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PLANT DISEASE FEATURE

Cacao Swollen Shoot Viruses in Ghana

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Abstract

Cacao swollen shoot virus causes cacao swollen shoot disease of *Theobroma cacao* (cacao) plants. At least six cacao-infecting *Badnavirus* species—*Cacao swollen shoot Togo A virus*, *Cacao swollen shoot Togo B virus* (previously known as *Cacao swollen shoot virus*), *Cacao swollen shoot CE virus*, *Cacao swollen shoot Ghana M virus*, *Cacao swollen shoot Ghana N virus*, and *Cacao swollen shoot Ghana Q virus*—are responsible for the swollen shoot disease of cacao in Ghana. Each of these species consists of a multiplicity of strains. The New Juaben strain, the most virulent cacao swollen shoot virus strain in Ghana, belongs to the *Cacao swollen shoot Togo B virus* species, and is a commonly used strain in laboratory transmission assays. Infection of cacao trees with multiple strains of the virus is common and new evidence suggests that these coinfections may have resulted in the emergence of recombinant strains of the virus. The impact of these emerging recombinant strains on disease severity is uncertain. This review focuses largely on the discovery of cacao swollen shoot virus in Ghana, diversity of the virus strains, molecular characterization, propagation of virus infection in cacao plants, emergence of recombinant virus strains, vector-mediated transmission of the virus, and the management of the cacao swollen shoot disease in Ghana. It also contains sections on the botany and origin of the cacao tree, its introduction to Ghana, the role of cacao swollen shoot disease in facilitating Ghana's independence from Britain, and a brief history of chocolate.

Keywords: badnavirus, cacao swollen shoot virus, *Caulimoviridae*, mealybugs, pararetroviruses, pathogen detection, pathogen diversity, red vein banding, tropical plants, viruses

The Cacao Tree

Cacao is a medium-sized tree species, native to South America, in the family Malvaceae and genus *Theobroma*. Cacao grows best in wet, humid, tropical rainforests spanning latitudes 18° north and 15° south of the equator with temperatures between 20 and 30°C (Argout et al. 2011; Cuatrecasas 1964). A cacao tree starts its life as a recalcitrant seed, germinating into a seedling with long-petiolated leaves. Early-maturing cacao varieties flower and produce cacao pods (Fig. 1) in as little as 18 months after planting (Cuatrecasas 1964; Goodall 1950; de Almeida and Valle 2007; Greathouse and Laetsch 1969; Greathouse et al. 1971). Depending on the cacao variety, cacao pods may be globose, ovoid, fusiform, ellipsoidal, or oblong in form, with either smooth or ridged surfaces (Fig. 2). Inside the pod, the cacao seeds (cocoa "beans") are arranged in rows, stacking on top of each other, and covered with a thick layer of gelatinous, sweet-tasting, and aromatic pulp. Cocoa beans are the primary ingredient in manufacturing chocolate (Cuatrecasas 1964; de Almeida and Valle 2007).

Certain Cacao Varieties Produce Better Cocoa Beans for the Chocolate Industry

During the nineteenth century, the people of Venezuela started referring to their native cacao populations as criollo (native, original) cacao and referred to the imported cacao varieties as forastero (foreigner, stranger) cacao (Piñero 1994). These groupings have since then become one of the major means of differentiating one cacao variety from the other. True criollo varieties are assigned to *Theobroma cacao* subsp. *typica*. Criollo cacao are among the oldest cultivated varieties of cacao. They are exceptionally prized for producing the highest-premium cocoa beans for the chocolate industry (Argout et al. 2011; Ciferri and Ciferri 1957). The lower-quality, true calabacillo forastero cacao are assigned to *T. cacao* subsp. *leiocarpum*. Between the true criollo cacao and the true calabacillo cacao are various classes of cultivated cacao varieties which are sometimes grouped solely on morphological characteristics. These include, in the order of decreasing cocoa bean quality, commercial criollo cacao varieties, superior forastero cacao varieties, average forastero cacao varieties, low-grade forastero cacao varieties, and almost calabacillo cacao varieties. The most commonly cultivated commercial cacao varieties for the chocolate industry are the superior forastero, average forastero, and low-grade forastero (Ciferri and Ciferri 1957; Howes 1929; Piñero 1994).

The Use of Cacao Products Dates Back to as Early as 1900 B.C.E.

Cacao cultivation, processing, and use were already deeply engrained in the customs, politics, gastronomic, and social lives of the Aztec people by the time the Spanish conquistador Hernán Cortés entered the imperial Aztec capital city of Technochtitlan in 1519 (Bergmann 1969; Brinkerhoff 2016). The Aztec King Moctezuma Xocoyotzin was served with several jugs of frothing chocolatl (Nahuatl word for chocolate) drinks on a regular basis (Brinkerhoff 2016). Cocoa beans were also used as currency by the Aztecs. For example, in 1545, the price of one tamale on the Aztec market sold for one cocoa bean. A porter was paid 100 cocoa beans for his daily labor and a turkey was sold for 200 cocoa beans (Edgar 2010).

However, the Aztecs were far from being the first indigenous Americans to use cocoa. Some forms of cocoa preparations were

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being served at Puerto Escondido, a small village in present-day northern Honduras, as early as in 1,400 to 1,100 B.C.E. (Henderson et al. 2007). Even earlier, the pre-Olmec Mokaya people of the Soconusco region of southern Mexico and western Guatemala were already using cocoa. At Paso de la Amada on the southern Pacific coast, traces of theobromine, a marker for the presence of cocoa, were found in jars dating as far back as 1,900 to 1,500 B.C.E. In San Lorenzo, the first capital of the Olmec indigenous Americans, cocoa was used in the mortuary rituals for sacrificial victims. Vessels dating back to 1,650 to 1,500 B.C.E. and containing traces of theobromine were also found at the Olmec site of El Manati. Cacao use among the Mayas has also been documented (Powis et al. 2008, 2011; Terry et al. 2022).

Fig. 1. Pruned cacao tree with cacao pods attached directly to the tree trunk and branches.

Fig. 2. Unit of field staff at the Cocoa Research Institute of Ghana, Tafo, loading harvested ripened cacao pods into the trailer of a tractor. In the background are cacao trees growing beneath tall trees that provide shade for the cacao trees.

A Brief History of Chocolate

The earliest chocolate beverages in Mesoamerica were made by, first, harvesting the ripened cocoa pods from the cacao tree (Fig. 2). The cocoa beans were then removed from the pods and fermented. Cocoa beans only acquire their characteristic chocolaty flavor during the process of fermentation. After fermentation, the cocoa beans were dried (Fig. 3), then roasted. The roasted cocoa beans were then ground into a paste and diluted with water and whisked continuously into a frothing chocolatl drink. Other additives such as ground corn, honey, and chili pepper could be added before the drink was served (Edgar 2010; Graziano 1998). A more sweetened version of this chocolate drink was first brought to the Western world primarily as a medication rather than a confectionery. Chocolate was prescribed in Europe for cheering the faint-hearted, expelling sorrows, and all perturbations of the mind. It was also used in treating alopecia, burns, coughs, dry lips, snake bites, pneumonia, scarlet fever, typhoid fever, rickets, atrophy rachitis, scrofula, and rheumatism (Bergmann 1969; Graziano 1998). In 1739, Spain listed cocoa in its pharmacopeia. This was followed by Britain in 1772, The Netherlands and Germany in 1801 to 1811, Sweden and Denmark in 1821, and France in 1818 (Graziano 1998). In 1727, Sir Hans Sloane, a physician to both British monarchs Queen Anne and King George II, begun adding dairy to his chocolate preparations. His recipe was later adopted and marketed by others, including Nicholas Sanders, William White, and the Cadbury brothers. Approximately two cubes (28 g) of Sir Hans Sloane's graded chocolate was placed in 2.5 cups of boiling milk and stirred until fully dissolved. Sugar could then be added to the milk chocolate elixir to taste (Graziano 1998). Today, chocolate has developed in its own right to become not a medicinal compound but, arguably, the sovereign of the confectionery industry. The desire for chocolate is almost ubiquitous. Chocolate can be purchased practically anywhere in the world in the form of plain chocolate bars, milk chocolate bars, chocolate-filled wafers or biscuits, chocolate-flavored liquors, chocolate spreads, hot chocolate, chocolate sweets, and many others (Januszewska 1996).

As of 2018, the estimated value of the chocolate industry was US$131.7 billion while that of the cocoa bean market upon which the chocolate industry depends was worth merely US$2.1 billion. This implies that, from the time cocoa beans are shipped from cacao plantations in the tropics to the time trademarked chocolate bars are displayed on the shelves of retail shops, the value of the cocoa would have appreciated by 6,261.90%. Cocoa bean shipping merchants, chocolate manufacturers, and other stakeholders in the chocolate value chain, rather than cacao farmers, are the main beneficiaries of these enormous profits (Mintah 2019). In 2019, 5.6 million metric tons (t) of cocoa beans were produced globally. The majority of the produce (67.60%) came from Africa, with Cote D'Ivoire and Ghana alone contributing 2.2 million t and 811,700 t of cocoa beans, respectively, to the global production (FAO 2019).

From South America to West Africa: How Cacao Came to Ghana

In 1822, Portuguese travelers from Bahia, Brazil took forastero Amelonado cacao on their journey to the Central African Portuguese colony of Sao Tome and Principe (Fig. 4). By 1840, cacao had moved from Sao Tome to the nearby island of Fernando Po (Fig. 4) (modern name, Bioko Island). It was at Fernando Po that a blacksmith, Tetteh Quarshie, from the Gold Coast (the British colonial name for the southern coastal portion of modern Ghana) came into contact with cacao while working as a Ghanaian (Gold Coastian) immigrant on the island. The 36-year-old blacksmith smuggled some of the cocoa pods in his toolbox from Fernando Po to the Gold Coast (Ghana) in 1879 (Muojama 2016). The cacao plantation he established at Akwapim Mampong, in the Eastern Province of the Gold Coast in the nineteenth century, is still active.

Ghana, Previously the World's Leading Exporter of Cocoa Beans

Once an area with suitable soil, relative humidity, rainfall, temperature, and a good amount of overhead shade is obtained (de Almeida and Valle 2007), cacao is fairly easy to grow, requiring less maintenance and producing yield recurrently for over four decades after it reaches fruit-bearing stage. Many Ghanaian farmers considered this to be an opportunity for a long-term investment and ventured into cacao farming. The British colonial government also

Fig. 3. After the fermentation of fresh cocoa beans, they are dried, in this case, in the sun, until they are bagged and sold to the cocoa exporting companies or processing facilities.

promoted cacao farming as a means of obtaining income for the people and for government expenditure. For instance, in the Ashanti Kingdom (a nation outside of the British Gold Coast colony but merged with the Gold Coast colony and two other regions to form Ghana after independence from Britain) (Fig. 5), any farmer who planted 1,000 cacao seedlings was given one Dane gun, a keg of gun powder, and two lead bars (Leiter and Harding 2004).

