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Phytopathology™ REVIEW

Chocolate Under Threat from Old and New Cacao Diseases

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ABSTRACT

Theobroma cacao, the source of chocolate, is affected by destructive diseases wherever it is grown. Some diseases are endemic; however, as cacao was disseminated from the Amazon rain forest to new cultivation sites it encountered new pathogens. Two well-established diseases cause the greatest losses: black pod rot, caused by several species of *Phytophthora*, and witches' broom of cacao, caused by *Moniliophthora perniciosa*. *Phytophthora megakarya* causes the severest damage in the main cacao producing countries in West Africa, while *P. palmivora* causes significant losses globally. *M. perniciosa* is related to a sister basidiomycete species, *M. roreri* which causes frosty pod rot. These *Moniliophthora* species only occur in South and Central America, where they have significantly limited production since the beginnings of cacao cultivation. The basidiomycete *Ceratobasidium theobromae* causing vascular-streak dieback occurs only in South-East Asia and remains poorly understood. Cacao swollen shoot disease caused by *Cacao swollen shoot virus* is rapidly spreading in West Africa. This review presents contemporary research on the biology, taxonomy and genomics of what are often new-encounter pathogens, as well as the management of the diseases they cause.

Keywords: disease control and pest management, mycology, virology

The international trade in chocolate is valued at USD 103 billon per annum (Zion Market Research 2018). The main ingredient in chocolate is seed (beans) from the fruit of *Theobroma cacao* L., an understory tree domesticated by indigenous people in the Amazon about 5,300 years ago (Zarrillo et al. 2018). Cacao production represents the major source of income for millions of small farmers worldwide. Up to 38% of the annual global cacao harvest (4.7 million metric tons in 2017) (ICCO 2017) (Table 1) is lost due to diseases. Some of the causal agents, such as *Phytophthora palmivora*, are globally distributed but others, such as *P. megakarya* and *Cacao swollen shoot virus* in West Africa, *Moniliophthora perniciosa* and *M. roreri* in Latin America and the Caribbean, and *Ceratobasidium theobromae* in Southeast Asia and the Pacific, have geographically restricted distributions (Table 1). Human activities, whether intentional or accidental, pose the biggest threat for the spread of these pathogens. Additionally, threats that are presently localized or have minor impacts may become worse with climate change or in new environments. This review describes contemporary research on black pod rot, witches' broom, frosty pod rot, vascular steak dieback, and cacao swollen shoot virus, old and emerging threats to cacao cultivation.

BLACK POD ROT, STEM CANKER, LEAF, AND NURSERY BLIGHTS

Background. Black pod rot, stem canker, leaf and nursery blights, caused by species of *Phytophthora*, are responsible for greater losses than any other disease of cacao. The two major species causing disease on cacao, *P. palmivora* and *P. megakarya*, originated outside the cacao center of diversity, and are thus "new encounter" pathogens. *P. palmivora* (E. J. Butler) E.J. Butler 1919

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has several hundred recorded hosts and is of universal importance in cacao, causing annual global yield losses up to 20 to 30% and tree deaths of up to 10%, although individual farms in wetter cacao-growing areas may suffer total loss (Drenth and Guest 2013). *P. megakarya* Brasier & M.J. Griffin 1979 is the most important cacao pathogen in Central and West Africa, frequently causing total loss of pods due to the large number of zoospores the species produces (Akrofi et al. 2015; Bailey et al. 2016); it is endemic to Equatorial Guinea, Gabon, Cameroon, Togo, Nigeria, and Ghana, and is still in an invasive phase in neighboring Côte d'Ivoire. Pod rots caused by *P. capsici* Leonian 1922 and *P. citrophthora* R.E. Sm. & E.H. Sm. Leonian are considered common in Central and South America and may cause significant losses under favorable environments.

Taxonomy. *Phytophthora* is a waterborne heterokont stramenopile related to the chlorophyll c-containing algae (Surujdeo-Maharaj et al. 2016). Although both mating types of *P. palmivora* and *P. megakarya* are present in some production areas, clonal populations usually affect cacao (Maora et al. 2017; Mfegue et al. 2012).

P. megakarya and *P. palmivora* are closely related among the *Phytophthora* spp. causing diseases in cacao, and are both members of clade 4 of the 10 clade system based on the internal transcribed spacer (ITS) sequences of genomic rDNA commonly used in the genus (Cooke et al. 2000). Ali et al. (2016) developed a PCR primer-based protocol for distinguishing the species. This can be critical since *P. megakarya* is considered more damaging to cacao justifying greater efforts in its management (Akrofi et al. 2015; Bailey et al. 2016). This technique however has proven difficult to reproduce. Although genetic diversity exists among isolates of *P. megakarya* in Africa and *P. palmivora* around the world (Ali et al. 2016; Djeumekop et al. 2018), the consequences of this variability in the disease process are poorly understood.

Biology. The infection cycle of *Phytophthora* spp. on cacao is complex due to the number of tissues affected and the existence of numerous infection routes (Fig. 1). At the beginning of the season, infection occurs from primary inoculum present in soil and plant parts, where it survives the dry season. Multiple infection foci appear simultaneously, often in the same spot as the previous year (ten Hoopen et al. 2010).

In young trees, soilborne inoculum must be dispersed into the canopy where it causes the most conspicuous damage. Early investigations identified rain splash, ants and insects, rodents and cultural practices as means by which inoculum reaches into the canopy (Ristaino and Gumpertz 2000). Pod-boring insects that breed in piles of pod cases on the ground have also been identified as vectors (Konam and Guest 2004).

In the canopy, primary inoculum (mycelium and chlamydospores) survives in infected stems, flower cushions, leaves, alternate hosts, and pod mummies (Fig. 1) (Opoku et al. 2002; Purwantara 2008). Sporangia that develop under wet conditions can each release 30 to 40 zoospores that can encyst, germinate and initiate new infections within 1 h (Drenth and Guest 2004; Sarria et al. 2016). Alternatively, in the absence of water, sporangia can germinate directly (Clerk 1972).

Germ tubes penetrate the host, either via the cuticle and epidermis following the formation of appressoria, or through stomata (Fig. 1) (Ali et al. 2016). Hyphae spread throughout the plant tissue and secondary inoculum is produced within 3 to 5 days. Pods rot completely within weeks and mummify in the canopy, providing an inoculum reservoir that remains viable for several years and initiates new epidemics in rainy seasons (Surujdeo-Maharaj et al. 2016). Stem and branch cankers reduce productivity and occasionally kill the tree. Root infections may not be economically important but are an inoculum source (Akrofi et al. 2015; Opoku et al. 2002). Interestingly, black pod incidence increases with the number of interplanted non-cacao trees (Gidoin et al. 2014).

