Chapter

Oil Palm Fatal Yellowing (FY), a Disease with an Elusive Causal Agent

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Abstract

Fatal yellowing disease (FY) is a bud rot-type disease that severely affects oil palm plantations in Latin America. Since 1974, when it was first reported in Brazil, this disorder has been responsible for severe economic losses in the oil palm industry; and, for nearly 50 years, several studies have tried to identify its causal agent, without success. The etiological studies regarding FY in oil palm explored either biotic and abiotic stress scenarios, in a single or combined manner. Most recently, the hypothesis in favor of one biotic cause has lost some grounds to the abiotic one, mainly due to new insights regarding deficient aeration in the soil, which reduces the potential for oxy-reduction, causing changes in the ionic composition of the soil solution. This review presents an overview of the history of this disease and the several efforts done to fulfill Koch's postulates over the last 40 years, besides discussing recent studies that revisited this subject using some omics technics. We conclude by discussing further uses of omics via a multi-omics integration (MOI) strategy to help finally find out what is really behind the genesis of FY. Finding this elusive causal agent of FY out will allow either the development of a more efficient diagnostic tool and the advance in studies trying to find out the source of the genetic resistance hidden in the genome of the American oil palm.

Keywords: *Elaeis guineensis*, palm oil, epidemiology, tropical diseases, etiology, abiotic stress, biotic stress

1. Introduction

The African oil palm (*Elaeis guineensis* Jacq.) is a palm tree originally from the West Coast of Africa and currently distributed in three regions of the equatorial tropics; Africa, Southeast Asia, and Central and South America, where it exists in the wild, semi-wild, and cultivated form [1]. Among the oilseeds, it is the one with the highest average yield, producing 4 to 6 tonnes per year of vegetable oil [2]. The fruit palm oil (mesocarp) and the palm kernel oil (almond) are the raw material for several products in the food industry, in the cosmetic and personal hygiene industry, and the biofuels industry [3, 4]. Palm oil provides 36% of the global supply of vegetable oils with a considerable increase, from crude palm oil (CPO) production from 5.3 million tonnes in 1981 to 71.45 million tonnes in 2018 [2].

The Asian continent concentrates most of the CPO production, led by Indonesia and Malaysia, which together accounts for 85% of the world's CPO production [4]. However, the limited availability of areas for cultivation in Southeast Asia has opened new frontiers for expansion, culminating in the growth of Latin America's share in the global production of oil palm [5]. Latin America has the largest suitable area for oil palm cultivation, notably led by Brazil (2,283,000 km^2), Peru (458,000 km^2), and Colombia (417,000 km^2) [5]. Among Latin countries, Colombia is the world's fourth-largest producer of CPO and the first in the Americas, with an estimated production of 1.67 million tonnes in 2020, followed by Guatemala with 852 thousand tonnes and Honduras with 580 thousand tonnes [4].

Unfortunately, oil palm plantations in this geographical area are affected by a wide variety of pests and diseases that negatively affects productivity and discourage investment in this sector [1]. Notably, "bud-rot type" diseases pose the greatest threat to oil palm plantations in Latin America [6]. Among them, *Pudrición del Cogollo* (PC) and Fatal Yellowing (FY) are the diseases that cause most of the damage, both presenting a common symptom: rotting of the spear leaf that evolves until reaching the apical meristem system leading to the death of individuals [6, 7]. By far, the FY exhibits the most dramatic scenario because, in contrast to PC, its causal agent remains unknown, hindering effective sanitary control practices [8].

Fatal Yellowing was first identified by Reiking in 1928 in oil palm plantations in Panama, with cases reported in Colombia, Ecuador, Peru, Costa Rica, Venezuela, Suriname, Nicaragua, and, reportedly, in Central Africa, after that [6]. In Brazil, it was only in 1974 that the first symptomatic individuals were identified and, from the epidemiological explosion that occurred in the 1980s, FY started to represent the greatest threat to oil palm in the country [9]. As a result, several studies began to search for the possible biotic causal agent behind it and its putative vectors [8]. However, the research efforts made for more than 30 years have not exactly pointed out organisms directly linked to FY's cause [10–16]. Some studies also looked for possible abiotic causes, with inconclusive results so far [17–19]. Recently, techniques such as metabolomics, proteomics, and metagenomics started to be applied to provide insights into the possible FY etiology, initiating a new phase in the process to solve this problem [20–22].

Although Brazil has more than 30 million hectares with an aptitude for oil palm production, it currently has less than 1% of this area destined for this purpose [23]. Fatal Yellowing is the main contributor to hinder the expansion of the oil palm industry in Brazil, and the attempts to control the emergence of sick plants have not been successful, and its nature remains a mystery [10]. This review intends to analyze descriptively the studies carried out to investigate the FY problem in Brazil, besides pointing out new strategies employed for understanding the development of the disease, confirm the real cause behind it, and develop tools for early diagnostics.

2. The oil palm industry: social & economic importance

2.1 In the world

Oil palm is originally from West Africa and adapted to the intertropical areas of Africa, Asia, South and Central America [1]. It is the most profitable oil crop, as it presents a higher yield with a lower production cost [24]. Its oil yield is of the order of 4-6 tonnes per hectare per year of CPO, much higher than that presented by other crops, such as rapeseed (0.69 t), sunflower (0.69 t), and soybeans (0.44 t) [3]. Another positive point is that this crop uses only 6% of the area to produce 36% of the global oil supply, while soy, for example, occupies 40% of the land to generate 26% [4, 24]. Because of that, oil palm stands out as a player fundamental to help the world meet the growing global demand for vegetable oil in 2050 that will be around 240 million tonnes [25, 26].

The expansion of the oil palm industry has been strongly encouraged by governments and private sectors in Southeast Asia [27]. It is by far the most productive region in the world, supplying 85% of the CPO produced, reflecting the rapid expansion of the cultivated area that started in the middle of the last century [25]. The commercial oil palm plantations in Indonesia, for instance, went from 70 thousand hectares in 1961 to 6.78 million hectares in 2018, with a considerable increase of 9.582% during this period [2]. As a result, Southeast Asia production rose to 63.26 million tonnes in 2018, or a 22,378% increase in the period [2, 3].