Fig. 4. Forastero cacao was shipped from Bahia, Brazil, to the Portuguese Central African colony of Sao Tome and later to the nearby Fernando Po island of Equatorial Guinea. It was from Fernando Po that Tetteh Quarshie smuggled cacao pods to the Gold Coast in 1879. (Figure used with permission, https://d-maps.com/carte.php?num_car=5868.)

The Basel missionaries also established experimental cacao plantations at Aburi and Akropong in the Eastern Province of the Gold Coast colony to teach the inhabitants about cacao husbandry (GCBS 1891). In 1830, the major centers of cocoa production and export were Venezuela, Ecuador, and Trinidad (Bekele 2004). Ghana exported only 36 kg of cocoa beans in 1891, rising to 536 t by 1900. In 1911, Ghana was the world's largest single producer of cocoa. In 1939, cocoa accounted for 80% of Ghana's total export. During the 1964–65 season, Ghana, still the world's largest exporter of cocoa, shipped 566,000 t of cocoa abroad (Chauveau 1997; Hill 1959; Leiter and Harding 2004). Currently, cacao is grown in the Western, Western North, Central, Eastern, Volta, Oti, Ahafo, and Bono regions of Ghana (Fig. 5B). However, some areas are more suitable than others for cacao cultivation (Fig. 6). Presently, Ghana's neighboring country, Cote d'Ivoire is the world's leading producer of cocoa, with Ghana taking the second spot (FAO 2019).

A New Plague on Cacao Farms in Ghana

In 1930, there was a widespread dieback on cacao farms at Nankese in the Eastern Province of the Gold Coast. However, the affected trees seldom produced stem swellings. Six years later, a cacao farmer at Effiduasi, also in the Eastern Province, noticed that some of his cacao shoots were swollen and that plant vigor and yield were dropping, followed by dieback and death of the trees (Fig. 7) (Stevens 1936; Strickland 1948). The farmer sent symptomatic shoots to the nearby Department of Agriculture district office for a solution (Danquah 2003; Greenwood 1943). This emerging condition on cacao farms was also associated with leaf vein banding, leaf vein clearing, leaf chlorosis, and swollen roots. Soon, there were more reports of the cacao plague from other parts of the Eastern Province. In 1935, Frank Stockdale, an agricultural advisor to the British Secretary of State for the colonies, visited the Gold Coast and recommended that a cacao research station should be established there to, among other objectives, find the cause of the deterioration on cacao farms. A Central Cacao Research Station was then founded in June 1937 at Tafo (Danquah 2003; Hodge 2009; Greenwood 1943).

Prior to the establishment of the Central Cocoa Research Station, W. F. Stevens, the Department of Agriculture plant pathologist who first alerted the world about the nature of the emerging cacao plague

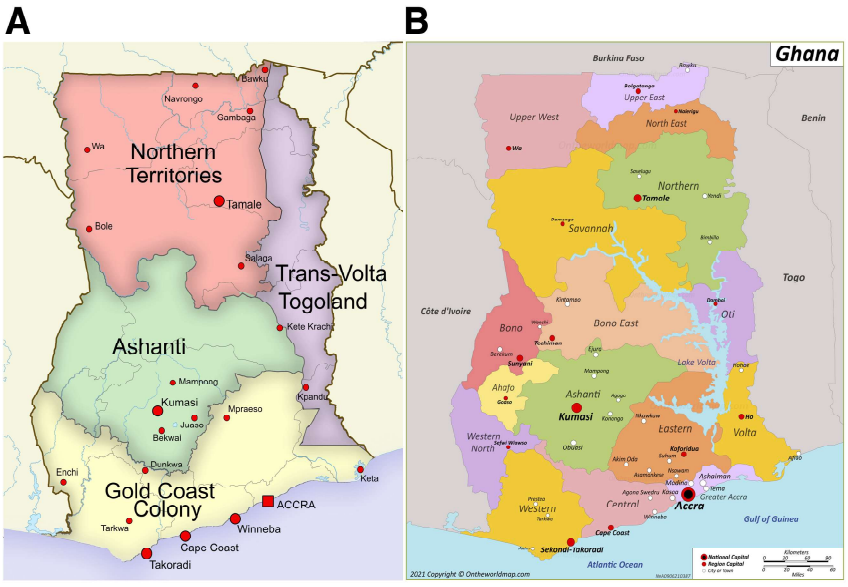

Fig. 5. A, Historical and **B,** modern maps of Ghana. The preindependence historical map of the future Ghana shows the Gold Coast colony of Britain, the Ashanti Kingdom (it temporarily became a British protectorate at some point in time), the British-administered Northern Territories, and the British-administered Trans-Volta Togoland (used with permission under the CC-BY-SA license, https://commons.wikimedia.org/wiki/File:Pre-independence_regions_of_Ghana.svg). The modern map of Ghana shows the 16 administrative regions (used with permission, https://ontheworldmap.com/ghana/).

in the Gold Coast, encouraged the Department of Agriculture to remove 81,000 affected cacao trees from 300 cacao farms. In 1937, however, roguing ceased when Henry Arthur Dade, a mycologist from the International Mycological Institute, Kew, was brought to the Gold Coast to find the cause of the cacao deterioration. After unsuccessful attempts to isolate a pathogen from the affected cacao trees, Dade concluded that the condition was caused by soil nutrient deficiency coupled with unfavorable environmental conditions. Based on his conclusions and other similar conclusions from fellow scientists, the Gold Coast government passed the Plant Pest and Diseases Ordinance of 1937 that, among other things, promoted the planting of more shade trees and windbreaks on cacao farms. Farmers who were caught destroying or removing cacao trees with the swollen shoot condition were to either pay fines or face imprisonment, because the cacao plague was now assumed to be caused by a physiological disorder and not a pathogen (Greenwood 1943, Hodge 2009).

In 1939, scientists at the Central Cacao Research Station observed that healthy cacao seedlings reproduced the swollen shoot symptoms when grafted with buds excised from cacao trees with the swollen shoot condition. These observations and subsequent experiments at the research station led to the conclusion that the swollen shoot condition of cacao was actually an infectious disease caused by a virus, which they named cacao "swollen shoot" virus (Posnette 1940).

The Pioneering Days of Cacao Swollen Shoot Virus Research in the Gold Coast

The Gold Coast became the center of cacao swollen shoot disease research after it reported the first cacao-infecting virus to the world in 1940 (Posnette 1940). Right from the early days of cacao swollen shoot virus (CSSV) research, it was recognized that the extensive variations in symptoms observed among diseased cacao trees,

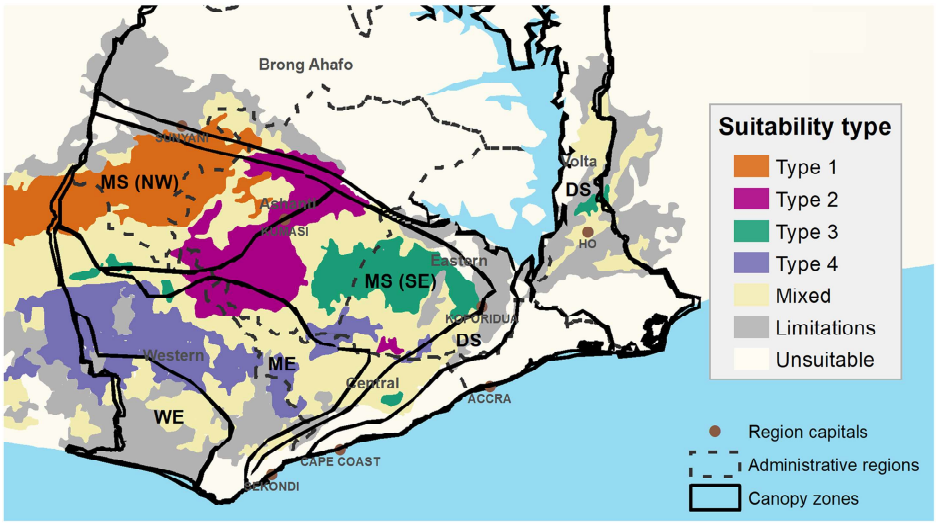

Fig. 6. Cacao cultivation suitability zone types in Ghana. Segments of Ghana that are not color-coded in the map are not suitable for cacao cultivation. Segments that are color-coded in gray may still be used for cacao cultivation but may not provide optimum conditions for the cacao plant. Photo credit: Bunn et al. 2019, used with permission.

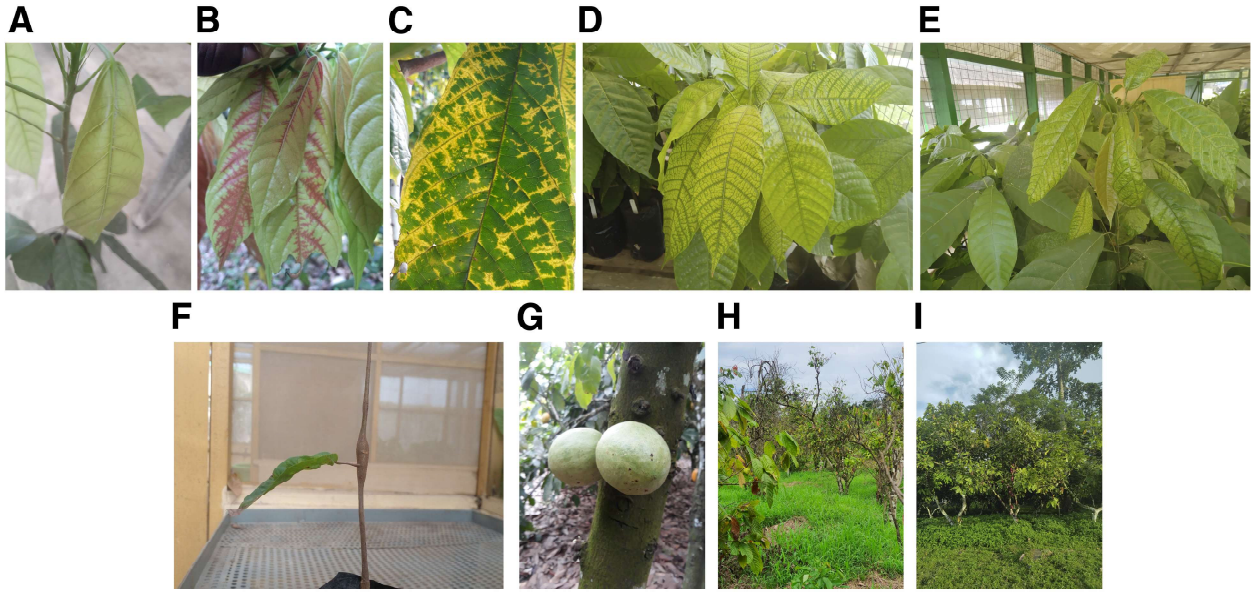

Fig. 7. Cacao swollen shoot virus infected cacao plants showing **A and B,** red vein banding of flush leaves; **C,** yellow vein banding of mature leaves; **D,** extensive chlorophyll depletion with green vein banding; **E,** leaf malformation (puckering and cupping); **F,** stem swelling; **G,** deformed cacao pods with smooth surface; **H,** dieback of diseased trees; and **I,** healthy cacao farm for comparison. Photo credits: Owusu Domfeh, Cocoa Research Institute of Ghana (CRIG) (B, C, G) and Adolf Boakye, CRIG (H).

especially in the different geographical regions of the Gold Coast, were largely due to the wide diversity of the badnavirus strains causing the disease (WACRI 1946).