Long-distance dispersal occurs through the movement of contaminated soil, flood, and river water, pruning tools, and infected pods (Djeumekop et al. 2018; ten Hoopen et al. 2010). In new plantations, secondary inoculum is most significant, as new infections occur near diseased trees. Primary inoculum becomes established throughout the plantation over the years, leading to more disease foci appearing at the beginning of the season in older plantations (Nkeng et al. 2017).

Genomics and molecular pathology. Most recently, draft genomes for *P. megakarya* and *P. palmivora* have been released (Ali et al. 2017). These assemblies included only Illumina sequence and significant changes seem likely once long read technologies are applied due to their obvious complexity and large size. Currently the *P. megakarya* genome is estimated at 126.9 Mbp and the *P. palmivora* genome is estimated at 151.2 Mb. Both genomes carry large gene numbers (42,036 and 44,327 for *P. megakarya* and *P. palmivora*, respectively), compared with other *Phytophthora* species, a clear indication of their apparently complex genome compositions. *P. megakarya* shows evidence of large scale gene amplification by retroelement activity and *P. palmivora* by whole genome duplication. In addition, both species were found to harbor large sets of gene family members typically associated with the plant–pathogen interactions: pectinases, proteases, elicitins, Crinklers, necrosis-inducing proteins (NLPs), RXLRs. Most importantly, the species were found to carry the largest set of RXLR effectors of any *Phytophthora* species known to date: 1,181 in *P. megakarya* and 991 in *P. palmivora*. Machine learning techniques grouped *Phytophthora* genes into 24 classes based on their expression patterns in zoospores, in vitro-grown mycelia, and in planta expression. The pectinases, NLPs, and RXLRs showed a strong preference for enhanced expression in zoospores and/or in planta compared with crinklers and elicitins which were more divergent in

TABLE 1
Losses caused by the major cacao diseases between 2001[a] and 2016

Disease	Annual losses in 2001 (×1,000 metric tons)	Annual losses in 2016 (×1,000 metric tons)[b]
Phytophthora spp.	450	873
Witches' broom (*Moniliophthora perniciosa*)	250	492
Cacao swollen shoot virus	50	96
Vascular streak dieback (*Ceratobasidium theobromae*)	30	61
Frosty pod (*Moniliophthora roreri*)	30	76
Cocoa pod borer (*Conopomorpha cremerella*)	40	81
Total	850	1,679

[a] From Bowers et al. 2001.

[b] Losses in 2016 were estimated based on the incremental production in the cacao producing regions affected between 2012 (Ploetz 2016) and 2016.

their expression patterns. As such, a large set of genes was identified needing further functional analysis for their importance in supporting disease establishment. Fortunately, transformation techniques are much better developed for *Phytophthora* including RNAi (Whisson et al. 2005) and CRISPR (Gumtow et al. 2018).

Management. Phytophthora diseases on cacao are managed using genotype selection, cultural, and chemical controls. Sanitation practices, including removal and composting or burying of diseased pods and pod husk piles, reduces the level of primary inoculum and insect vectors (Akrofi et al. 2015; Doungous et al. 2018). Phytosanitary harvests performed every 4 days can limit secondary inoculum (Fomba et al. 2018). Improved drainage and shade management through pruning, weeding, and spacing, increases ventilation and light penetration, reduces inoculum in humid areas, but has less effect in dry regions (Abdulai et al. 2018). Pod rot incidence has also been reduced using physical barriers such as plastic pods sleeves that prevent access by insect vectors and infective propagules (Ali et al. 2016; Rosmana et al. 2013). Pod rot and cankers are effectively managed using protectant sprays of copper-based products and systemic fungicides including annual trunk injections of phosphonates (McMahon et al. 2010). In terms of genetic resistance, a meta-analysis of previous published reports (Lanaud et al. 2009) identified 13 consensus quantitative trait loci (QTLs) for resistance to *Phytophthora* in cacao. Most recently, a study in Brazil identified 12 minor QTLs for resistance to *P. palmivora*, *P. capsici*, and *P. citrophthora* using a leaf discs assay (Barreto et al. 2018). Current research aims at finding the source of pod resistance using detached pod inoculations.

WITCHES' BROOM AND FROSTY POD

Background. The somewhat convoluted histories of witches' broom disease and frosty pod rot have been well documented (Bailey et al. 2018b; Evans 2002, 2016a, b; Evans et al. 2013). The