Africa has not seen an expansion of the oil palm industry as significant as Southeast Asia in the last 60 years [3, 28]. The area occupied by oil palm increased from 3.55 million hectares in 1961 to 4.55 million hectares in 2018 in the African continent, representing an increase of only 33% (**Figure 2**) [2]. Meanwhile, the Americas now occupy 6% of the international market, producing around 4.89 million tonnes of palm oil in 2018, a 273% increase in the last two decades [2].

The considerable increase in oil palm production was supported mainly by the advances in genetic breeding programs that increased oil productivity more than 2 folds since 1960 [1].

Most of the CPO and its derivatives produced stays in the Asian markets that absorb 51% of the total, led by India, which imports 19.4%, and China 13.0% [29]. The European markets, which import 26%, have the Netherlands (6.1%) and Italy (4.3%) as the leading importers [23]. Africa (12%), the Middle East (4%), and Latin and North America (7%) also have a consumer market for vegetable oils, and palm oil from Southeast Asia helps to supply the demand [29]. The global vegetable oil market allocates 70% of total production to food and 30% to non-food industrial purposes, such as, for example, the production of cosmetics and personal hygiene products (24%) and as a raw material for the production of biofuels (5%) [26].

2.2 In Latin America

The increase in global palm oil production in the 21st century is due mainly to new plantations in producing countries, especially in Malaysia and Indonesia [27]. However, due to a reduction in the areas available for expanding cultivation in Southeast Asia, an opportunity opened up to expand to new frontiers to meet the growing global demand for palm oil [5]. As a result, Latin America became one of the most promising regions for oil palm cultivation, as it has one of the largest areas suitable for cultivation, notably represented by Brazil, Peru, and Colombia [5].

Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Mexico, Peru, and Venezuela produce together 4.65 million tonnes of palm oil, representing 6% of world production in 2020 [2]. Colombia is the largest oil palm producer in this region and ranks 4th in the World, with 1.61 million tonnes produced in 2018, or 33% from the annual palm oil of Latin America (**Figure 1**) [2, 30]. Guatemala produced a total of 875 thousand tonnes in 2018 what places the country in the 2nd position in Latin America [2, 31]. Honduras is in the 3rd, followed by Ecuador, Brazil, Costa Rica, and Venezuela [2].

2.3 In Brazil

The first oil palm plants arrived in Brazil in the 16th century, adapting very well to the Northeast region of the country [32]. The oil palm industry in Brazil

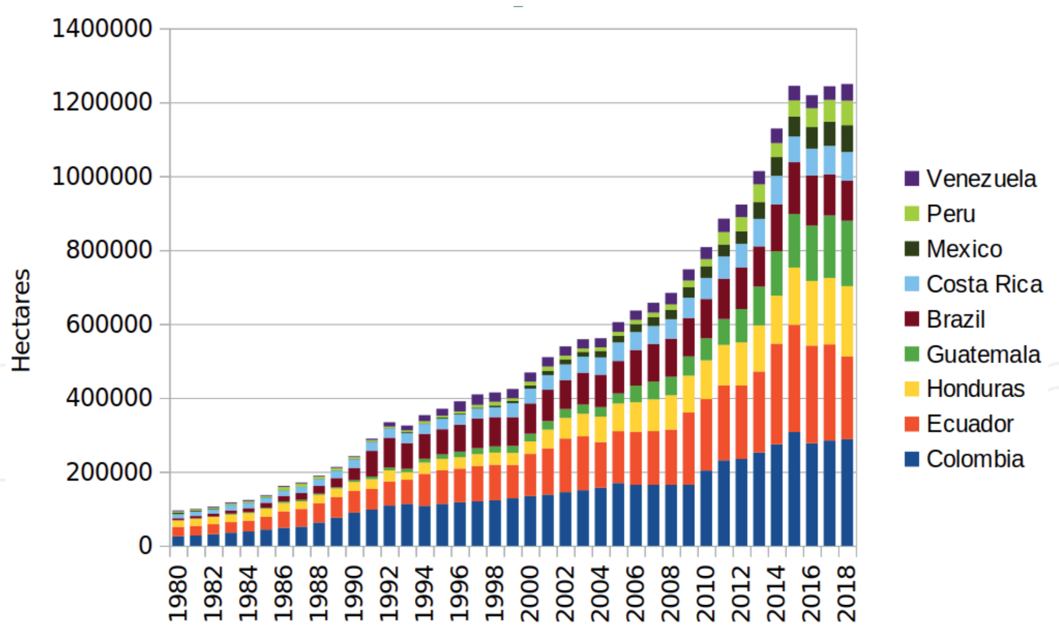

Figure 1.
Land use for oil palm cultivation in central and South America since 1980, in hectares. Source: adapted from our wold in data [2].

Figure 2.
Fatal yellowing (FY) disease in oil palm. (a) Oil palm plantation affected by FY; (b) individual showing signs of yellowing and necrosis of the intermediate leaves; (c, d, e) evolution of yellowing and dryness of the spear leaf with the presence of necrotic tissue, and (f) root section of an individual with signs of rot. Source: by authors.

stayed as a small industry until 1960, when, due to increasing demand for oil for cooling steel sheets in the national steel park, it started to experience significant growth [33]. In 1967, the oil palm cultivation expanded to the Pará State, in the North region of Brazil, with the first commercial-scale plantations comprising about 3.000 hectares [32].

Driven by technical advances and growth in demand for vegetable oils, there was a significant increase in the cultivated area of oil palm in Brazil, which went from 11 thousand hectares in 1980 to more than 236 thousand hectares in 2008 [3]. Brazil has large areas with the aptitude for oil palm production, favored by climatic conditions similar to the most productive regions in the world [1]. However, until 2014, less than 1% of this area was occupied by commercial plantations [34, 35]. Brazil's position as the 13th, and 23rd, in palm oil production and on the productivity scale, respectively, in the world, is due mainly to this under-utilization of available areas [3, 32].