Cacao swollen shoot badnavirus isolates were grouped based on the geographical region where they were first reported. For instance, Ashanti Province strains, Togoland strains, Eastern Province strains, and Western Province strains were originally discovered in those provinces (WACRI 1953b). The relationships between the various strains of the virus were determined through cross-protection assays. Strains that could protect against a subsequent infection by another strain were said to be immunologically related (WACRI 1950). Strains occurring within the same geographical zones were further categorized based on the nature of symptoms they induced in the infected cacao plants. For example, the Western Province strains were grouped into mosaic class, yellow mosaic class, yellow class, and clearing class. Viruses belonging to the mosaic class strains include Amafie, Bosumuoso1, and Wiasi (Supplementary Table S1). Their infection is characterized by fine vein banding, uniform close flecking, and fern patterns on leaves of the affected plants. Bosumuoso 2 and Jamesi are members of the clearing class strains, which produce prominent transparent lesions along the larger leaf veins (WACRI 1949). Many strains within their native geographical zones caused only mild symptoms in comparison with the very severe New Juaben strain (WACRI 1947). For instance, cacao plants infected with strains such as Bisa are symptomless in the chronic phase, except for development of shoot swellings. In the acute phase, Bisa-infected cacao plants may show only temporary, mild foliar chlorosis. In contrast, a New Juaben strain (severe A strain)-infected cacao plants display prominent red vein banding, extensive chlorosis, vein clearing, leaf crinkling, and leaf mosaic during the acute phase of the infection. In the chronic phase of the infection, yellow vein banding, leaf necrosis, reduced leaf size, root swelling, shoot swelling, and stunting are common. Subsequently, the New Juaben strain-infected cacao plants begin to die back and die within 3 years of infection (WACRI 1946).

Current Characterization of Cacao-Infecting Badnaviruses in Ghana

Cacao swollen shoot disease in Ghana is caused by at least six cacao-infecting *Badnavirus* spp.: *Cacao swollen shoot Togo A virus*, *Cacao swollen shoot Togo B virus*, *Cacao swollen shoot CE virus*, *Cacao swollen shoot Ghana M virus*, *Cacao swollen shoot Ghana N virus*, and *Cacao swollen shoot Ghana Q virus* (Chigandu et al. 2017a, b; Muller et al. 2018). Each of these virus species consists of a multiplicity of strains (Supplementary Table S1).

Cacao swollen shoot Togo B virus (CSSTBV) strains are the most widely distributed badnaviruses associated with swollen shoot disease of cacao in Ghana, and are found in all of the cacao-growing regions of the country (Abrokwah et al. 2016) (Fig. 6). Cacao swollen shoot Togo A virus (CSSTAV) strains have been reported in the Western, Western North, Volta, Oti, and Ashanti regions, whereas cacao swollen shoot CE virus (CSSCEV) strains are found in the Eastern, Bono, and Ahafo regions (Fig. 5). Cacao swollen shoot Ghana Q virus (CSSGQV) usually occurs as a component of mixed infections (Abrokwah et al. 2016; Muller et al. 2018).

The Cocoa Research Institute of Ghana (CRIG) houses cacao swollen-shoot-diseased plants and virus isolates collected from the various cacao-growing regions of Ghana. These disease isolates do not necessarily correspond to individual virus strains and may represent mixed infections (Posnette and Todd 1955). However, some of the curated disease isolates, such as Kofi Pare, are infected with only a single virus species, the *Cacao swollen shoot Togo B virus* (Muller 2021; Muller et al. 2018).

Origin and Characteristic Features of CSSV Strains

The common ancestor of all CSSV strains is thought to be of West African origin, because indigenous plant species in the subregion have been shown to harbor these badnaviruses. It is assumed that these West African endemic viruses jumped from their wild native plant species onto cacao after its introduction to the African subregion (Muller 2016; Tinsley 1971).

Viruses that cause cacao swollen shoot disease belong to the genus *Badnavirus* and are members of the virus family *Caulimoviridae* (Chigandu et al. 2017b; ICTV 2019; Uhde et al. 1993). They possess circular, double-stranded, 7.2- to 9.2-kb monopartite DNA genomes encapsidated in bacilliform, nonenveloped virions that, depending on the species, measure approximately 120 to150 nm in length and 30 nm in diameter (Fig. 8). In a few instances, the encapsidated DNA molecules occur in knotted forms (Adomako et al. 1983; Bhat et al. 2016; Ménissier et al. 1983). In infected plants, virions accumulate in the cytoplasm and vacuoles of phloem companion cells and in xylem parenchyma cells but are also distributed unevenly elsewhere in the host plant. The swollen shoot symptoms are a result of phloem proliferation (Jacquot et al. 1999). In the nuclei of these infected cells, cacao swollen shoot badnaviral genomes exist as independent chromatin-like nucleoprotein complexes. Leaf flushes from systemically infected plants tend to contain more virions than older symptomatic leaves (Bhat et al. 2016; Ménissier et al. 1983; Muller et al. 2001).

Once isolated from the host plant, the virions are fairly stable and can be stored at room temperature for up to 2 weeks at pH 6 to 8 without losing infectivity. However, storage at 50°C for 10 min is adequate to abolish infectivity (Brunt et al. 1964; Lot et al. 1991).

Groupings of CSSV Strains in Ghana Based on Conserved Nucleotide Identity and Genomic Organization

CSSV strains are also classified based on their genomic organization and the extent of conserved nucleotides in specific segments of the virus genome. They are classified as group 1, group 2, or group 3 strains based on the number of long open reading frames (ORFs) they possess. Group 1 CSSV strains possess four long ORFs: I, II, III, and Y. CSSTAV strain Gha25.15 (MF642716) and CSSCEV strains GH64 (KX592572) and GH67 (KX592571) belong to group 1 (Fig. 9). CSSCEV strains have the greatest intraspecific divergence among all of the CSSV groups in Ghana (Chigandu et al. 2017a, b; Ramos-Sobrinho et al. 2020). They are associated with very severe disease symptoms and death of cacao trees in the Western and Western North regions of Ghana (Chigandu et al. 2017b; Ramos-Sobrinho et al. 2020).

Group 2 CSSV strains possess five long ORFs: I, II, III, X, and Y. The CSSTBV strains New Juaben/severe 1A (AJ608931) and N1A (AJ609020) are members of this group. Although both the New Juaben/severe 1A strain and the N1A/attenuated 1A strain share 93.1% nucleotide identity, they differ immensely in their virulence. The former is the most severe CSSV strain in Ghana while the latter is known for its mild or asymptomatic infections (Chigandu et al. 2017a; Kouakou et al. 2012; Muller and Sackey 2005; Muller et al. 2018). The first CSSV strain to be sequenced, the Togolese Agou1 strain, belongs to the same species and groups as the

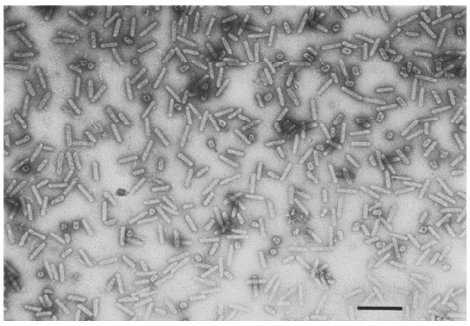

Fig. 8. Electron micrograph of purified cacao swollen shoot virus particles. Scale bar: 200 nm (Lot et al. 1991).

Ghanaian New Juaben strain based on the ORFs they both possess (Hagen et al. 1993; Muller et al. 2018). The genomic organization of the group 2 CSSTBV New Juaben strain is shown in Figure 10A. Group 3 CSSV strains possess six long ORFs: I, II, III, IV, X, and Y. The CSSTBV Peki strain belongs to group 3 (Fig. 10B) (Chigandu et al. 2017a). CSSV strains are also classified based on the degree of nucleotide identity in specific segments of the ORF III (Fig. 11); specifically, regions of the ORF III encoding the movement protein or the reverse transcriptase/RNase H proteins (ICTV 2019). Sequenced virus genomes are aligned to estimate the phylogenetic relationships between the various strains and virus strains assigned to groups based on their degree of shared nucleotide identities. A strain may be classified as A, B, C, D, E, F, G, J, K, L, M, N, P, Q, or R. Group members that share at least 80% nucleotide identities in their reverse transcriptase/RNase H region are classified as a species (Abrokwah et al. 2016; Chigandu et al. 2017a; ICTV 2019; Muller et al. 2018). Nucleotide alignments in the RNase H domain and a random segment of the reverse-transcriptase domains of some Ghanaian CSSV strains are shown in Figure 11.

Propagation of Infection in Cacao Plants

Mealybugs (family Pseudococcidae) are the major vectors for CSSVs. However, inoculation can also occur through mechanical bruising, grafting, or budding (Box 1945; Brunt and Kenten 1960; Posnette 1940). When a CSSV virion enters a cell, a nuclear localization signal in the capsid protein targets and subsequently docks the virion onto the nuclear membrane of the host cell (Karsies et al. 2002). Herpesviruses may provide a model for how badnaviruses discharge their DNA into the host cell nucleus (Kann et al. 1997, 1999; Karsies et al. 2002; Ojala et al. 2000). Herpes simplex virus 1 docks in a distinctive orientation, such that the capsid portal aligns with the cytoplasmic filaments of the nuclear pore complex, followed by release of viral DNA into the nucleus of the host cell through the capsid portal, leaving behind an empty capsid (Kann et al. 1997, 1999; Ojala et al. 2000). A similar mechanism may be employed by CSSV strains in releasing their DNA into the host cell nucleus (Fay and Panté 2015; Hohn and Rothnie 2013).