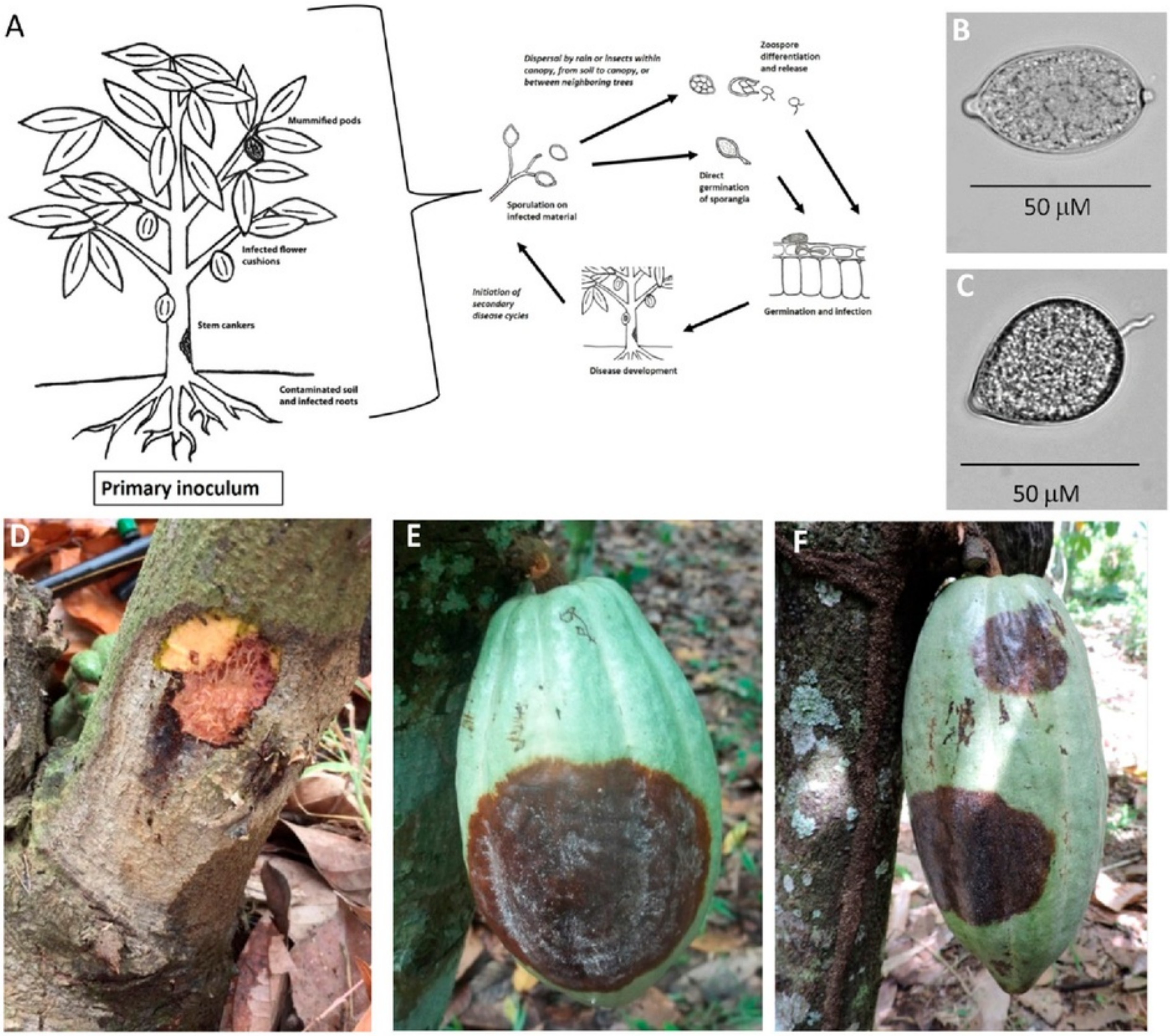

FIGURE 1

Phytophthora disease cycle and symptoms. **A,** Phytophthora disease cycle. **B,** Sporangium of *Phytophthora megakarya*. **C,** Sporangium of *P. palmivora*. **D,** Trunk canker caused by *P. palmivora* in Ecuador. **E,** Lesions on pods caused by *P. magakarya*. **F,** Lesions on pods caused by *P. palmivora*.

causal agents of these South American diseases of cacao are recognized as sister basidiomycetes in the genus *Moniliophthora*. Both diseases are still on an invasive front and pose an ever-present threat to unaffected regions in the Americas, as well as to those in the Paleotropics.

Taxonomy. *Moniliophthora* was erected to accommodate the frosty pod pathogen *Monilia roreri*, which had previously been considered an ascomycete (Evans et al. 1978). ITS and small mitochondrial rDNA sequences from *M. roreri* revealed an extremely close match with *Crinipellis perniciosa* (Evans et al. 2002). Aime and Phillips-Mora (2005) analyzed five nuclear gene regions and recognized that *M. roreri* and *Crinipellis perniciosa* are sister taxa within the Marasmiaceae, making the new combination *M. perniciosa*. More recently, it was recognized that the generic diagnosis was based solely on the purported asexual stage of *M. roreri*. As doubts still exist about the mitotic versus meiotic nature of sporogenesis in *M. roreri* (Bailey et al. 2018b; Evans 2016a), the genus was amended to accommodate taxa with agaricoid fruit bodies or basidiomata (Evans et al. 2013).

Several agaricaceous species from the Paleotropics have since been added to the genus, including three species from Southeast Asia (Kerekes and Desjardin 2009). There is also a more recent report of a new species, *M. aurantiaca*, from Polynesia (Kropp and Albee-Scott 2012), showing close alignment to *M. roreri*. Current research concentrates on analyzing the phylogeny of isolates that produce *M. perniciosa*-like basidiomata, collected from a range of hosts in Brazil, including species that are hosts to *M. perniciosa*–the Bignoniaceae, Malpighiaceae, Malvaceae, and Solanaceae (Fig. 2A and B).

A provisional phylogenetic tree (Supplementary Fig. S1) shows those isolates with *M. perniciosa*-like fruit bodies group in clades according to the plant-host families, with the isolates from the malpighiaceous host *Heteropterys acutifolia*, previously described as *Crinipellis brasiliensis* (de Arruda et al. 2005), as a distinct taxon. Sitting within or between these clades are isolates from unknown and symptomless liana hosts found in the litter or understory layers of tropical forests, often with large basidiomata (Fig. 2C and D). Most of the isolates from *Solanum* species group into a separate Solanaceae clade. In the forest tree, *Allophyllus edulis* (Sapindaceae), fruit bodies are produced directly from the main stem in regular flushes, without any symptoms of disease. This confirms the long-held opinion that some strains of *M. perniciosa*, possibly the ancestral types, exist as benign endophytes within woody plant hosts (Evans 2002, 2016b; Evans et al. 2013).

Biology. Both *M. perniciosa* and *M. roreri* are hemibiotrophs with well-defined and prolonged biotrophic phases associated with severe hypertrophy and hyperplasia of host tissues. A switch to necrotrophy follows changes in host nutrient levels (Evans 2016a, b). Basidiomata of *M. perniciosa* may take up to 12 months to develop on necrotic brooms, while the diagnostic snow-white pseudostroma and powdery spores of *M. roreri* develop rapidly on the pod surface following necrosis (Fig. 2E). There is increasing evidence that *Moniliophthora* is typified by taxa with an endophytic habit (Evans et al. 2013), and it has been suggested that both *M. roreri* and *M. perniciosa* act as vectors of an unidentified infectious microorganism, the causal agent of the growth disorders, within a tritrophic relationship (Evans 2016b; Evans et al. 2013).

Genomics and molecular pathology. One of the most interesting aspects of *M. perniciosa* biology is the transition from biotroph to necrotroph associated with the shift from haploid to dikaryotic mycelia (Calle et al. 1982; Meinhardt et al. 2006; Rincones et al. 2008; Teixeira et al. 2014). Of 433 genes differentially expressed in planta, candidate effector genes were identified in addition to many genes whose homologs had known potential for enhancing disease processes in other plant microbe interactions (Teixeira et al. 2014). The molecular signals involved in establishing infection and initiating the shift from biotrophy to necrotrophy remain elusive and speculation continues as to whether plant tissue death precedes, follows, or is even involved directly with this process.