Oil palm production is concentrated in Pará state, which accounts for 97.19% of the cultivated area and 97.99% of the national palm oil production, followed by Bahia (1.98%), Roraima, and Amazonas [36]. The expansion of cultivation to already deforested areas in the Amazon and other regions in Brazil is an opportunity to reduce pressure on forests and supply the palm oil demand from the food and energy sectors [35]. To make the plantations more environmentally sustainable, the Brazilian Government launched the agro-ecological zoning (ZAE) program in 2010, a legal mechanism to delimit the oil palm cultivation area [37]. This area include Acre, Amapá, Amazonas, Mato Grosso, Pará, Rondônia, Roraima and Tocantins, part of Maranhão and five municipalities in Goiás state, comprising about 59% of the Brazilian territory [35].

3. The fatal yellowing (FY) disease

3.1 History in the world

Several fatal syndromes of bud-rot severely affect plantations of oil palm in South and Central America [6, 38]. Bud-rot type disease was reported for the first time on oil palm plantations in Suriname in the 1920s, followed by another incidence in Panama reported by Reinking in 1927 [6]. In general, symptoms of bud-rot type diseases initiate with chlorosis of the youngest leaves and later necrosis that rapidly reach immature tissues of the meristem, causing a collapse of the spear leaf and plant death [9]. Bud rot diseases can take two forms: a lethal form found in Ecuador, Brazil, and in certain zones of Colombia and Suriname, and a non-lethal one, with a high recovery rate, found mainly in the Colombian Llanos [6]. The disease is synonym to a few other names such as "pudrición del cogollo" (PC) in most Spanish speaking countries, "PC típica" (PCt) or "PC diversa" (PCd) in the plantation Palmeras del Ecuador (PDE) in Eastern Ecuador, "amarelecimento fatal" (AF) in Brazil, "spear rot "in Suriname [1, 6, 7].

The first large-scale bud rot damage on oil palm plantations in Latin America was due to the PC disease in northern Colombia, where a field of 2,800 hectares located in the Turbo region was virtually devastated by PC in 1965 [9]. In Suriname, the spear rot was first registered in the Victoria region in 1976 on four-year-old oil palms in a plantation of 1,700 hectares. Despite the phytosanitary practices applied to control the disease, an exponential progression reduced the original area by 85% [39]. In Ecuador, the first PC cases happened in 1976 on four-year-old oil palms on the Pacific slopes of the Ecuadorian cordillera [1], and, like other regions, the plantation was decimated by the disease in a few years [6]. Recently Martinez et al. [7] carried out a study in Colombia to isolate microorganisms and reproduce PC in healthy oil palm plants and, in conclusion, they postulate that the oomycete *Phytophthora palmivora* is associated with the emergence of PC.

Fatal yellowing exhibits, by far, the most dramatic scenario among the bud-rot type diseases of oil palm in the Americas. The factors linked to the emergence of this disease in some countries remain unknown after experiencing more than 50 years of outbreaks in Brazil, Ecuador, Panama, Suriname, Costa Rica, Nicaragua, Honduras, Peru, and Venezuela [6, 9, 10].

3.2 History in Brazil

The FY disease first appeared in Brazil in 1974, with sporadic occurrences in a field established in 1967 near Benevides, a city in the Pará State [8, 12]. The disease progressed slowly in the following years, from 25 symptomatic plants in 1978 to 125 in 1981. In 1984, ten years after the first report, the number of plants diagnosed with FY was 465 [11]. In the first ten years after its first appearance, the disease progressed in a linear model, and the numbers of affected plants remained more or less the same per unit of time. This mode of progress indicated that the contamination did not occur from plant to plant. However, the numbers of affected plants rose to 9,968 in 1986 and 32,673 in 1987, starting a period of exponential increase [11]. In the first two decades after its first occurrence in Brazil, approximately 100 thousand oil palm trees died from this disease, resulting in the loss of entire plantations [11, 40].

Roguing was then put in place to maintain the source of the inoculum of a possible pathogen to a minimum, eliminating all plants showing symptoms up to one month after the discovery [40, 41]. The oil palm industry promoted training on the fast and precise recognition of FY symptoms to guarantee the effectiveness of this phytosanitary measure [42]. Despite it, the disease kept on occurring in plants between the 15th and the 16th year after planting, making FY one of the main problems of this crop in Brazil. Not surprisingly, this discouraged the expansion of oil palm cultivation in the affected regions [11]. As the inability to identify the causal agent and promote effective control of FY persists, the oil palm industry remains in a state of insecurity to expand in the regions affected by FY [42].

3.3 Symptomatology and diagnosis

Proper and early disease diagnosis is vital for applying control practices at the right moment. Without an efficient and effective early diagnosis of the disease and the disease-causing agent, any control measures will be inefficient [43]. Until the FY etiology is known and diagnostic systems developed, the only way to find out that a plant has this disease is by checking for characteristic symptoms and signs. Once a plant is diagnosed with FY, it must undergo roguing. In Brazil, symptoms identification in the field is still the only diagnostic system used for FY [8, 12].

An oil palm plant affected by FY shows necrosis or dryness of the spear leaf that evolves towards the base, then the region of the meristem rots, and a foul odor is felt in some cases (**Figure 2**) [12, 44]. The process of rotting of the meristem region, frequently observed in rainy seasons, motivated the initial designation of the disease as spear leaf rot [8, 40]. After losing the spear leaf, there is a general decline leading to plant death; however, some individuals during this process may temporarily re-issue a new one [12, 18]. In plants affected by FY, chlorosis appears in leaflets at the base of the intermediate leaves, which advances towards the extremity, followed by necroses frequently observed in younger leaves [6]. There is no synchronism between the spear leaf necrosis and the chlorosis of the leaflets. The FY symptoms always begin with leaflets chlorosis, which led to the Fatal

Yellowing disease name [1]. In Brazil, the oil palm tree usually dies 7 to 10 months after the onset of the first symptoms, but it can vary depending on the region [41].