Once the badnavirus genome is deposited in the nucleus, discontinuities in the double-stranded viral DNA are repaired (Hohn and Rothnie 2013) and the circular double-stranded DNA associates with histones, forming supercoiled chromatin-like nucleoprotein complexes (Ménissier et al. 1983). In the majority of instances, badnavirus genomes exist as episomal elements in the nuclei of the infected host cells, remaining separate from the host genome. However, just like retroviruses and certain other plant pararetroviruses such as the florendoviruses, badnavirus genomes that do not belong to CSSV species group have been found integrated into the cacao genome (Muller et al. 2021). The host DNA-dependent RNA polymerase II transcribes these viral minichromosomes into more than genome-length pregenomic RNA molecules which are then shuttled into the cytoplasm, where they function as both polycistronic messenger RNAs (mRNAs) for the various virus proteins and templates for reverse transcription to regenerate viral DNA (Bhat et al. 2016; Geering et al. 2014; Pooggin et al. 1999).

The 5′-Leader Regulatory Sequence of CSSV Pregenomic RNAs

The long intergenic region that precedes the major ORFs of the CSSV pregenomic RNA contains various regulatory elements. This *Caulimoviridae*-wide conserved 5′-leader sequence putatively forms a stable stem-loop secondary structure (Fütterer et al. 1988; Pooggin and Ryabova 2018; Pooggin et al. 1998, 1999). The presence of this

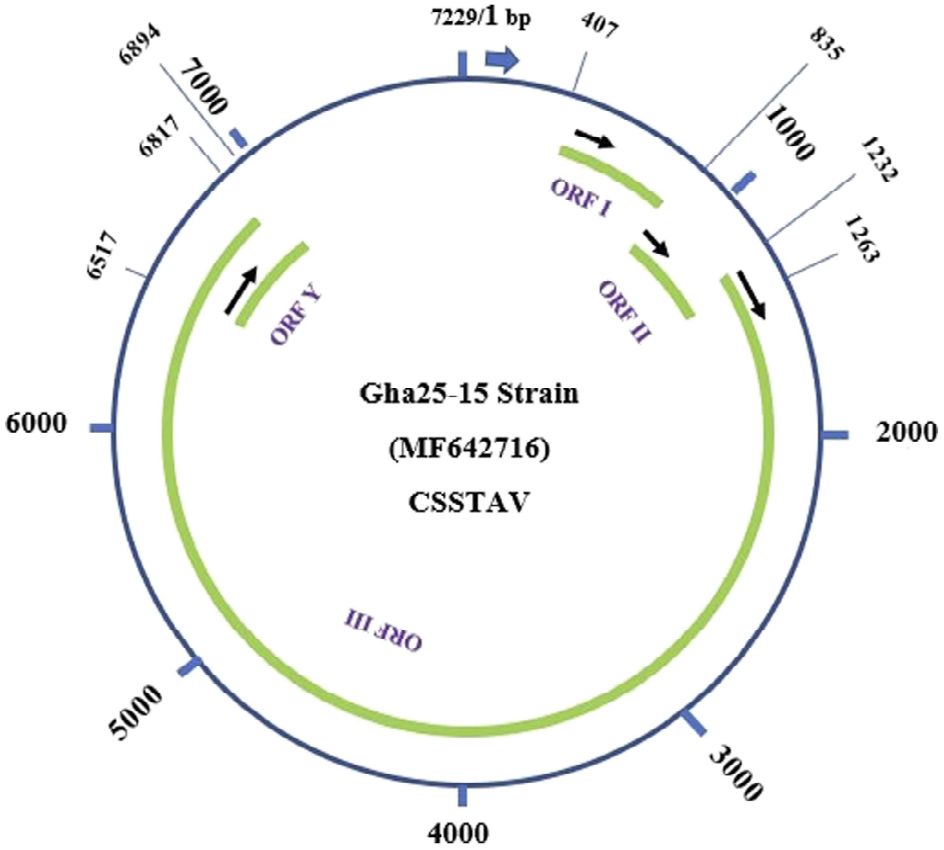

Fig. 9. Schematic representation of the genomic organization of cacao swollen shoot Togo A virus (CSSTAV) Gha25-15 strain. Specific locations of the various open reading frames (ORFs) are marked in base pairs. Black arrows indicate the location of the initiation codon.

secondary structure in the 5′-leader sequence of cauliflower mosaic virus 35S RNA has been confirmed experimentally (Hemmings-Mieszczak et al. 1997, 1998). In CSSV strains, however, its formation is only predicted based on computer modeling. It is purported to be formed by base pairing between specific nucleotides of the long intergenic region and a few nucleotides of the CSSV ORF I, such that the start codon for the virus ORF I is embedded within the stable stem-loop structure. Preceding the stem-loop structure are a series of short ORFs (sORFs); the third sORF terminates just a few nucleotides upstream of the stem-loop structure (Pooggin et al. 1999). A schematic representation of the 5′-leader sequence of CSSV strains is shown in Figure 12.

Other regulatory elements upstream of the stem-loop structure are a conserved sequence complementary to the nucleotides of plant methionyl-transfer RNA (met-tRNA) (Fig. 12), a purine-rich conserved sequence, and a CU-rich conserved sequence that has been implicated in promoting translation of virus long ORFs via ribosome shunting. A few nucleotides downstream of the stem-loop structure is a conserved AU-rich sequence putatively serving as a landing sequence for ribosomes shunting the secondary structure (Fütterer et al. 1988; Pooggin et al. 1999).

ORFs of CSSV Strains

CSSV strains encode four to six long ORFs, all of which are located on the +sense DNA (Hagen et al. 1993). ORF I of the New Juaben CSSTBV strain encodes a protein of unknown function, 143 amino acids (aa) long. In *Commelina yellow mottle virus*, the type species of the *Badnavirus* genus, an ORF I encoded protein is associated with previrions (Cheng et al. 1996; Hagen et al. 1993).

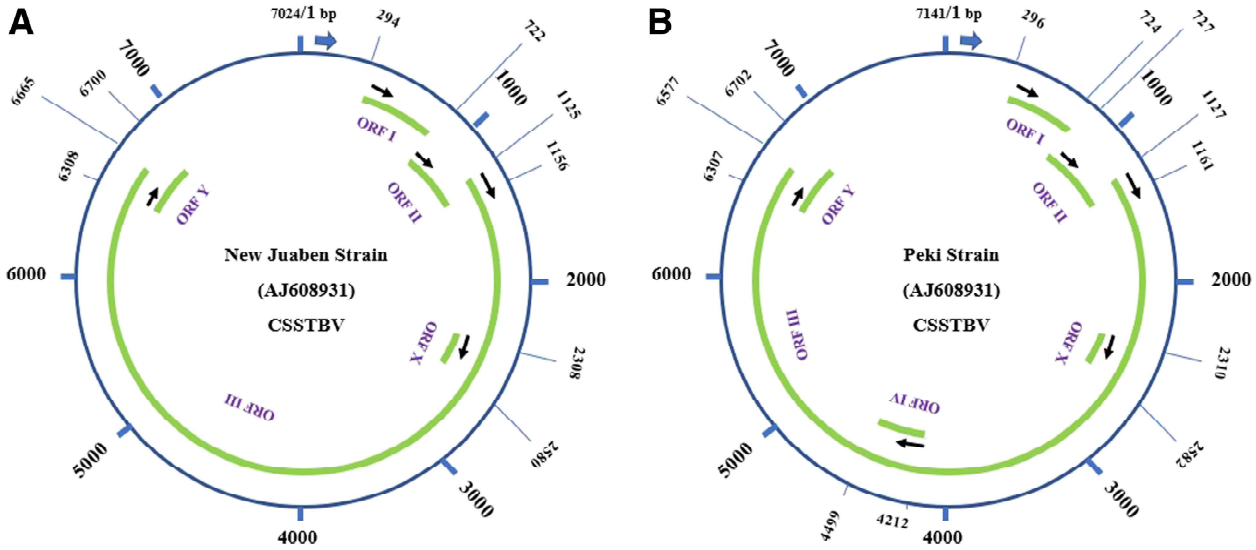

Fig. 10. Schematic representations of the genomic organizations of a group 2 (New Juaben) strain **(A)** and a group 3 (Peki) strain **(B)** of Cacao swollen shoot Togo B virus (CSSTBV). Specific locations of the various open reading frames (ORFs) are marked in base pairs. Black arrows indicate the location of the initiation codon.

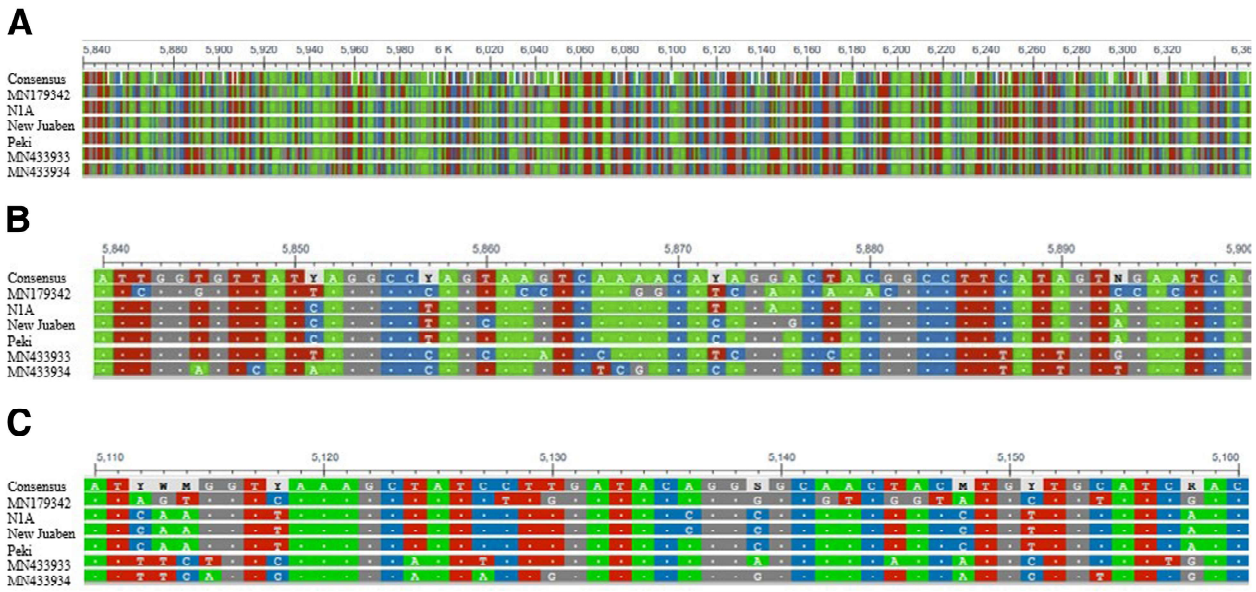

Fig. 11. A, Alignment of nucleotides in segments of the open reading frame III of the MN179342, N1A (AJ609020), New Juaben (AJ608931), Peki (AJ609019), MN433933, and MN433934 strains corresponding to the **A,** full-length RNase H domain; **B,** close up on a random 60 nucleotides in the RNase H domain; and **C,** close up on a random 50 nucleotides in the reverse-transcriptase domain. Consensus sequences for each alignment are shown in the first row of each diagram. Nucleotide A are color-coded green, nucleotide G are color-coded gray, nucleotide C are color-coded blue, and nucleotide T are color-coded red. Cacao swollen shoot virus nucleotide sequences were aligned using the National Center for Biotechnology Information Multiple Sequence Alignment Viewer.