Garcia et al. (2007) identified three copies of necrosis-inducing, Nep1-like (NLP) proteins in the *M. perniciosa* genome, one that is preferentially expressed during the biotrophic phase and another that is expressed during both phases; both have necrosis-inducing activity in cacao. Ceita et al. (2007) and da Silva et al. (2011) suggested that oxalate contributes to ROX production in NLP-induced necrosis, offering a potential novel source of resistance through the activity of oxalate decarboxylase. A second protein family with necrosis inducing potential, the cerato-platanins, has also been detailed in the *M. perniciosa* genome (de O. Barsottini et al. 2013; de Oliveira et al. 2011; Zaparoli et al. 2009). Gene sequences encoding at least five cerato-platanin-like proteins were identified, one of which caused necrosis on its own when produced transgenically and acted synergistically with MpNep2 (Zaparoli et al. 2009). Other potential functions of the *M. perniciosa* cerato-platanins were later detailed (de O. Barsottini et al. 2013; de Oliveira et al. 2011). The thaumatin-like protein encoding gene family is expanded in *M. perniciosa* with suggested functions in competition with other fungi and pathogenesis (Franco et al. 2015). *M. perniciosa* also carries a large gene family encoding pathogenesis related 1-related genes with several proposed functions including actions as virulence factors (Teixeira et al. 2012). The AOX-dependent respiratory pathway was shown to be critical to development of biotroph-like mycelia potentially regulating the biotroph/necrotroph shift (Thomazella et al. 2012). More detailed review of these and other *M. perniciosa* genes/gene families can be found in Mondego et al. (2016).

In *M. roreri*, the biotroph/necrotroph shift is not associated with the shift from haploid to dikaryotic mycelia found in *M. pernicisoa*, but is coordinated with the shift from green to necrotic pods (Bailey et al. 2018a). Although multinucleated cells have been observed in mycelia, they are not associated with spore formation in *M. roreri* (Bailey et al. 2018a). Spores and those cells associated with their immediate formation and germination are the only cells commonly observed to be multinucleated. Unlike *M. perniciosa*, the establishment of slow growing biotroph-like mycelia is common regardless of the growth media that is used. *M. roreri* propagates clonally (Bailey et al. 2018a; Díaz-Valderrama and Aime 2016; Mora 2003) and remains in a primarily haploid state throughout its life cycle.

In an initial study dissecting the molecular interaction between *M. roreri* and cacao, genes associated with transcriptional and translational processes or metabolite modification were shown to be repressed during the biotroph/necrotroph shift while genes associated with substrate breakdown, metabolite transport, glyoxylate cycle and plant and fungal cell wall modification were induced (Bailey et al. 2013). Meinhardt et al. (2014) highlighted secretome transcripts targeting modification of plant and fungal cell walls, many of which showed phase-specific expression. Both phases employ unique secretome gene sets, including many hydrolases, to modify and adapt to the intercellular environment during the long biotrophic phase and rapid necrotrophic phase shift. Bailey et al. (2014) found that when *M. roreri* attacked and successfully initiated infections on tolerant cacao clones in the field, gene expression shifted early in the process shortening the biotrophic phase and mimicking gene expression patterns normally observed in the necrotrophic phase. In tolerant genotypes, sporulation was limited.

A transcriptome study result indicated that Nep1-like proteins, cerato-platanins, Pr1-related proteins, thaumatin-like proteins, and hydrophobin-encoding gene families share similar numbers and expression patterns in *M. roreri* and *M. perniciosa* (Bailey et al. 2018b). In *M. roreri*, genes associated with the glyoxylate cycle (Bailey et al. 2013) and alternative oxidase (Bailey et al. 2014) pathways were somewhat specific to the necrotrophic phase.

FIGURE 2

Moniliophthora spp. life cycle and signs. **A,** Witches' broom of *Moniliphthora perniciosa* on a flower cushion of cacao. **B,** Witches' broom developing on inflorescence of liana host (Malpighiaceae), Mato Grosso, Brazil. **C,** *Moniliophthora perniciosa*-type basidiomata developing on main stem of *Allophyllus edulis* (Sapindaceae), 2 to 3 m above ground, in Atlantic rain forest, Minas Gerais, Brazil. **D,** *Moniliophthora perniciosa*-type basidiomata developing on necrotic broom of *Solanum swartzianum*, Rio de Janeiro State, Brazil. **E,** *Moniliophthora roreri*-infected pod, showing the snow-white pseudostroma diagnostic for frosty pod rot.

M. roreri carries a large set of candidate effectors, generally small secreted proteins high in cysteine targeted toward the apoplast or plant cell nucleus (Bailey et al. 2014; Barbosa et al. 2018). Unfortunately, direct comparisons between the two available transcriptomes have not been made. Detailed studies of individual effector candidates in *M. roreri* need to be conducted. Although transformation techniques have been developed for *M. perniciosa* (Lima et al. 2003; Lopes et al. 2008) including RNAi (dos Santos et al. 2009), efforts with *M. roreri* have so far failed.

Using the S-biotype strains of *M. perniciosa*, Marelli et al. (2009) developed a model system using tomato to accelerate the study of molecular host–microbe interactions. Since then, this model has helped understand how this pathogen alters the host metabolism and growth (Deganello et al. 2014; Pierre et al. 2017; Scotton et al. 2017).

Management. Currently, promising genetic material is being assessed for resistance to both witches' broom and frosty pod diseases (Bailey et al. 2018b; Evans 2016a, b). QTLs contributing to resistance to witches' broom disease or frosty pod rot have been identified (Lanaud et al. 2009; Royaert et al. 2016). However, short-term options are needed, particularly where the diseases are recent arrivals. In addition, action plans are needed for areas that are presently free of the diseases.

Undoubtedly, good crop sanitation throughout the season, with removing infected pods and brooms not only from the trees but from the cacao ecosystem, is the most effective way to reduce inoculum and the consequent development of disease (Evans 2002; Soberanis et al. 1999). However, this is labor intensive and often not cost-effective. A compromise recommendation is for a one time removal during the intercropping period restricting inoculum sources at the beginning of the next season delaying disease build-up and has proven to be particularly effective for *M. roreri* (Leach et al. 2002; Evans 2016a).