Once the oil palm plant gets affected by FY, the developed bunches can reach the maturation stage and are not affected. However, the immature ones rot, and the inflorescence abort [40, 41]. The root system is visibly affected, and emission of new primary roots reduced, leading to a total cease of roots growth. FY kills the tips of the roots generating new false primary ones. In addition, the root tissue is usually necrotic at the beginning of the appearance of symptoms in the aerial part [45, 46]. On the other hand, no apparent internal symptoms are observed, such as rot or necrosis of the stipe and vascular system, a characteristic that is also seen in PC [41].

4. A genetic source of resistance to FY

The causal agent of FY is still unknown, but a possible genetic solution for this problem exists. This genetic solution resides upon the fact that the American oil palm (*Elaeis oleifera* (Kunth) Cortés) and the interspecific hybrids between this species and the African oil palm are considered resistant to this disease [47].

The genus *Elaeis* (from the Greek *Elaion* that means oil) belongs to the class Liliopsida (Monocotyledones), order Arecales (Palmales), family Arecaceae (Palmae), subfamily Arecoideae, tribe Cocoseae (Cocoinaea) and, subtribe Elaeidinae [48, 49]. This genus consists of two species, *E. guineensis* and *E. oleifera*, with a pantropical distribution and two distinct diversity centers, Nigeria and South America, respectively [50–52]. The former is the African oil palm, the predominant species in commercial plantations Worldwide, and known in Brazil as "Dendê"; and the latter is the American oil palm, which originated from Central and South America, and is known as "Caiaué" [53].

The American oil palm is endemic to Equatorial America, with natural populations distributed from Central America to northern South America, including the countries of Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Honduras, Mexico, Nicaragua, Panama, Peru, Suriname, and Venezuela [1]. In Surinam, there are dense stands on poor, sandy soil, while in Colombia, it can grow in damp or even swampy situations near or on the banks of rivers [1].

The American oil palm also has a history of use as a source of vegetable oils and other products, but its most important value to the oil palm industry is its capacity to hybridize with the African oil palm [1]. The interest in the germplasm of this species is due to valuable characteristics for breeding programs of the African oil palm, such as slow growth, oil quality (mainly unsaturated oil) [54], and disease resistance, including FY [47].

These two species can sexually cross and generate fertile interspecific hybrids with intermediate characteristics to the two parental species [55]. Some interspecific hybrids between these species are already commercially available, and the Brazilian genetic group of *E. oleifera* is parental to most of them — Manicoré (BRS Manicoré from Embrapa, and [Mangenot × Manicoré] × La Mé from PalmElit SAS), Manaus (Amazon from ASD Costa Rica), and Coari (Coari × La Mé, Coari × Yangambi) [47].

Independent whether the origin of FY is biotic or abiotic, or a combination of both, once it is finally known, new studies will be necessary to confirm this genetic resistance and gain insights on possible strategies to transfer this resistance to the African oil palm more efficiently and effectively, besides the use of interspecific crosses followed by backcrosses.

5. The search for the causal agent

5.1 Biotic stress

5.1.1 Insects

After the epidemiological explosion of FY in 1986, Embrapa (the Brazilian Agricultural Research Corporation) started conducting studies on insects as a possible vector of the FY causal agent [8]. As the spread of the disease followed the direction of the prevailing winds, while natural barriers - such as roads, rivers, and glades - were not sufficient to prevent it supported this hypothesis [8, 56]. This hypothesis on a possible entomological role in the spread of FY also resided in the fact that this disease has similarities with the lethal yellowing-type disease that affects other palms. This disease that affects several other palms is due to insect-transmitted phytoplasmas [57]. Initially, from inventory obtained in plantations affected by FY in the municipalities of Alvaraes, in the Amazonas State, and Benevides, in the Pará State, the main insects suspected of being responsible for the transmission corresponded to *Persis* sp. and *Myndus crudus* because they are commonly found in oil palm plantations and depend on palm oil for nutrition [15].

Initially, an inventory of insects captured directly on the oil palm plantations located inside and outside areas with FY occurrence was generated. Healthy oil palm plants, isolated in cages made of wood and nylon canvas, received populations of the inventoried insects, and the plants monitored for symptoms appearance. After using almost one million insects in the FY transmission test, no symptomatic plant appeared, and there was no relationship between the affected areas with the collected insect fauna [15, 58]. Additional studies have attempted to establish a link between the insects *Contigucephalus* sp., *Omolicna* sp., and *Myndus crudus* and this disease, but they all gave negative results. Consequently, the authors discarded a Homoptera as the FY vector and suggested new studies on possible very active and rare insect species [8, 56].

Another study attempted to investigate the relationship between the presence of homopterans in the vegetation cover in oil palm plantations and the occurrence of FY [12]. No relation between the vegetation cover and FY occurrence appeared as the disease manifested itself either in an area covered with *Pueraria* spp. as in areas where there were grasses, especially *Brachiaria* spp. [25]. Studies using a series of chemicals in areas where FY occurs - such as insecticides, fungicides, and bactericides - did not reduce the appearance and development of FY [40].

5.1.2 Phytoplasmas

Phytoplasmas are prokaryotes of the Class Mollicutes that cause diseases in several plant species, including several economically important ones [59]. As biotrophic parasites, they colonize the elements riddled with the phloem and can also be found inside the vectors [60]. These organisms are responsible for Lethal Yellowing (LF), a fatal disease that affects the coconut (*Cocos nucifera* L.) and at least 36 other palm species in the Americas [61, 62].

Insects from the Homoptera order, popularly known as leafhoppers, are the vectors for most phytoplasmas causing disease in plants [63]. Biological characteristics, symptoms, and specificity of the insect vector were the focus of the first studies aiming to associate phytoplasmas with plant diseases [64, 65]. Later, new and more accurate DNA-based techniques started to dominate these studies, leading to the production of specific oligonucleotides for diagnosis [65].