ORF II of the CSSTBV Agou1 strain encodes a 132-aa-long p2 protein. The p2 protein interacts and binds with both single-stranded and double-stranded DNA or RNA in a sequence-nonspecific manner (Hagen et al. 1993; Jacquot et al. 1996). The ORF II encoded 145-aa-long protein of the New Juaben strain is slightly longer than that of strain Agou1 (Muller et al. 2018).

The full-length New Juaben ORF III encoded polyprotein is 1,847 aa long. The polyprotein is subsequently cleaved into a movement protein, a capsid protein, an aspartyl proteinase, a bi-functional reverse transcriptase, and ribonuclease H protein. The aspartyl proteinase is responsible for processing the virus-expressed polyprotein into the individual functional proteins, the movement protein is required for cell to cell and systemic movement of the virus, and the capsid protein is required for encapsidating the viral nucleic acid to protect the genome and enable the virion to target the nuclear membrane. The reverse transcriptase is required for converting the pregenomic virus RNA into genomic virus DNA. Ribonuclease H activity is required for degrading the virus pregenomic RNA following reverse transcription. ORFs IV, X, and Y encode individual proteins of unknown functions (Hagen et al. 1993; Karsies et al. 2002; Muller et al. 2018).

Translation of the Polycistronic Pregenomic RNA of CSSV Strains

In the cytoplasm, CSSV translation, reverse transcription, and assembly may occur in viroplasms (Haas et al. 2002, 2005). The caulimovirus P6 multifunctional protein is known to shuttle between the nucleus and the cytoplasm and may act in transporting virus-associated transcripts from the nucleus of the host cell into the viroplasms, where translation or assembly of pregenomic RNA into virions may occur (Haas et al. 2005; Hohn and Rothnie 2013).

The host translation preinitiation complex containing the 40S ribosomal subunit binds at the 5′ end of the capped virus pregenomic RNA and starts to scan the 5′-leader sequence toward the 3′ direction (Kozak 1989a; Pooggin and Ryabova 2018). The upstream sORFs preceding the stem-loop structure, the stem-loop structure itself, and the proximity or the overlapping nature of the virus long ORFs prevent translation of any ORFs downstream. For example, the ORF X and ORF Y of the New Juaben strain of the CSSTBV species overlap the ORF III. Employing host translation machinery in translating these overlapping viral ORFs could pose a challenge (Abrokwah et al. 2016; Kozak 1989a, b).

To overcome this, some pararetroviruses encode proteins that adapt the host's monocistronic translation machinery to translate the polycistronic mRNAs of viruses (Pooggin et al. 2012; Ryabova et al. 2006). For example, the cauliflower mosaic virus expressed P6 multifunctional protein acts, among other things, as a translational transactivator/viroplasmin (TAV) protein that interacts with the host translation machinery to enable translation of the multicistronic 35S RNA (Fig. 13). Specifically, P6 reengages the eukaryotic initiation factors to the ribosomes, such that they are available for reinitiating translation of downstream long ORFs even after terminating translation of the first long ORF (Bonneville et al. 1989; Park et al. 2001; Pooggin and Ryabova 2018). However, CSSV strains are not known to encode a TAV (Hagen et al. 1993). RNA splicing has also been implicated in aiding in the translation of downstream ORFs on polycistronic virus mRNA (Froissart et al. 2004; Fütterer et al. 1994; Kiss-László et al. 1995).

Translation by ribosome shunting and leaky scanning are of central importance in enabling pararetroviruses to translate ORFs downstream of inhibitory secondary structural elements (Pooggin and Ryabova 2018; Pooggin et al. 1998, 2008, 2012; Ryabova et al. 2006). The 5′ proximal short ORF closest to the base of the stable stem-loop structure of the 5′-leader sequence is vital to ensure successful ribosome shunting (Fig. 14). Subsequent to translation of the 5′ proximal sORF closest to the base of the stem-loop structure, the translation initiation complex shunts over the stem-loop structure onto a receiving landing sequence downstream of the stem-loop structure, after which scanning is resumed. Without successful ribosome shunting of the stem-loop structure, the translation initiation complex may not reach the downstream coding regions of the virus pregenomic RNA (Fütterer et al. 1993; Kozak 1989b; Pooggin et al. 1998, 2008; Ryabova et al. 2006).

Because the start codon for ORF I is predicted to be embedded in the shunted stem-loop structure, translation of ORF I may start from a non-AUG initiation codon, resulting in a truncated protein. A GUG triplet in a moderate context a few nucleotides from the stem-loop structure could possibly serve as an alternative non-AUG start

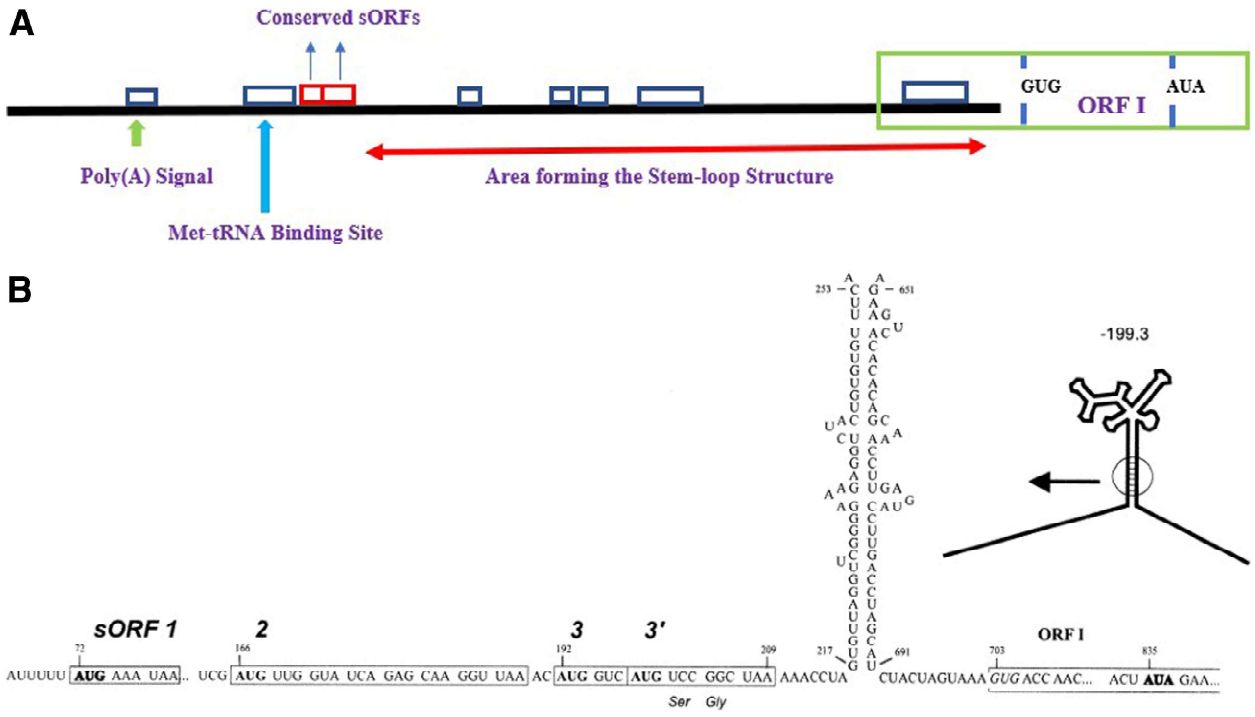

Fig. 12. A, Schematic representation of the 5′-leader sequence of cacao swollen shoot virus RNA. sORF = short open reading frame and met-tRNA = methionyl-transfer RNA. **B,** Nucleotide composition of the cacao swollen shoot Togo B virus Agou1 (L14546) leader (Pooggin et al. 1999).

codon for ORF I (Fig. 14). A number of the shunting polysomes may recognize the GUG as the start codon for ORF I and initiate translation (Pooggin et al. 1999; Ryabova and Hohn 2000). The remaining polysomes may continue scanning the pregenomic RNA until the weak start codon for ORF II is reached. Similarly, some of the polysomes may recognize the weak start codon of ORF II and initiate translation, while the remaining polysomes continue scanning until the start codon of ORF III is reached and translated into the polyprotein. ORF IV, ORF X, and ORF Y may be translated in a similar manner (Fig. 14). Once the newly synthesized polyprotein has been processed into the various mature viral proteins, the enzymes needed for the assembly and maturation of virions become available (Pooggin and Ryabova 2018; Pooggin et al. 1999, 2008).