A little-explored option, at least for *M. roreri*, is biological control and, specifically, the use of coevolved natural enemies from the centers of origin or diversity of the pathogens. A study of the natural enemies of a variant of *M. roreri* occurring on pods of *Theobroma gileri* in the Chocó forest ecosystem in Colombia, has revealed a guild of invertebrates and mycoparasites not previously recorded from cacao plantations (Evans 2016a). Similarly, novel mycoparasites have been identified on witches' brooms collected from the natural range of cacao, in a region of Pará State (Amazonia, Brazil) with an unusually low incidence of the disease (Evans 2016b). *Cladobotryum amazonense* (Bastos et al. 1981) and *Trichoderma stromaticum* (Trichovab) (Samuels et al. 2000) have demonstrated potential as biocontrol agents against *M. perniciosa* (Samuels et al. 2012).

The recent report of *M. roreri* in Jamaica and the alarm that it caused at government level (Bailey et al. 2018b), and the socio-economic, as well as ecological chaos that ensued when *M. perniciosa* reached the Bahia region of Brazil (Evans 2002), demonstrate the fear that these pathogens provoke among cacao growers. That the latter outbreak resulted from the intentional introduction of the pathogen to Bahia (Evans 2007, 2016b; Evans et al. 2013) suggests that future events of agro- or bioterrorism with these or other cacao pathogens are possible (Caldas and Perz 2013).

VASCULAR-STREAK DIEBACK

Background. Vascular-streak dieback (VSD) was first identified in Papua New Guinea (PNG) in the 1960s and initially caused epidemic death of mature trees and seedlings. The disease was shown to be caused by a basidiomycete originally named *Oncobasidium theobromae*, now known as *Ceratobasidium theobromae* (Samuels et al. 2012).

By the 1970s the most susceptible genotypes had succumbed to VSD. VSD has since been described on cacao across most of South and Southeast Asia and PNG, from New Britain in the east to Hainan Island, China in the north and Kerala State, India, in the west, where it causes local yield losses up to 80% and regional losses of 14%. Global losses are estimated to be around 60,000 metric tons annually (Table 1).

Together with cocoa pod borer (*Conopomorpha cramerella*) (Table 1), VSD contributed to the decline of large commercial plantations in West Malaysia and Sabah. It is widespread in Indonesia, including in the fine flavor cacao plantations in East and West Java, and in the large newer cacao plantings in Sulawesi. Symptoms appear to be most severe at lower elevations (McMahon and Purwantara 2016).

The only known host other than cacao is avocado, which is also an exotic plant in Southeast Asia and the Pacific (Anderson 1989). It is believed that the fungus originated on an as yet unidentified indigenous host in Southeast Asia/Melanesia. Thus, VSD is another new encounter disease of cacao.

Biology. The most characteristic initial symptom of VSD is the chlorosis of a single leaf with scattered islets of green tissue 2- to 5-mm in diameter, usually on the second or third flush behind the shoot apex (Fig. 3A). Chlorotic symptoms typically appear in the early dry season. Affected leaves are shed within a few days and symptoms progressively develop in adjacent leaves up and down the stem, causing dieback. Lateral buds may proliferate and die, causing "broomstick" symptoms to develop. Atypical leaf symptoms of expanding necrosis beginning at the margins of leaves appeared, apparently simultaneously and without explanation, in many areas in 2004 (Guest and Keane 2007; McMahon and Purwantara 2016; Fig. 3B). Unlike the classic symptoms, abscission of necrotic leaves is delayed (Fig. 3C). Lenticels on infected trees usually become enlarged, causing roughening of the bark (Fig. 3D). Three blackened vascular traces are visible when the dry surface is scraped off leaf abscission scars, and dark streaks indicate blockage and necrosis of xylem vessels and parenchyma (Fig. 3E).

The fungus may spread through colonized xylem vessels to other branches and kills the tree if it reaches the main stem. For this reason, the disease is most damaging in seedlings. Only the most susceptible genotypes are killed by infections beginning in outer branches on older trees. The pathogen has typical *Rhizoctonia*-like, binucleate hyphae with dolipore septa and hyphal constrictions adjacent to right angled branches when growing in infected xylem vessels. Hyphae can be observed asymptomatically colonizing xylem vessels several centimeters beyond the visible streaking.

When an infected leaf falls during wet weather, hyphae emerge from the leaf scar on which to basidiophores develop, visible as white, flat, velvety coatings over the leaf scar and adjacent bark (Fig. 3D). Basidiophores associated with atypical symptoms also appear on cracks in the central vein and petiole of infected, attached leaves. Basidia develop after evening rainfall and basidiospores are forcibly discharged after midnight until after dawn but lose viability when exposed to morning sunshine. Basidiophores remain viable for about a week on attached branches, but only for a day or two on cut branches. Extended periods of wetness are required for basidiospore production, explaining the link between rainfall peaks, infection periods and the high rate of disease spread in regions where annual rainfall exceeds 2,500 mm.

Basidiospores germinate and hyphae penetrate unhardened leaves at branch termini by growing directly through the cuticle, above leaf veins (Prior 1979). While basidiospores are assumed to be uninucleate, infective hyphae are binucleate. It is not known when plasmogamy takes place. Infected leaves do not show symptoms for 3 to 5 months, by which time the pathogen has ramified through the xylem in the adjacent stem. This incubation period explains why disease symptoms often occur several months after infection during seasonal rainfall peaks and coincide with dry season drought stress (McMahon et al. 2018).

Pathogen population studies have been limited due to difficulties collecting samples, the near-obligate parasitism of the pathogen, and challenges in purifying high-quality pathogen DNA from infected plant tissue. Samuels et al. (2012) identified three lineages of the pathogen based on ITS haplotype analysis, one widespread across Indonesia and Malaysia and more restricted populations from Papua and Vietnam. Unidentified indigenous host(s) are believed to be the inoculum source in newly planted areas (Keane and Prior 1991).

Management. Because natural spread of *Ceratobasidium theobromae* is limited, quarantine measures that restrict human transport of the fungus effectively reduce long-distance spread of the disease. Plants should be clear of symptoms for at least 6 months before export and be monitored for a further 2 months after arrival and before distribution to farmers.

It is highly unlikely that live seeds or fermented and dried beans pose a threat to importing countries since the beans formed on the mature pod are not infected and there is no evidence the pathogen moves from vascular tissue to placenta and colonizes the bean. Extensive tests of seed collected from pods on infected branches have not demonstrated transmission of the disease through seed (Keane and Prior 1991). Mature pods rarely form on diseased branches, and when they do, colonization of the placenta or embryo has not been observed.

