insect herbivory, pathogen attack, or nutrient shortage. Therefore, the chemical composition of root exudates influences the metabolism of rhizosphere microbial taxa while the recruited microbiota assists plant homeostasis by encoding functionalities that extend the plant genome [36]. For example, a study with the model plant *Arabidopsis thaliana* and inoculated with the Gram-negative pathogen *Pseudomonas syringae* demonstrated that subsequent generations of *A. thaliana* subjected to inoculation with the pathogen were able to modulate the root exudation profile, alter the composition of the rhizosphere microbial community, and increase the disease suppressive response [37].

Above ground, there is also an important plant compartment that hosts microorganisms; it is called the phyllosphere and refers mainly to the leaves. However, there are also other important plant sub-compartments above ground, such as the anthosphere (external environment of flowers), caulosphere (environment of the plant stem), carposphere (external surface of fruits), and spicosphere (niche formed in plants with spikes) [38–39]. The phyllosphere is an oligotrophic environment subject to severe modifications in a short period of time (temperature, humidity, and radiation fluctuations); despite being disconnected from the soil, this environment indirectly influences the phyllosphere. Dust particles from the soil can be dispersed by the wind and deposited in the above-ground plant compartments and thus provide nutrients for the microbial communities of the phyllosphere. In addition, microorganisms can be dispersed from the soil to the above-ground part of the plant by wind or colonize the phyllosphere after being recruited in the rhizosphere and systematically translocated to the above-ground part via the xylem. Microorganisms that can colonize plant tissues internally or translocate via the xylem to different tissues of the plant above ground without causing disease are called endophytes. These microorganisms may live part of their life cycle associated with the root endosphere region or translocated to plant tissues above ground. Furthermore, as in the rhizosphere, the bacterial communities of the phyllosphere are dominated by the taxa Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria [40].

The phyllosphere is an open environment where the arrival and departure of taxa are constant [41]. The phyllosphere generally consists of microorganisms that are dispersed mainly from the soil and reach the surface of leaves and stem through rain, wind, and insects (**Figure 1**). The survivability and colonization of these microorganisms depend on several factors, such as host genotype and health (since both modulate the chemical composition of leaves), water availability, temperature fluctuation, UV exposure, chemical applications (i.e., fertilizers and pesticides), and inter- and intraspecific microbial competition [41–43]. Although several factors influence microbial communities, it is recognized that environmental conditions are the key factors that determine population structure in microbial communities of the phyllosphere. For instance, a recent study revealed that the microbial community

composition of the phyllosphere of spruce trees was influenced by seasonal changes, whereas the bacterial and fungal communities of the rhizosphere of these same trees were influenced by anthropogenic nutrient availability [44].

Because it is an environment with harsh conditions, many microorganisms have evolved and developed survival strategies to colonize the phyllosphere. Thus, to colonize niches in the phyllosphere, some microorganisms can alter the chemistry of the leaf surface or use specialized cellular structures to increase their ability to compete. For example, the bacterium *P. syringae* (an important plant pathogen) releases surfactants that increase mobility and local water availability on leaf surfaces. In addition, this microorganism has flagella that favor bacterial motility on the leaf surface. In the case of *P. syringae*, these strategies are related to its virulence [41]. Nonetheless, characteristics of the host plant's aerial part structures also influence the successful colonization of the phyllosphere. One study evaluated the bacterial functional diversity in the phyllosphere of different tree species in a Neotropical forest and found evidence suggesting an adaptive correspondence between phyllosphere microorganisms and their tree hosts [40]. The authors demonstrated that tree characteristics, such as leaf morphology and leaf metal contents (copper, manganese, and zinc), are correlated with phyllosphere microbial community composition [40].

4. Functions of the host-associated microbiota

Plant-associated microorganisms can assist the health of the host in different ways. Many endophytic microorganisms can make previously scarce nutrients available in the soil or increase host tolerance to stressors. Mycorrhizae play an important role in maintaining tree health among endophytic microorganisms. The role of these microorganisms is related not only to making phosphorus and nitrogen available to the host but also to increasing plant tolerance to water stress and pathogen attack. In one experiment, the authors evaluated the effects of inoculation of an AMF (*Glomus etunicatum*) on pistachio (*Pistacia vera*) seedlings subjected to water stress. They observed that plants inoculated with the fungus had an increased tolerance to water stress compared to the control (seedlings subjected to water stress and not inoculated) [45]. Among the mechanisms related to increased stress tolerance and possibly induced by the mycorrhizal association, the authors highlighted a greater accumulation of osmotic adjustment compounds (i.e., soluble sugar content), increased activity of antioxidant enzymes (i.e., catalase and peroxidase), secondary metabolite production (i.e., flavonoids), and nutrient accumulation (nitrogen and calcium) [45].