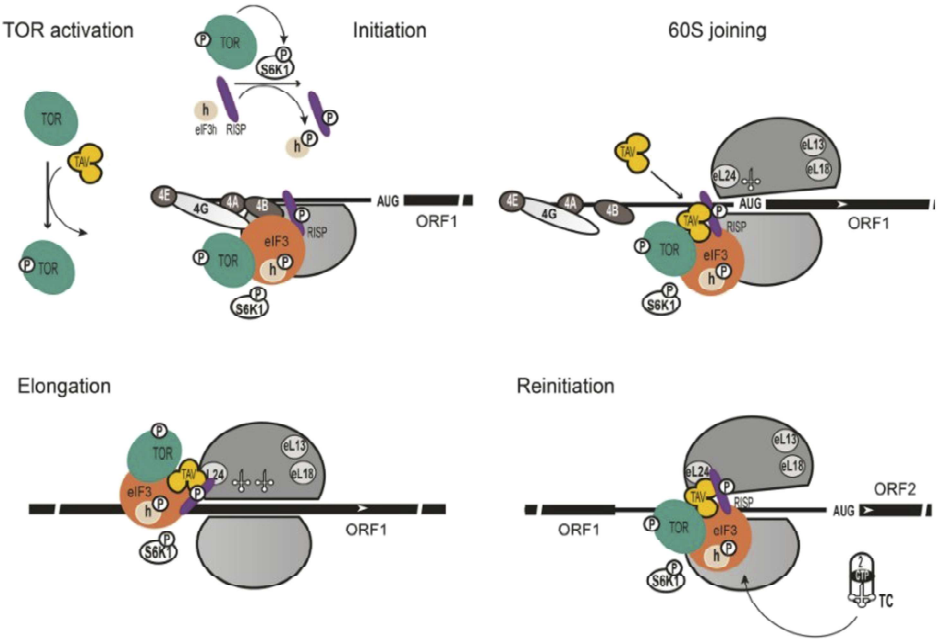

Fig. 13. Transactivator/viroplasmin (TAV)-mediated reinitiation of translation of downstream caulimovirus long open reading frames (ORFs). During translation of caulimovirus polycistronic messenger RNA (mRNA), the virus encoded TAV binds and activates target of rapamycin (TOR). Subsequently, the activated TOR interacts with the eIF3-40S preinitiation complex at the 5′ cap of the virus mRNA. These interactions lead to the activation of S6 kinase 1 (S6K1), reinitiation supporting protein (RISP), and eIF3. However, the 40S-bound TAV can also interact and bind the eL24 ribosomal protein associated with the 60S. Thus, during the 60S-joining phase of the caulimovirus polycistronic mRNA translation event, TAV acts to secure the incoming 60S to the 40S by binding specific ribosomal proteins associated with each of the ribosomal subunits on the 80S. This TAV-mediated complex formation ensures that the postterminating 60S ribosomal subunit does not dissociate from the 40S ribosomal subunit even after termination of translation, and that it can be reused for successive reinitiation of translation events on the same polycistronic mRNA (Pooggin and Ryabova 2018).

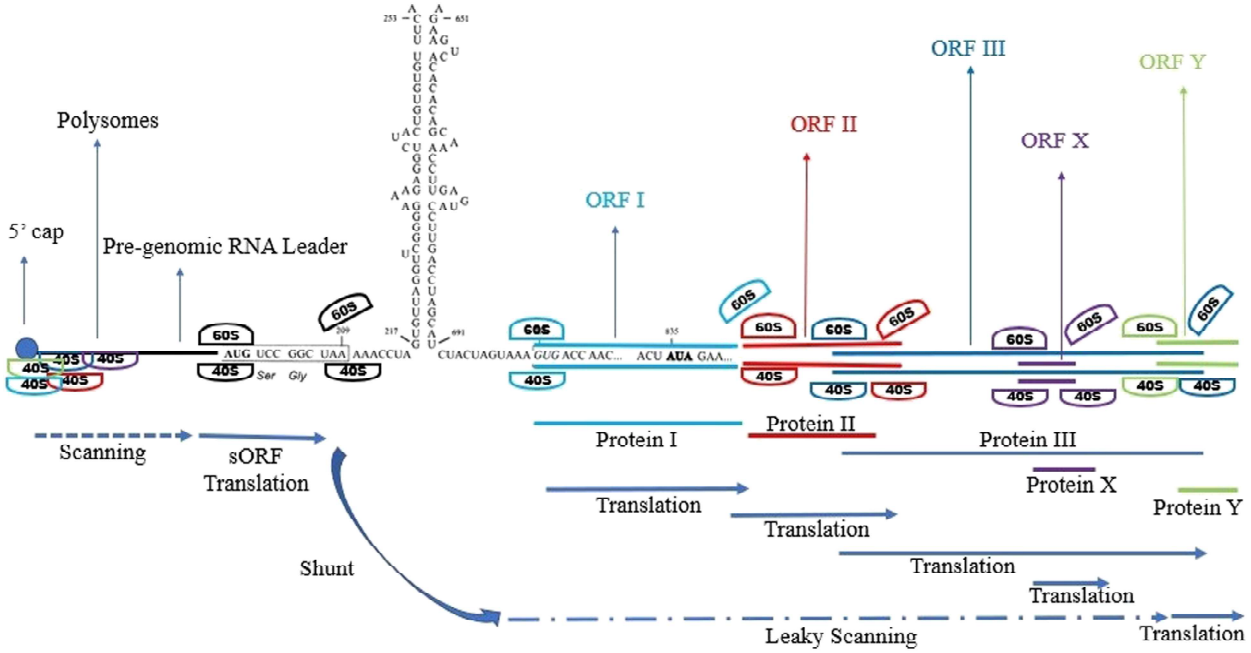

Fig. 14. Translation of cacao swollen shoot virus open reading frames (ORFs) by ribosome shunting and leaky scanning. sORF = short ORF.

Virion Assembly and Systemic Movement

The capsid precursor protein of CSSV strains contains several RNA- and DNA-binding domains. Through these domains, the precapsid protein may interact with the packaging signal located in the 5′-leader sequence of the virus pregenomic RNA. Such an interaction could result in the sequestration and packaging of virus pregenomic RNA into previrions (Hohn and Rothnie 2013). Unlike mature capsid proteins that target the nucleus, capsid precursor proteins localize in the cytoplasm. This may provide ample time for a proper cytoplasmic assembly of virions and also ensure that only viable virions are sorted to the nuclear membrane of the host cell (Karsies et al. 2002).

Once the pregenomic RNA is packaged into previrions, a purine-rich cleavage product from viral RNase H directed pregenomic RNA digestion is used in priming the synthesis of the +single-stranded DNA while the host met-tRNA primes the synthesis of the −single-stranded DNA. The RNase H is required for degrading RNA components of the DNA-RNA duplexes that form during reverse transcription (Hohn and Rothnie 2013; Pfeiffer and Hohn 1983). The nuclear localization signal is then activated after the maturation of the precapsid protein. The newly assembled matured virion may then be acquired and transmitted by a feeding insect vector or may accumulate in the infected cell. It may also spread systemically via the plasmodesmata into neighboring cells with the aid of the movement protein, as observed in the badnavirus commelina yellow mottle virus (Cheng et al. 1998). Plant cells are linked to each other via a continuous connection of plasma membranes that traverse plasmodesmatal pores. Once at the plasmodesmatal pore, the accumulating movement protein forms tubular structures, and the P6 multifunctional protein has been implicated in delivering virions into these tubular structures to facilitate their transport between cells (Carluccio et al. 2014; Rodriguez et al. 2014). In commelina yellow mottle virus, the badnavirus-encoded movement protein forms a 50-nm-diameter tubule that delivers virions to neighboring cells via the plasmodesmata (Fig. 15). A similar mechanism is employed by other badnaviruses, making it likely that this is the means of cell-to-cell movement of CSSV strains (Cheng et al. 1998; Guerra-Peraza et al. 2005). Once a virion arrives in a new cell, it will repeat the process of targeting and docking to the nuclear pore system, depositing its genomic double-stranded DNA into the nucleus, and perpetuating the infection cycle (Bhat et al. 2016; Karsies et al. 2002).

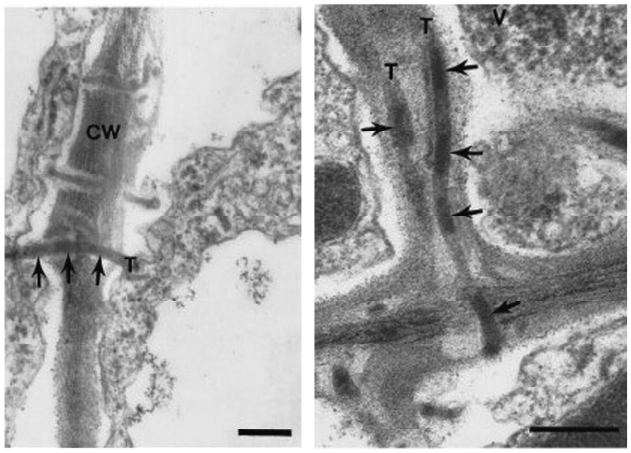

Fig. 15. Commelina yellow mottle virus associated tubular structures (T) observed traversing adjacent cell walls (CW) in commelina yellow mottle virus-infected *Commelina diffusa* plant tissue. Arrows indicate commelina yellow mottle virions found within these tubular movement protein structures. Scale bar: 100 nm (Cheng et al. 1998).

Mixed Infections, Recombination, and Emerging CSSV Strains

Mixed infections have been recorded in the field and at the cacao swollen-shoot-virus-infected plant resources museum (Abrokwah et al. 2016; Muller et al. 2018; Posnette and Todd 1955). For instance, a cocoa plant belonging to the Gavekpe Todzi disease isolate is associated with three different CSSV species: *Cacao swollen shoot Ghana R virus*, *Cacao swollen shoot Ghana N virus*, and *Cacao swollen shoot CE virus*. When two or more virus strains or species coinfect a host simultaneously, there is a high possibility that the coinfecting virus strains may interact with each other (Hammond et al. 1999). In certain pathosystems, a mixed virus infection is a prerequisite for a specific disease to manifest (Azzam and Chancellor 2002; Fondong et al. 2000; Hull 1996). In mixed infections, the possibility of one virus strain being encapsidated in the coat protein of another coinfecting virus is realistic. Such *trans*capsidation may alter the range of vectors that could transmit the virus (Hammond et al. 1999). Coinfecting virus strains may also exchange genetic

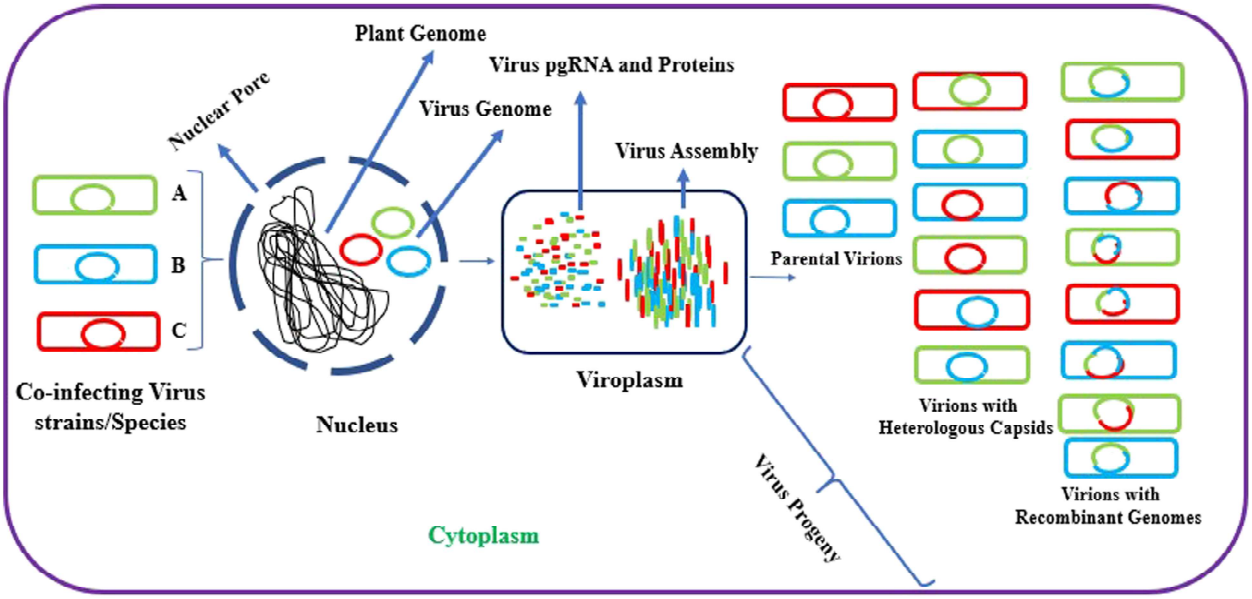

Fig. 16. Mixed virus infections create opportunities for heterologous encapsidation, recombination, and the emergence of new virus variants. pgRNA = pregenomic RNA.