Transmission electron microscopy was, for many years, the tool used for the detection and study of the cytological interaction between phytoplasmas and the hosts [66]. Studies using this tool were not successful in associating phytoplasma with FY, been replaced by new molecular techniques for the same purpose [8]. Studies carried out by Brioso et al. [67, 68] using nested-PCR in oil palm plants symptomatic for FY found just a very few samples positives for the presence of phytoplasmas from the SrI and 16SrI groups, which do not allow to associate these phytoplasmas to FY. An attempt to reproduce the disease was carried out by grafting intermediate leaf tips with FY into healthy seedling petioles and, during the period of two years, healthy individuals did not show symptoms characteristic of FY and, thus, the hypothesis proposing phytoplasma as the causal agent was discarded [12].

5.1.3 Fungi, bacteria, and nematodes

In the attempt to establish a causal relationship between plant pathogenic fungi, bacteria, and nematodes with FY, some studies tried to reproduce the symptoms in healthy young and adult oil palm plants inoculated with some of these microorganisms previously isolated from symptomatic plants [69, 70].

A pathogenicity test focused on studying the growth, reproductive and developmental habits of microorganisms, included one-year-old nursery plants with individual inoculations and a mixture of three fungi (*Fusarium* sp., *Pythium* sp., and *Coprinus* sp.) isolated from symptomatic plants; and again, the inoculum was unable to reproduce the disease in healthy oil palm trees [69]. The possibility of mechanical transmission between symptomatic and asymptomatic individuals by some microorganisms was also tested, with no success [69]. The chemical control attempts using fungicides or antibiotics failed to link fungi and bacteria to FY in oil palm [11].

Interestingly, some authors have observed similarities between the disease PC in Colombia and FY in Brazil. Furthermore, the oomycete *Phythophtora palmivora* was reported to be the PC causal agent [7]. The strategy used by Martinez et al. [7] was to remove tissue from oil palm plants exhibiting early symptoms of PC disease to inoculate fruit traps. Once microbial growth was observed in the fruits, tissue was transferred to culture media and pure cultures were obtained. Using the DNA isolated from the pure culture, amplification of the ITS region was performed and sequence analysis showed 99.9% homology to *P. palmivora*. The same study reported pathogenicity tests where sporangia were inoculated into the base of the spear of 150 oil palm nursery plants. After 3 to 4 days, the first symptoms of PC were observed in 85% of the plants [7]. However, full PC symptom development occurred in 15% of inoculated oil palm plants, and depended on environmental conditions. In another experiment, 20 immature spear leaves were inoculated with *P. palmivora,* and 3 days later all tissues were disintegrated, displaying a characteristic odor. Microscopy experiments showed the presence of *P. palmivora* in these tissues, and it was re-isolated using the fruit trap technique.

Nematodes are typically wormlike invertebrates able to live in the soil or inside plant structures such as roots, stems, leaves, and flowers and can cause morphological and developmental changes in their hosts [71]. The hypothesis of a nematode as a causative agent of FY came from observations of FY and the red ring disease - caused by the nematode *Bursaphelenchus cocophilus* - in the same area. Ferraz [72] did not observe this nematode in necrotic tissues or young leaves. Some studies found nematodes in the spear leaf rake and young leaves of symptomatic plants and the soil of oil palm plantations with a history of FY but were unable to link it to the appearance of this disease [24, 72].

5.1.4 Viruses and viroids

Other plant pathogens studied as potential causal agents of FY in oil palm were viruses and viroids. Several methods, including mechanical transmission, grafting, pollen-mediated dispersion, transmission electron microscopy, nested RT-PCR, RCA - rolling circle amplification, and electrophoresis, were used to test the hypothesis of a virus or a viroid as the causal agent of FY, without success [8, 10].

Lin et al. [73] evaluated extracts from plants with and without FY using the polyacrylamide gel electrophoresis technique, and the band patterns generated in both samples did not reveal any apparent difference. The same author also carried out a study to purify virus particles via separation with a fractional density gradient with no success [74]. Kitajima [75] evaluated ultrafine tissues from roots, leaves, and spear leaf of symptomatic and asymptomatic individuals by transmission electron microscopy, but no pathogen could be associated with FY.

Other studies have directed their efforts towards viroids, which are the smallest known phytopathogens, consisting basically of a single-stranded, circular RNA molecule not encapsulated [76, 77]. Beuther et al. [13] searched for viroids and viroid-like RNAs in oil palm plants using two-dimensional gel electrophoresis and return gel electrophoresis of nucleic acid extracts, with no success in showing a link between this type of pathogen and FY.

5.2 Abiotic stress

The initial pieces of evidence of a possible abiotic cause for FY came from observations made about the indefinite dissemination pattern in affected areas, with an exponential growth form not observed in the case of biotic stresses [78, 79]. Among the possible abiotic causes linked to the appearance of FY, there are lower and higher amounts of water, high or low temperature, high content of soluble salts in the soil, soil pH unsuitable for oil palm, nutritional deficiencies or excesses, presence of toxic organic compounds and intensity and balance of nutrients [78].

The regions with oil palm plantations and FY occurrence located in the North region of Brazil have soils with patches of quartz sand interspersed with patches of lateritic concretions and are subject to prolonged floodings, 5 to 6 months per year [41]. Thus, studies started aiming to understand the composition of the soil and its influence on FY emergence.

The concentrations of Cu, Fe, Mn, and Zn in the leaves of healthy and symptomatic oil palm plants and resistant interspecific hybrids were determined and found out that their concentrations were below the ideal range, suggesting their involvement in the appearance of FY [80]. Compact soils that stay temporarily saturated by rainfall suffer oxidation by anoxia, making it impossible for plants to absorb Fe [80]. Based on these observations, applications of ferrous sulfate were carried out on plants under different stages of FY, but after 120 days of the experiment, there was no regression of the disease in the evaluated oil palms [80].