Further findings have also suggested that association with mycorrhizal fungi improves host resistance to pathogens [46]. For instance, a recent study used amplicon sequencing to determine the presence of a wide taxonomic range in the rhizosphere of apple (*Malus domestica* Borkh) rootstocks [47]. The authors observed that roots of the G.890 rootstock (which is tolerant to apple replant disease—ARD) harbored a significantly higher percentage of AMF species, indicating a possible active role of endophytic fungal communities in apple tree tolerance to soil pathogens (including *Rhizoctonia* spp., *Phytophthora* spp., and *Pratylenchus penetrans*) that cause ARD [47]. In another study [25], the authors evaluated the effects of inoculating ECM species (*Suillus bovinus* and *Amanita vaginata*) and dark septate endophytes (DSE, *Gaeumannomyces cylindrosporus* and *Paraphoma chrysanthemicola*) on the tolerance of pines (*Pinus tabulaeformis*) to PWD caused by the pine nematode *Bursaphelenchus xylophilus*. The authors demonstrated that inoculating pines with ECM/DSE reduced

disease severity caused by *B. xylophilus* and increased the recruitment of beneficial bacterial and fungal groups in the rhizosphere of pines [25]. In addition to improving nutrition and stimulating defense mechanisms in their hosts, ectomycorrhizal fungi can assist their host against pathogen attacks by forming a thick fungal mantle that acts as a mechanical barrier against the penetration of soil pathogens [48].

Ectomycorrhizae are known to benefit their host trees in different ways. This mutualistic symbiosis is especially useful for forest plantations intended for the production of wood, cellulose, or the recovery of degraded areas. However, mutualism between trees and ECM species can stimulate another highly profitable economic activity: truffle farming. Truffles are the reproductive structures of hypogean ectomycorrhizal fungi and are appreciated in haute cuisine [49]. A recent study evaluated the effect of mycorrhization of pecan trees under subtropical conditions in Brazil [50]. The authors demonstrated that inoculation of pecan seedlings with the ectomycorrhizal species *Tuber aestivum* and *T. brumale* increased plant growth and, in addition, ECM produced high-value edible structures (truffles) [50]. These results indicate the possibility of developing a highly profitable economic activity associated with pecan orchards in subtropical regions.

Recruitment of plant growth-promoting bacteria (PGPB) is another important strategy trees adopt to increase their resistance to pathogen attacks; PGPB is known to promote plant growth through different mechanisms, including producing or stimulating plant hormones (i.e., gibberellins, auxins, and cytokinins), nitrogen fixation, phosphate solubilization, among others. In addition, PGPB may exhibit biocontrol activity against plant pathogens; numerous mechanisms have been described as being responsible for the biocontrol ability of PGPB, such as direct competition for space, emission of volatile compounds, siderophore production, lytic enzymes (i.e., proteases, chitinases, and lipases), antibiotics (i.e., amphisin, 2,4-diacetylphloroglucinol, and oomycin A), and induction of systemic resistance of the host plant [51–53].

The ability of the plant to recruit partner microorganisms indicates that the rhizosphere is a reservoir of beneficial microorganisms amenable to selecting and applying disease biocontrol programs. For example, an endophytic PGPB (*Bacillus velezensis* OEE1) was isolated from olive (*Olea europaea*) roots, and its disease biocontrol ability was tested against fungal (*Fusarium solani*, *Botrytis cinera*, etc.) and oomycete (*Phytophthora* spp.) pathogens [54]. The authors observed that the biocontrol ability of fungal pathogens and oomycetes by the isolate *B. velezensis* OEE1 was related to a wide range of competitive characteristics, including phosphate solubilization and producing siderophores, extracellular hydrolytic enzymes (amylases, cellulases, and pectinases), biosurfactant (surfactins), and secondary metabolites [54]. In another study, bacteria were isolated from the rhizosphere of avocado trees that survived root rot infestations caused by the oomycete *Phytophthora cinnamomi* [55]. The authors evaluated the antagonistic activity of the isolates against *P. cinnamomi* and selected a potential PGPB (*Bacillus acidiceler*) that could inhibit the growth of the oomycete by producing volatile compounds, indicating the potential use of this PGPB in biocontrol programs for pathogenic oomycetes [55].

5. Conclusions

The microbial communities of the rhizosphere and phyllosphere are essential to the health of their hosts. Researchers are currently seeking to understand the active role of tree-associated microorganisms and the mechanisms related to the

recruitment of beneficial taxa. Knowledge about tree-associated microorganisms is increasingly more important in the face of the current scenario of increasing diseases of forest declines. Therefore, microbial ecology studies applied to tree-associated bacteria and fungi communities will enable researchers to bioprospect new microorganisms for use in plant growth promotion and disease control.

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Conflict of interest

The authors declare no conflict of interest.

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