information during replication, resulting in recombinant viruses with altered virulence (Fig. 16). Conceivably, recombination events might enable a virus to combine counter-defense factors of all the coinfecting virus strains in the shared host into a single resistance-breaking variant capable of evading the host's resistance mechanisms (Pérez-Losada et al. 2015; Rojas et al. 2005; Rubio et al. 2001; Wainberg 2004; Zhou et al. 1997). Using recombination detection software, evidence of at least six recombination events occurring among CSSV strains have been detected (Ramos-Sobrinho et al. 2020). Recombination breakpoints are predicted in the long intergenic region and also the ORF II of CSSV strains, particularly among CSSTBV, cacao swollen shoot CD virus, and CSSTAV. The Togolese CSSTBV strains Kipi7b and Kipi10b are putatively recombinant strains with DNA segments obtained from the Togolese cacao swollen shoot CD virus Kipi7a strain. Intraspecific recombination events have also been predicted within certain cacao-infecting badnaviruses such as the genetically diverse *Cacao swollen shoot CE virus* species (Muller et al. 2018; Ramos-Sobrinho et al. 2020).

Mealybugs, the Primary Vectors of Cacao-Infecting Badnavirus Strains

Numerous mealybug species, including *Formicococcus njalensis* (cacao mealybug), *Planococcus citri* (citrus mealybug), *Pseudococcus bukobensis*, *Dysmicoccus brevipes* (pineapple mealybug), *P. concavocerarii*, *P. longispinus* (long-tailed mealybug), *Ferrisia virgata* (striped mealybug), *Phenacoccus* sp., and *Paraputo ritchiei*, can transmit one or more strains of cacao-infecting badnaviruses (WACRI 1950). Long-tailed mealybugs can transmit the Kpeve and Mampong strains but do not transmit the New Juaben strain, whereas striped mealybugs transmit several strains of the virus, with the exception of the Kpeve and Mampong strains. The most efficient mealybug species acting as vectors of the virus on cacao is the cacao mealybug (Fig. 17), which can transmit several strains of the virus (WACRI 1950).

The Cacao Mealybug

Aside from cacao, the mealybug *Formicococcus njalensis* also feeds on several plant species including *Ceiba pentandra*, *Theobroma bicolor*, and *Cola chlamydantha*, which are also susceptible to specific strains of the cacao swollen shoot badnaviruses (WACRI 1948, 1949). Mealybugs are cryptic insects and are usually hidden in the tree canopy, on tree barks within cracks, and as discrete colonies on cacao pods, almost always in association with attending ants of the genus *Crematogaster*. These mealybug-attending ants build protective tents around the mealybug colonies, boosting their wellbeing and size (Fig. 17C). Nevertheless, there are ants such as those belonging to the subfamily Pseudomyrmecinae that prey on mealybug colonies (WACRI 1949).

Life Cycle of the Cacao Mealybug

Before reaching adulthood, *F. njalensis* undergoes three stages of molting. The first-instar crawlers emerge from eggs a few hours after being laid. A week or so later, the first instar goes through its first molt to become a second instar crawler. The second instar goes through a second molting after approximately 5 days to become a third-instar nymph. The third-instar nymph undergoes a final molt in approximately 7 days to become a young adult capable of mating and oviposition 22 days later. Once female mealybugs start ovipositioning, they may live for up to 10 days. Unlike adult female mealybugs, adult male mealybugs go through a 6- to 8-day-long pupation to emerge as winged adults. The males emerge at approximately the same time that the third-instar female nymphs emerge as adults. Adult male mealybugs may only live for an average of approximately 48 h due to their inability to feed. Mating commences immediately after the adult male mealybugs emerge. Adults female mealybugs possess functional mouthparts and are able to feed but can survive 10 days without access to food source (WACRI 1948, 1949).

Cacao Mealybugs as Vectors of CSSV

All life stages of *F. njalensis* can acquire and transmit specific strains of the virus from cacao leaves, shoots, bark, and roots within 4 h of feeding. However, optimum virus acquisition and transmission are carried out by adults feeding for a minimum of 48 h on flushes of newly emerged symptomatic cacao leaves (WACRI 1947).

Mealybugs lose their ability to transmit the virus after prolonged periods of starvation. In the laboratory, before mealybugs are used in virus acquisition access assays, they are starved to enhance faster settling and feeding on the virus source plants. Also, by starving

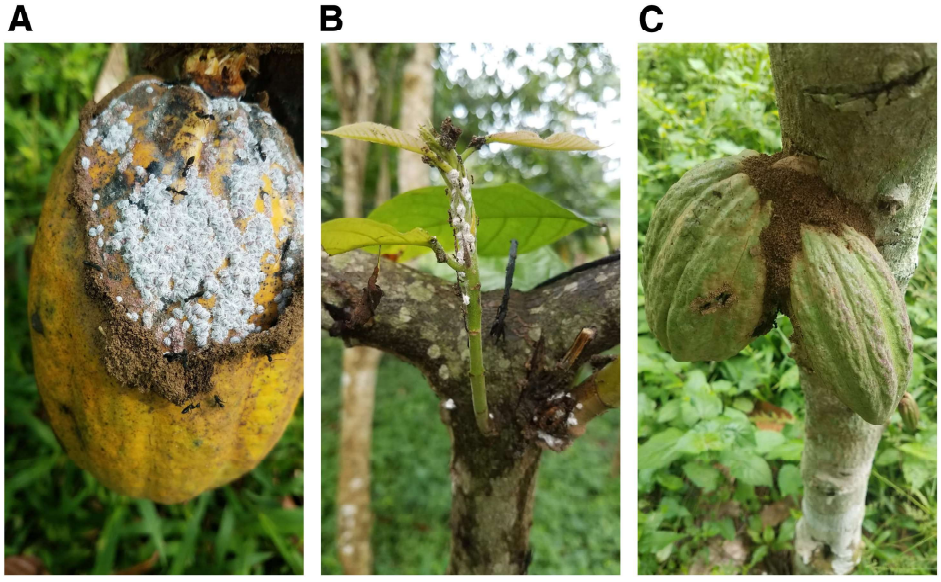

Fig. 17. Cacao swollen shoot viruses in Ghana are primarily vectored by *Formicococcus njalensis*. They can be found on **A**, cacao pods; **B**, cacao shoots; and usually under protective tents built by the attending ants (**C**). Photo credit: Adolf Boakye.

them, they may lose any virus they might have acquired from the field, because these mealybugs are directly collected from the nearby plantations for such assays. The probability of a successful virus acquisition and transmission by a single *F. njalensis* insect is approximately 0.103; hence, 30 viruliferous mealybugs are recommended per test plant in the virus inoculation access assays (Fig. 18) (WACRI 1948, 1949).

Once a mealybug arrives on a host plant, it attempts to thrust into host plant tissue with its rostrum, which then begins to retract once the stylet establishes contact. The stylet then penetrates the host tissue through a series of short discrete movements of approximately 0.5 mm/sec. During this process, the mealybug intermittently secretes saliva into the host tissue; it may pause briefly, partially withdrawing its stylet for a possible rerouting. Virus transmission occurs during salivation, because the virions are found in the salivary gland of viruliferous mealybugs. It takes approximately 30 min for an adult mealybug to fully extend its stylet into the phloem or xylem tissues of a host plant. Nymphs and crawlers require shorter settling time due to their shorter stylets (WACRI 1949).

Controlling Mealybug Populations on Cacao Farms

In the wild, mealybugs have many natural predators and parasites that could potentially keep their population under control. However, the ants that attend mealybugs meticulously shelter the mealybugs, driving away natural enemies. Hence, the presence of natural parasitic and predatory insect pests of mealybugs generally have no significant effect on the virus vectors (WACRI 1950). Aside from being protected by ants, mealybugs are also covered in waxy substances which make the use of liquid contact insecticides ineffective. This protection can sometimes be mitigated by supplementing the liquid contact insecticides with wetting agents. For contact insecticides, applications through dusting seems to be more effective than liquids. Diethoxy-(4-nitrophenoxy)-sulfanylidene-λ5-phosphane (Parathion), benzene hexachloride, and nicotine have all been tested on mealybugs in the past. Parathion application produces good results but has high mammalian toxicity, and it also kills the natural enemies of mealybugs. Unlike *F. njalensis*, *Ferrisia virgata* seems to possess some level of resistance against the effect of nicotine. Among the insecticides used in the past, the systemic insecticide N-[dimethylamino(fluoro)phosphoryl]-N-methylmethanamine (Dimefox) was particularly effective. It killed more than 99% of mealybugs when the insecticide was applied around the base of cacao trees for root uptake. Dimefox worked particularly well on mealybugs feeding on the vegetative parts of infested cacao tree compared with mealybugs feeding on the cacao pods. Nevertheless, Dimefox was very toxic to humans and it was also economically impractical to apply on cacao fields. There were also incidences of phytotoxicity and reports that systemic insecticides alter the flavor profiles of cocoa beans (Danquah 2003; WACRI 1953b). Currently, the use of Dimefox is prohibited. The research station has also, in the past, imported series of predatory and parasitic insect pests into the country to prey on mealybugs. For instance, approximately 35,000 coccinellids (predatory pests of mealybugs) were imported from California, United States into the Gold Coast. There has also been an importation of approximately 21,000 *Leptomastix dactylopii* (endoparasitoid wasps) in the past (Fig. 19). However, mortality among the imported predatory and parasitic insect pests was high. For example, 90% of imported endoparasitoid wasps could not survive. Typically, these imported insects are either wiped out by torrential rain or ingested by the native predatory insects (Danquah 2003).