The physical properties of the soil from areas with the occurrence of FY revealed that they were naturally well-drained and deep but had a thickening or compacting between the depths of 30 cm and 60 cm, as well as the occurrence of speckles in this depth, which results in soil saturation in the superficial layer during the rainfall season [81]. Bernardes [82] carried out chemical analysis on roots of symptomatic plants, and the results did not allow to pinpoint any element imbalance that could be responsible for FY. Another fact that needs consideration as possibly linked to a potential cause for the disease is the fact that at the moment when the first symptoms appear in the aerial part, the root system is severely impaired, which explains the plants' lack of response to fertilization and other interventions [82].

A series of field observations made in the heart of the oil palm production area in Brazil led to new hypotheses for a possible abiotic cause for FY [83]. The main field observations taken into consideration were: a higher occurrence of flooding in oil palm plantations, in comparison to the previous level, observed under native vegetation cover; the layers close to the soil surface without vegetation cover or with oil palm tend to stay close to water saturation for periods much longer than in the native forest; the presence of mottled-iron reduction in the profile of the oil palm plantations, and the redox-potential values (Eh) below −200 mV; and the presence of reduced iron ions on the soil surface in oil palm plantations during periods of intense rain [83].

The new hypotheses were brought together and summarized as: Deficient aeration reduces the potential for oxy-reduction in the soil, causing changes in the ionic composition of the soil solution (reduction of Fe^{3+} ions; NO^{3+}; Mn^{3+}). The soil solution with a high concentration of reduced ions initially causes damage to the root system (**Figure 3**) predisposing the oil palm plant to physiological disturbances (passive poisoning and attacks of secondary pathogens) whose symptoms are known as FY [84].

To gain insights into the idea of oxygen deficiency (hypoxia) in the origin of FY, a study by Encinas [85] evaluate the influence of land use and temporal variations on the dynamics of nutrients in the solution of soil and water at an oil palm plantation and a nearby area still with primary forest. Another by Muniz [83] compared the changes in water flow at an oil palm plantation and a nearby area still with native vegetation cover and evaluated its effects on iron dynamics and the structure of the soil. These two studies gathered additional shreds of evidence to further support this hypothesis, such as the electrical conductivity increased during a long flooding period (95 days), indicating that ions from the aggregates migrate to the solution; the soil pH increases after the initial flooding period, reaching values close to neutrality, with a subsequent reduction, but above the values found in aerated soil; the soil redox potential decreases during the flooding period, forming a highly reducing environment; the total carbon contained in the macroaggregates reduced

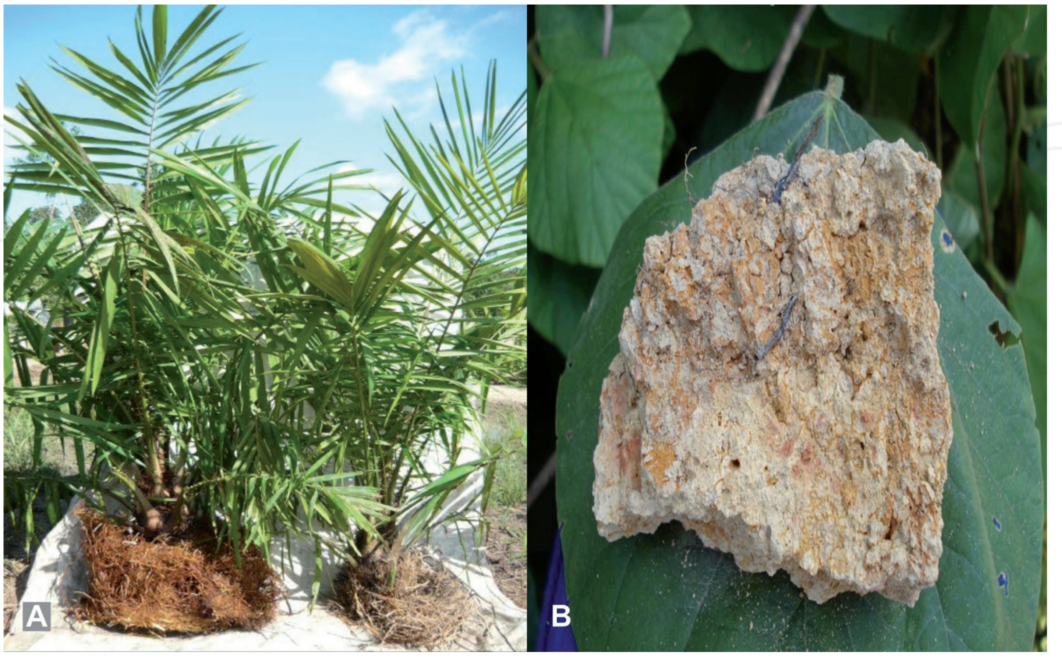

Figure 3.
Oil palm plant showing reduction of the root system in hypoxia conditions (A), and soil clouds showing the typical reductimorphic or oximorphic color mottles caused by stagnating soil environment (B). Source: Wenceslau Teixeira.

after flooding for a period of 11 days; the iron contained in the aggregates of Yellow Latosols with medium texture migrates to the soil solution under flooding conditions; there is a high negative correlation between the iron in the flooding solution and the DMG of the aggregates in the Yellow Latosols, and flooding for a period of 11 days promotes the destabilization of aggregates of Yellow Latosols with medium goethite texture.

6. New technologies to gain insights on the FY causal agent

The so-called 'omics' techniques (**Figure 4**) provide new opportunities to study oil palm FY. To get insights on FY possible causal agent, different research groups in Brazil have used metagenomics, metabolomics, and proteomics analysis [20–22]. To our knowledge, no work focusing on transcriptomics and FY has been published yet. The most commonly used approach in these studies is to compare healthy plants (without symptoms of FY) to those showing disease symptoms at different stages of progression. In contrast to more traditional non-molecular studies of FY, these techniques provide a global glimpse of the organism by looking at the associated microbiota (metagenomics), the complete protein content (proteomics), or metabolite content (metabolomics) of cells.