Very high levels of infection and death have been observed in plants raised near or under diseased cacao. Thus, nursery stock should be raised away from diseased cacao. Nursery stock can be further protected by growing under a shelter that keeps leaves dry for all but a few hours following watering. Budwood from infected branches does not form basidiocarps, and grafts prepared from infected budwood are unsuccessful thus it is highly unlikely that infection can spread through grafting.

Since the pathogen cannot be maintained in culture and field-collected spores are not always available, studies of resistance are difficult. Nonetheless, selection has occurred among seedlings in the heterogeneous, partly-out breeding Trinitario population of cacao and from hybrid progeny of Upper Amazon and Trinitario parents, each of which produce useful resistance (Epaina 2012; McMahon et al. 2018). These genotypes become infected but initiate an intense host response indicating incompatibility; pathogen growth is slower and restricted to smaller branches, sporulation is rare, and the resulting symptoms are less severe. Symptoms appear in the outer branches but rarely kill trees. Resistance is durable and inherited additively and quantitatively; however, VSD resistance must be bred into genotypes with good agronomic characteristics and tolerance to other pests and diseases

FIGURE 3

Symptoms of vascular-streak dieback (VSD) of cacao. **A,** Defoliation and "green island" symptoms appearing on several leaves behind the stem tip. **B,** Marginal necrosis and delayed leaf fall characteristic of atypical symptoms. **C,** Tree affected by VSD showing leaf yellowing, necrosis and dieback. **D,** Basidiophores of *Ceratobasidium theobromae* forming on leaf abscission scars and raised lenticels. **E,** Vascular streaking and necrotic vascular traces on leaf scars on a cacao branch affected by vascular-streak dieback.

(McMahon et al. 2018). In PNG, breeding with surviving genotypes has been successful, and VSD is of minor importance in most years.

Pruning about 30 cm below discolored xylem prevents further extension of infections and reduces inoculum levels by removing potential sporulation sites. In a mature planting in Java, highly trained teams detected and pruned out infected branches every 2 weeks for nearly 2 years, keeping the incidence of infected trees below 1%. In an unpruned planting, disease incidence increased from about 30 to 90% over 10 months (Soekirman and Purwantara 1992). Shade and canopy management to increase aeration and sunlight penetration is important as sporulation and infection require moist conditions. These practices are best integrated with other management practices designed to control *P. palmivora*, CPB, and other pests and diseases.

Systemic ergosterol biosynthesis inhibiting fungicides (DMIs), including flutriafol, hexaconazole, propiconazole, tebuconazole, and triadimenol, have been successfully used to control VSD under experimental conditions. However, none of these fungicides are commercially viable in established cacao plantations (Guest and Keane 2007) (D. Guest, *unpublished data*). The potential exists that epiphytic microbes may reduce leaf infection, endophytic fungi and bacteria may protect against vascular colonization, and that hyperparasites could be developed to target the basidiophore.

CACAO SWOLLEN SHOOT DISEASE

Background. The cacao swollen shoot disease (CSSD) was first reported in Ghana in 1936 (Steven 1936), then Nigeria (Murray 1945), Ivory Coast (Mangenot et al. 1946), Liberia and Sierra Leone (Attafuah et al. 1963; Posnette 1940; Steven 1936), and Togo (Oro et al. 2014). Although not reported in Cameroon, the close proximity to the CSSD-affected areas in Nigeria suggests that the CSSD is present there or will be in the near future.

The hallmark symptom of the disease is the unusual swelling of woody plant parts above and below the ground, including vegetative suckers sprouting from below, at, or above the base of the tree, the roots, and/or tree branches. The swollen shoot phenotype is highly variable with respect to extent of swelling, location, and shape, particularly when it occurs on shoots, which may be rounded on the end or show necrosis and death. Sometimes the swelling and necrosis is followed by bud-break below the necrotic tip, from which a stem will grow for some time before dying, stimulating the next lower bud to break (Fig. 4E and F).

Foliar symptoms of CSSD can be variable, persistent or ephemeral. However, they are associated with the new growth or "flush" leaves that develop during and subsequent to seasonal rainfall. Foliar symptoms include vein-clearing, red vein-banding, vein-etching, fern-like patterns, and green, pink, red, white mosaic and/or mottling (Fig. 4A to D). The most severe symptoms include the rapid decline and sudden death of trees and stems without foliar symptoms (Chingandu et al. 2017a).

Pods produced on infected trees are smaller in size, can be rounded instead of oval, and may develop discolored areas. Infected trees produce fewer pods and bean quality is reduced. Three to five years after symptom onset, trees show reduced vigor and decreased pod set, and often shed leaves, exposing the tree to sunburn, followed by slow or rapid decline and death (Adegbola 1975; Posnette 1950; Steven 1936) (Fig. 4).

CSSD is caused by a diverse set of badnaviruses. Prior to genomic sequencing, symptom phenotypes were used to differentiate severe, mild, and intermediate "strains" of these pathogens. These terms referred to differences in the severity of symptom development, with the most severe strains characterized as either causing foliar symptoms and stem swelling, with root swellings or shoot dieback (Brunt et al. 1964; Posnette 1940; Thresh et al. 1988). Unfortunately, whether the diverse range of CSSD symptoms is associated with host plant genotype, tree age, nutritional and/or environmental factors, or are specifically related to the virulence of a particular strain of the pathogen has not been resolved. Since Koch's postulates have been completed for only one species, *Cacao swollen shoot virus*, using an infectious clone (Jacquot et al. 1999), additional studies are required to link viral genotype to symptom type, distribution, and host genotype and age.

The lack of adequate diagnostic tools for studying the phylodynamics of the causal badnaviruses has limited the understanding of the epidemiology of CSSD. In addition, diverse cacao germplasm required for the development of resistant or highly tolerant germplasm has been generally unavailable outside of the center of origin of cacao (Padi et al. 2013). Recent breakthroughs in breeding programs established in West Africa may provide a temporary reprieve, but new germplasm is expected to become infected due to the ubiquitous distribution of infected endemic hosts (Posnette 1950; Tinsley 1971; Todd 1951), and the great potential for badnaviral species to evolve.

Taxonomy. CSSD-viral genome sequences indicate that a complex of badnavirus species are associated with this disease (Supplementary Fig. S2) (Chingandu et al. 2017a, b). The *Cacao swollen shoot virus* (CSSV), *Cacao swollen shoot cd virus* and the newly discovered *Cacao red vein virus* have been identified in Cote d'Ivoire and Ghana (Chingandu et al. 2017a, b; Kouakou et al. 2012), and *Cacao swollen shoot Togo A virus* has been detected in Togo (Muller 2015).