6.1 Metagenomics

Koch's postulate was fundamental to the identification of disease-causing microorganisms [86]. In short, the strategy of isolating and cultivating the potential pathogen, and inoculating it into a healthy organism to confirm the symptoms of the disease, brought many advances to the study of infectious diseases [87]. More recently, due mainly to the advent of next-generation sequencing (NGS) technologies, the frontiers of microbiology expanded to those microorganisms that we cannot cultivate by classical microbiology techniques. That has opened the possibility to test the hypothesis that a microorganism not grown in vitro easily is the cause of FY [88]. If this is the case, metagenomics would be the technique to study FY.

Metagenomics is a culture-independent approach to study microbial communities. A metagenomics strategy allows one to skip the step of isolation and cultivation of microbial species. Metagenomics studies can contribute to elucidate the identity

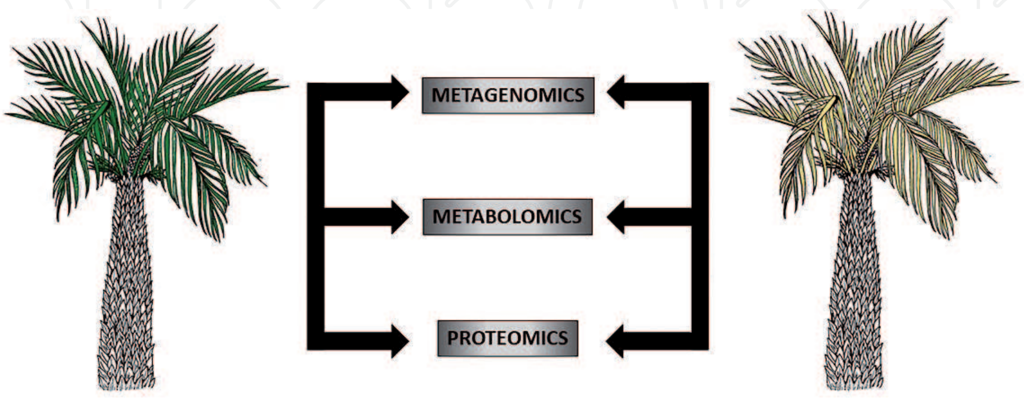

Figure 4.
Schematic showing a healthy oil palm tree (green leaves) and another one (yellow leaves) showing fatal yellowing (FY) symptoms. Different molecular techniques such as metagenomics, metabolomics and proteomics can be used to compare these contrasting biological situations. Metagenomics is a culture-independent technique that can be used to identify the microorganisms present. Metabolomics can used to identify and quantify cellular metabolites. Proteomics allows the identification of differentially expressed proteins. These 'omics' techniques are important high throughput tools that have been used to understand the biology of oil palm when challenged by FY disease. (credit: Clarissa Kruger).

and/or the genetic and metabolic capabilities of the microorganisms present in a sample, including any that are potentially pathogenic [89].

In this sense, metagenomics complements the classic techniques of isolation and cultivation of microorganisms, and one can apply it to study different classes of microorganisms (e.g., viruses, bacteria, fungi, archaea) [22, 90–92]. Metagenomics protocols begin with the extraction of total DNA from the sample of interest, which contains microorganisms. Samples can be many different ones, such as soil or plant parts with FY disease symptoms. There are distinct ways to study the microbial community from this DNA. Many studies in different plants use the ribosomal RNA (rRNA) gene or ITS amplification approach (i.e., PCR amplification with specific primers) to identify the microorganisms present, including a potential pathogen [93–95].

16S rRNA gene-specific primers amplify bacterial and archaeal sequences (16S rDNA). Similarly, the 18S rRNA gene and the ITS-specific primers amplify fungal sequences. The ITS refers to the internal transcribed spacer, the DNA situated between the small-subunit ribosomal RNA and large-subunit rRNA genes. The 16S rDNA, 18S rDNA, and the ITS regions are highly polymorphic, thus allowing taxonomical identification of the microorganisms present in a sample. The PCR-amplified DNA is then sequenced and submitted to bioinformatics analysis to compare the obtained sequences with sequence databanks, leading to a putative microorganism. In summary, this metagenomics approach that combines PCR amplification with NGS allows identifying microorganisms present in the community [96].

The first metagenomics work to use ITS amplification and high throughput NGS to study FY in Brazil was performed by Costa et al. [22], who evaluated fungal communities associated with leaves of oil palm plants, with and without symptoms of FY. Leaves from health plants and from plants showing FY symptoms in three different disease stages (stages 2, 5, and 8) were obtained. Because of the similarities between PC and FY, using primers specific to the genus *Phythophtora,* the authors attempted PCR-amplification of oil palm leaf samples showing symptoms of FY. Weak amplification was obtained in only one sample. Thus, this study provided preliminary evidence that DNA of the genus *Phytophtora* may not be commonly present in Brazilian FY, contrary to what has been reported in Colombia [7]. However, further experiments with more samples, and additional controls are needed to clarify the validity of this initial observation.

The Costa et al. [22] study reported the analyses of fungal diversity using the ITS region. Results showed that the fungal community in different healthy asymptomatic oil palm leaves are more similar to each other than those presenting FY disease symptoms. The fungal communities were not the same among all the symptomatic samples, and were not consistent even between samples at the same stage of FY disease. Importantly, no fungal taxon had its relative proportion increased in leaves across all the FY diseased oil palm plants. It was hypothesized that the changes observed in the fungal community composition could be a secondary effect of FY disease. Similar metagenomic studies to analyze the viral, bacterial and archaeal communities associated with FY are needed.