Cacao red-vein banding virus (CRVBV) has recently been described from cacao in Nigeria (Chingandu et al. 2019; Dongo et al. 2018), and it is likely that additional variants will be discovered in the future (J. K. Brown, *unpublished data*) (Muller et al. 2018). New surveys in West Africa, some utilizing next-generation sequencing (NGS) platforms for discovery and polymerase chain reaction (PCR) and Sanger DNA sequencing for verification, should increase knowledge of the distributions of these species, of which CSSV appears to be the most widely distributed (Chingandu et al. 2017a,b; Kouakou et al. 2012; Muller et al. 2018).

Biology. The cacao-infecting badnaviruses in West Africa are transmitted in a semipersistent manner by mealybug vectors, 14 species of which have been implicated in CSSD (Roivainen 1976). Virus transmission requires a minimal acquisition access period of ~30 min, and an inoculation access period of ~90 min for two unique species in Trinidad, only recently identified as of New World (non-African) origin (Chingandu et al. 2017c). Similar transmission parameters have been established for the CSSD-badnaviruses endemic to West Africa (Posnette 1940). The badnaviruses infecting cacao in West Africa are not transmitted through seed (Ameyaw et al. 2014; Quainoo et al. 2008), but are experimentally graft transmissible (Steven 1936). Graft and mealybug transmission have been used to conduct host range studies (Posnette 1950). PCR amplification with primers designed around available genomic sequences indicate that the CSSD viruses can be detected in cacao plants experimentally inoculated with viruliferous mealybugs (Abrokwah et al. 2016) (R. Aka and J. K. Brown, *unpublished data*).

Genomics and molecular pathology. Badnaviruses have a mostly double-stranded, circular DNA genome (referred to as a gapped genome because of the single-stranded region that is repaired by a host enzyme prior to replication) (Hohn et al. 1997), ranging from ~7.0 to 7.3 kbp in size. The genome is encapsidated in a nonenveloped bacilliform particle of about 128 × 28 nm (Brunt et al. 1964). A recent exception among the CSSD-badnaviruses is the CRVBV identified recently from Nigeria, that has a genome that is several hundred nucleotides smaller. All of the CSSD-badnaviruses harbor essential open reading frames (ORFs) (Chingandu et al. 2019).

The CSSD-associated badnaviruses encode from four to six ORFs, referred to as ORFs 1–4, X. The function of the badnaviral

16 kDa ORF1 protein is not known. ORF2 encodes a 15 kDa protein having DNA and RNA binding activity (Jacquot et al. 1999), and ORF-3 encodes a ~212 kDa polyprotein harboring domains that are cleaved into a movement protein (MP), coat protein (CP), aspartic protease (AP), and the Retro Transcriptase (RT) and ribonuclease H (RNase H) proteins. The ORFs 4, X, and Y overlap with ORF3, and encode proteins of 95, 13, and 14 kDa, respectively, with unknown functions (Hagen et al. 1993; Hohn et al. 1997; Jacquot et al. 1999; Lot et al. 1991). Several genome organizations are known for badnaviruses, but most of the genome arrangement is conserved. While most of the conserved predicted domains (CPDs) are similar, unique CPDs have been identified among the CSSD badnaviruses compared with the entire genus (Chingandu et al. 2017a).

Recent results using NGS sequencing have detected several putatively non-endemic cacao-infecting badnaviruses in germplasm maintained in Ghana's "museum collection" of living infected cacao trees (Muller et al. 2018). Given their predictive divergence from the previously described West African badnaviruses that infect cacao and non-cacao hosts there (Supplementary Fig. S2), it is plausible that they were introduced on cacao germplasm brought into the region for use in the breeding program. How widely spread these putative new species and strains are, and whether they contribute to the regional CSSD epidemic is not known.

Management. Accurate, early diagnosis is fundamental to developing programs to manage CSSD. While serological and PCR amplification assays have been developed, they do not detect virus in all symptomatic samples (Chingandu et al. 2017b, 2019; Kouakou et al. 2012; Oro et al. 2014). In an extensive study involving 124 symptomatic trees in Cote d'Ivoire, only half of the samples were positive for CSSD-badnaviruses by PCR amplification and DNA sequencing of the cloned amplicons (Chingandu et al. 2017b). The