A less common metagenomic approach that can also be used to study plant disease is to assemble genomes from the metagenome obtained from plants showing symptoms of disease. In this case, instead of using PCR to amplify a specific gene, one can completely sequence the DNA extracted from the samples of interest, and use bioinformatics tools to assemble genomes (metagenome-assembled genomes) of the microorganisms present. This type of methodology allows, in addition to identifying microorganisms present, access to their genomes. This creates the possibility of studying the genetic relationship among the species present, and predicting metabolic capabilities as well as the interactions between the organisms of the community [97]. One limitation to this method, however, is that the plant host genome sequence needs to be available and subtracted *in silico* from microbial community sequences. If possible, it is useful to find a way to selectively extract microbial DNA from the samples before sequencing to avoid or reduce the presence of the plant host DNA [98]. It should be noted that if the complexity of the microbial community is high or if a lot of host DNA is present in the sequenced samples, inadequate sequencing depth might be an important limitation to this method. To our knowledge this approach has not been used yet to search for the causal agent of FY.

6.2 Proteomics and metabolomics

Proteome designates the set of proteins expressed by a cell, tissue, or organism at any given time [99]. Proteomic tools make it possible to obtain a protein profile with precision and sensitivity with the aid of electrophoresis, chromatography, mass spectrometry, and bioinformatics [99]. Proteomics is more and more used nowadays to understand plant responses to different biotic and abiotic stress conditions [100, 101].

In this context, and based on the hypothesis that the primary stress behind FY was abiotic and present in the soil, proteomics was applied to study this disease [21]. This hypothesis is based on observations regarding symptoms seen in the root system before they appeared in the aerial part [83]. Soil compaction, which hinders drainage and subject the roots to long periods of flooding in a hypoxia condition, would be in the origin of the stress [83].

Nascimento et al. [21] carried out a proteomic analysis to compare the protein profiles from symptomatic and asymptomatic oil palm plants, employing the mass spectrometry technique. The study looked for proteins linked to tolerance induction to relate the different areas collected and the distinct stages of the disease, analyzing the roots of symptomatic plants in early, intermediate, and final stages.

Proteins involved in the metabolism of phenylpropanoids and lignins, with a recognized role in reducing the effects of biotic and abiotic stress, were negatively regulated in symptomatic individuals, aggravating FY symptoms. In asymptomatic plants, enzymes such as S-adenosylmethionine - with a crucial role in methionine's biosynthetic metabolism - showed a recognized action in response to the stress. Plants with FY symptoms showed some pathogen-related proteins positively regulated, implying a progression of infection by biotic agents [21].

The hypothesis of a possible physiological dysfunction caused by factors present in the soil was reinforced by the large accumulation of antioxidant proteins in asymptomatic individuals [21]. The participation of the antioxidant system may indicate some level of resistance, considering that this system is vital for plants in conditions of soil flooding [102]. In addition, the accumulation of aldehyde dehydrogenase may indicate that the root system is under an anaerobic condition as it converts the acetaldehyde, promoting plant survival in this condition [21, 103]. Thus, these results indicate that plants affected by FY are in abiotic stress conditions and, with the damages done to the roots, it becomes a gateway for several opportunistic organisms [21].

In contrast to proteomics, metabolomics refers to a comprehensive analysis to identify the set of metabolites present in a sample with the aid of analytical techniques, such as liquid chromatographies or liquid–gas, associated or not with mass spectrometry, among others [104].

Rodrigues-Neto et al. [20] performed the first metabolomics work to study FY in Brazil using an untargeted metabolomics strategy to prospect metabolites differentially expressed in the leaves of FY symptomatic and asymptomatic plants. A high throughput method based on metabolic fingerprinting MS, using UHPLC coupled to high-resolution mass spectrometry (HRMS), was employed, and chemometric analysis, PCA and PLS-DA, were used to evaluate metabolic differences. This study aimed at prospecting a biomarker for FY early diagnosis, besides gaining insights on pathways responsive to this disease valuable for future improvement studies.

Nine secondary metabolites were detected in a higher concentration in the healthy plants in comparison to the FY affected ones: Glycerophosphorylcholine, arginine, asparagine, paniculatin or apigenin 6,8-di-C-hexose, tyramine, Chlorophyllide, 1,2-dihexanoyl-sn-glycero-3-phosphoethanolamine, proline, malvidin 3-glucoside-5-(6″-malonylglucoside) or kaempferol 7-methyl ether 3-[3-hydroxy-3-methylglutaryl-(1–> 6)]-[apiosyl-(1–> 2)-galactoside]. These metabolites made possible to identify different metabolic pathways that have been affected by the FY, such as the glycerophospholipid metabolism, the isoquinoline alkaloid biosynthesis, the flavonoid biosynthesis, the tetrapyrrole biosynthesis and citrate cycle derivatives pathways.

Unfortunately, due to the fact that these metabolites are already described in the literature as linked to other types of stress, they are not good candidate for biomarkers; except for two of them, glycerophosphorylcholine and 1,2-dihexanoyl-sn-glycero-3-phosphoethanolamine [20].

7. Final considerations

Fatal yellowing disease represents a threat of great magnitude to the Brazilian oil palm industry. For decades, several studies attempted to identify its causal agent without success. As a result, no measures used today can effectively reduce the economic loss for the oil palm industry due to this disease. The only glimpse of hope in solving this problem still resides in the genetic resistance found in the American oil palm. However, the road to transfer this resistance through interspecific crosses and backcrosses is very long and has many uncertainties.

The search for the primary stress leading to FY must go on, whether it is of biotic or abiotic origin - or the combination of both. Only then might be able to block its occurrence, or, if not possible to do that, develop early diagnostic tools to reduce its spread to a minimum.

Recent studies using single omics analysis have shown that these new technics can take the etiological studies regarding FY in oil palm to another level. We postulate that transcriptomics should be the next step in using omics to gain further insights regarding this disease. Even more, we believe that it should be done under the scope of a multi-omics integration (MOI) strategy, together with metabolomics, proteomics, and ionomics, at least.

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Conflict of interest

The authors declare no conflict of interest.

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