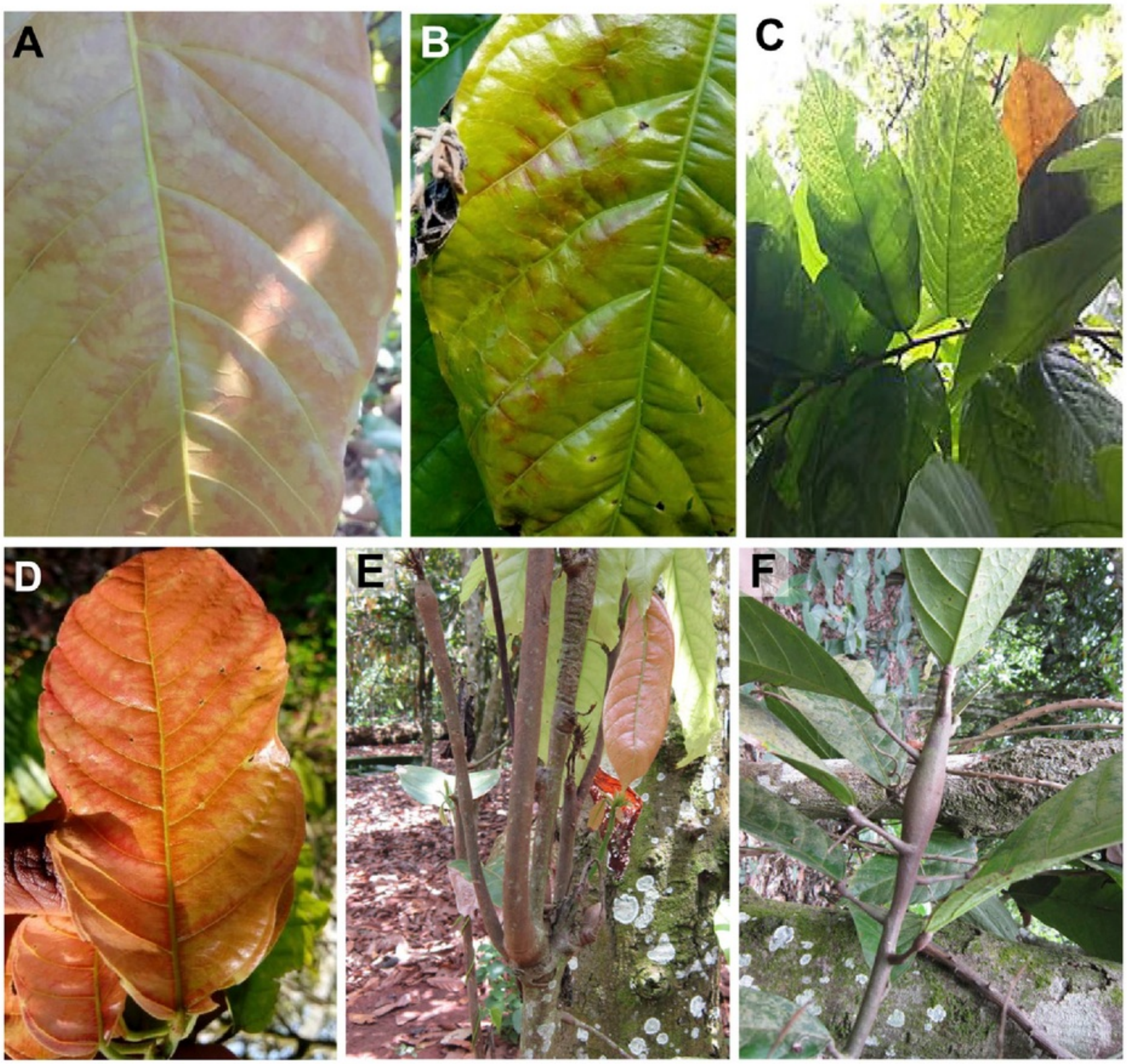

FIGURE 4

Cacao swollen shoot disease (CSSD) symptoms. **A to D,** Examples of symptoms observed in CSSD-affected cacao leaves. **E,** Swelling observed on branches. **F,** Swelling observed on and stems of CSSD-affected cacao trees.

PCR primers were designed based on five genomic regions among the seven available CSSD-badnaviral genome sequences from Cote d'Ivoire, Ghana and Togo (GenBank database), including viral CP, MP, RNase H, RT, and one noncoding region. Thus, genomic variability of the CSSD badnavirus complex was more extensive than anticipated. Clearly, new approaches are needed for CSSD diagnosis and to elucidate the genomic complexity and phylogeny of the CSSD badnaviruses (Chingandu et al. 2017b).

Beginning with the first recognition of CSSD, its management has relied on rogueing infected trees and replanting with presumed tolerant genotypes. However, this strategy has met with limited success (Ameyaw et al. 2014; Dzahini-Obiatey et al. 2010; Thresh et al. 1988). Since rogueing has been opposed by producers, it is often incomplete. Furthermore, selections from germplasm that was introduced into West Africa from South America have not resulted in disease-resistant genotypes (Padi et al. 2013; Posnette and Todd 1951). The Amelonado genotypes, which belong to the 10 genetic groups currently existing in cacao (Motamayor et al. 2008), are known to be more susceptible to the CSSD badnaviruses than the Upper Amazon and Trinitario genotypes, and only a limited number of those that exhibit some degree of tolerance have been made available to farmers. For example, in some Upper Amazon genotypes with Iquitos mixed with Calabacillo (IMC), Parinary (PA), or Nanay (NA) parentage, based on symptom development, rates of virus infection were only one-quarter less, compared with those for Amelonado (Nair 2010).

CONCLUSION

The cacao industry has been devastated in recent years due to the widespread, rampant decline and death of trees affected by emerging diseases. Production has only kept pace through unsustainable rainforest clearing and new plantings.

Diseases caused by *Phytophthora* spp. inflict chronic and serious losses to production in every growing region, but the emergence of the highly virulent *P. megakarya* in West Africa poses a significant threat to production in other regions. In West Africa threats posed by both the more "typical" and highly virulent CSSD badnaviral species have raised questions as to whether the CSSD crisis can be averted soon enough to save the cacao industry in West Africa where farmers face the difficult task of preventing black pod rot.

Moniliophthora diseases, while each endemic to limited parts of Latin America, have spread across the continent because of intentional and unintentional human activities. VSD is a new encounter disease affecting cacao across Southeast Asia and parts of Melanesia. Its origin and biology remain mysterious, although significant progress has been made in epidemiological and population studies as well as resistance breeding. Integrated management, including the production of disease-free plants in the nursery, canopy management and regular pruning of infected branches, and use of moderately resistant genotypes of cacao provides adequate management of frosty pod, witches' broom and VSD. However, the dominance of susceptible genotypes in regions currently free of these diseases poses a significant threat to global production.

A multitude of talents exist among outreach, research, and biotechnology partners. Among them, industry partners and national governments have committed resources and developed networks and programs with potential to inform and carry out rational prioritization of local and regional resource uses for disease management. Some hope may be on the horizon given new generation tools spanning gene silencing and/or gene editing technologies that can be harnessed and implemented in a timely manner. However, for these efforts to be translated into effective disease management and long-term solutions, they must be carried out concurrently with those devoted to developing genetic resistance and surveillance, with a plan for establishing sustained tree health and durability of the industry for the present and future.

Among the numerous immediate needs for informing trait improvement in commercial cacao genotypes, including virus and fungal resistance, are the establishment of a database of host genetic and pathogen genomic information, respectively, together with the development and implementation of effective molecular diagnostic platforms for pathogens and insect vectors/pests. These interconnected suites of resources are essential for facilitating the systematic, uniform characterization of phenotypic and genotypic responses to guide informed trait improvement in commercial cacao genotypes, including virus and fungal resistance, in breeding programs. Although there appears to be great potential for mitigating diseases, this goal will only be realized through the coordination and implementation of directed research, with immediate extension of the new knowledge and practices throughout the region. Signs pointing to a timely recovery are not promising given the current lack of capacity needed to ameliorate the many diseases over the short or the long term.

Disease spread and management are further exacerbated by the long lag-time between breeding, planting and economic pod production, and poverty faced by most smallholder cacao farmers. Cacao breeding is constrained by the overall low genetic diversity and the paucity of knowledge about the genomic variability of pathogens, availability of accurate and sensitive diagnostic tools and poor knowledge of epidemiology required to support resistance breeding, propagating improved planting materials and initiating rehabilitation and replanting programs.

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