CSSV Tolerance Among Cacao Varieties

Cacao plants growing under substantial shade usually produce milder symptoms than plants growing under full sun (WACRI 1953b). In a severe CSSV strain-inoculated cacao plant, the probability of transmitting milder variants of the virus seems to increase appreciably when the graft material is taken from the roots rather than the exposed aboveground tissues. Milder symptoms are also usually observable on susceptible cacao varieties that were inoculated from tolerant cacao varieties infected with virulent strains of the virus. These susceptible cacao varieties displaying milder symptoms, in many instances, also gain tolerance to a subsequent reinoculation

Fig. 18. Viruliferous mealybugs are placed in a cage and subsequently attached to the indicator plants that are being inoculated with the cacao swollen shoot virus (CSSV) virions for 3 days. During this period, the viruliferous mealybugs feed on and transmit the CSSV to the indicator plants.

with the virulent strain of the virus. However, it appears that tolerant cacao varieties sometimes attenuate the virulence of severe virus strains, such that mealybugs are only able to acquire mild strains of the virus from tolerant cacao varieties on many occasions for subsequent transmissions. Alternatively, it is possible that there is accumulation of some type of tolerance-inducing factors in the tolerant cacao varieties that are also accessible to mealybugs for transmission, such that, although the mealybug transmits the virus to the healthy but susceptible cacao varieties, it also transmits the tolerance-inducing factors that it acquired from the tolerant cacao varieties. In such a situation, the virus strains being transmitted may not necessarily be attenuated but the immunity of the susceptible cacao plants being inoculated may be primed, perhaps by faster-acting, mealybug-transmissible, tolerance-inducing factors that are transmitted with the virulent CSSV virions (WACRI 1948).

It is also fairly common to spot a susceptible cacao plant showing mild or no symptoms after being infected with a severe strain of the virus. Despite the apparent lack of symptoms of such an infected cacao plant, it may still be a source of the virulent strain of the virus for mealybug-mediated transmission. These classes of asymptomatic but infected cacao trees present a challenge to prophylactic removal of diseased trees because they are easily missed by visual inspection (WACRI 1948).

Cacao shoots emerging from buds that were formed during the chronic phase of the CSSV infection are also more likely to show only mild or no symptoms. In contrast, shoots emerging from buds that were formed prior to the cacao plant being infected are more likely to show acute symptoms, with extensive chlorosis, leaf crinkling, and leaf necrosis (WACRI 1949).

Mild Strain Cross Protection

Milder strains of a virus may offer protection against subsequent infection with a virulent variant of the virus with which it shares certain similarities in terms of symptom expression. Strains found in geographical zones that are farther apart are less likely to offer protection against each other. For instance, both the Western Province strain Wiasi and the Eastern Province strain severe 1A share similarities in their symptoms but Wiasi does not offer protection against the 1A strain (WACRI 1949). Preinoculation of cacao plants with either the attenuated A strain, Dawa strain, or Konongo strain offers complete protection against subsequent severe 1A reinoculations. This mild strain cross protection against the severe strain of the virus is not durable, however. Preinoculation of a cacao seedling with the Bisa strain significantly extended the latent period of a subsequent severe 1A strain reinoculation but the Bisa preinoculated plants subsequently succumbed to the severe 1A strain (WACRI 1948). Some strains do not provide protection against the severe strain of the virus but their presence in a shared cacao plant worsens disease symptoms. Examples of such strains are the attenuated A strain with the Bosomtwe strain and the attenuated A strain with the Mampong strain (WACRI 1948, 1949). The severe 1A strain accumulates and expresses the full complement of its symptoms in cacao plants preinoculated with Kpeve, Nkawkaw, Pamen, Dochi, Bosomtwe, Amafie, Jamesi, Dantano, Punekrom, or Wiasi but their mixed infections with 1A do not result in novel symptoms, as would have occurred in a mixed infection involving the Mampong strain and either the attenuated or 1A strains (WACRI 1949). Growth and yield data from cacao trees in the field that were inoculated with mild strains of the virus have given promising results over an appreciable length of time but their protective effect wears off in the presence of the virulent strains (Ameyaw et al. 2013, 2016; Domfeh et al. 2011, 2013, 2018; Dzahini-Obiatey et al. 2013).

Heat Treatment and Chemotherapy of Planting Materials

Incubation of inoculated cacao seedlings at 37.5°C tends to inhibit virus accumulation. However, virus accumulation and subsequent symptom expression resume if seedlings that had been subjected to 6 weeks of heat treatment are returned to ambient temperatures. Incubating inoculated seedlings at 42°C for 24 h had no impact on symptom expression after the heat treatment ceased (WACRI 1951).

Several chemicals—8-hydroxyquinoline (quinolin-8-ol), colchicine (N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5H-benzo [a]heptalen-7-yl]acetamide), urea, iodine, maltose, benzoic acid, calcium chloride, malachite green ([4-[[4-(dimethylamino)phenyl]-phenylmethylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylazanium; chloride), and zinc sulphate—have been applied to cacao plants for either root uptake or as budwood treatments in an attempt to inactivate the virus but none showed promising results (WACRI 1953a).

Roguing as a Management Practice

Removal (also known as roguing) of diseased cacao trees started at the Central Cacao Research Station in 1940. Initially, only trees that showed visible stem swellings were removed. A few months later, however, trees with chlorotic leaf symptoms were also included. In 1941, all apparently healthy trees in contact with any diseased cacao tree were also rogued out (Greenwood 1943).

Fig. 19. Endoparasitoid wasp (*Leptomastix dactylopii*) injecting its eggs and venom into a *Planococcus citri* mealybug. Photo copyright Hans Smid (www.bugsinspace.nl), used with permission.

Approximately 80% of coppiced CSSV-infected trees die shortly after removal of the trunks. The few diseased trees that are able to regenerate after coppicing often show clear disease symptoms and stunting. However, there are instances where a few coppiced diseased trees were able to regenerate and grow vigorously with very mild or no symptoms (WACRI 1948). Currently, coppiced trees are also treated with arboricides to reduce the chances of tree regeneration.

An outbreak is considered halted if no new symptoms are spotted 6 months after the cutting out. However, reinspection of a recently cut-out field for any new infections within a few months postcoppicing always results in better disease containment than neglecting a rogued field for a longer time (WACRI 1946, 1947).

A Spiraling Virus Epidemic and a Compulsory Disease Control Strategy

In spite of the early interventions that were adopted to contain the disease, the disease kept spreading to new cacao-growing regions in the country. In 1947, there were approximately 400 million cacao trees in Ghana, and 46 million of these cacao trees were already infected with the CSSV. Approximately 45 million of the 46 million infected cacao trees were in the Eastern Province alone (Danquah 2003; Greenwood 1943). Cacao yield in the Eastern Province also dropped drastically to just 70,000 t in the 1945–46 season, from 116,000 tons 8 years earlier. Between 1939 and 1944, the swollen shoot disease killed 74% of all of the cacao trees that were planted at Koransang in the Eastern Province from 1904 to 1914. Their yield had also dropped from 30 t in the 1926–29 season to just 6 t in 1943–44 season. The disease was now advancing steadily toward the Western Province and other cacao-growing regions of Ghana (Danquah 1994, 2003).

Considering the pace of the epidemic, in 1947 it was estimated that, each year, 15 million cacao trees were becoming infected with the virus. With the disease threatening to wipe out Ghana's cacao trees, the colonial government proposed the removal and rehabilitation of all CSSV-infected cacao farms in Ghana. All infected cacao trees and their immediate adjacent, apparently healthy-looking cacao trees were to be cut out to minimize the spread of the disease (Danquah 1994, 2003). In all, 114 million cacao trees were removed between 1946 and 1962. Approximately 93.5% of these destroyed cacao trees were in the Eastern Province. This implies that, in 1962, an estimated 23,700 t of cocoa beans were lost in the Eastern Province alone as a direct consequence of cutting out cacao trees (Bateman 1966).

How a Virus Disease of Cacao Trees and Its Eradication Policies Contributed to Ghana's Independence from Britain

To the Ghanaian cacao farmers, their farms were long-term investments intended to be passed on to their descendants. Therefore, any policy that appeared to obliterate these lifelong assets was destined to face severe opposition, particularly from cacao farmers who were yet to witness the devastation of the disease on their farms. To make things worse, this policy was being enforced by an occupying foreign authority, Britain. Because the farm sizes of many cacao farmers at that time rarely exceeded 3 acres, the removal policy meant some farmers lost their entire farms. Soon there were rumors among Ghanaians that the Europeans were establishing swathes of cacao farms in the Far East and, by destroying Ghana's cacao trees, they were securing their cacao investments elsewhere. The eradication gangs were also paid based on how many cacao trees they removed. Worst of all, the cutting out program was also happening at a time when cocoa prices were soaring after the end of the second world war in 1945 (Danquah 2003).

Cacao farmers opposed the eradication gangs. In 1947, when the British colonial government realized that the farmers were fighting the disease control measures, it made the program compulsory. Cacao farmers that resisted eradication of diseased trees were required to pay fines or face imprisonment. Farmers were to be compensated with £12 for every acre of cacao farm destroyed (Danquah 1994).

Due to the system of indirect rule by the British in the Gold Coast (Ghana), local chiefs held considerable power. The educated, urban middle- and upper-class Ghanaians that returned to the colony after their Western education did not always have the support of the local chiefs in power; thus, they could not enact their radical ideas against the colonial authority. However, the rural discontent spurred by the eradication program fueled broader opposition to British authority in Ghana (Danquah 1994). On 4 August 1947, Ghana's first political party, called the United Gold Coast Convention, was founded. At this stage, the cutting-out policy was an overwhelmingly politicized topic. The fatal shootings of three Ghanaian World War II veterans by the British colonial police force in 1948 created a crisis. Due to the pandemonium, the cutting out of diseased cacao trees was also to be suspended pending an investigation into the national discontent (Danquah 1994; Killingray 1983).

Upon recommendations from a British-led commission of inquiry, three plant pathologists from the Food and Agriculture Organization of the United Nations—Professor E. van Slogteren from Holland, Walter Carter from Hawaii, United States, and G. H. Berkeley from Canada—were invited to also independently assess the feasibility of the CSSVD eradication policy. The swollen shoot disease of cacao control regulation order was reinstated in December 1949. However, plans were also expedited to ensure that Ghanaians were accorded greater authority in matters pertaining to the governance of the country. In 1950, Dr. Kwame Nkrumah was elected as Leader of the Government Business and later the Prime Minister (Danquah 1994). In 1951, the newly organized African government suspended the CSSVD eradication policy in order to allow an all-African commission of enquiry to assess its feasibility. The African commission of enquiry agreed to reinstate the CSSVD eradication policy but also to increase the monetary compensations paid to the affected cacao farmers. On 6 March 1957, Ghana gained independence from Britain (Danquah 1994, 2003). The roguing of diseased cacao trees, together with the apparently healthy cacao trees surrounding the diseased trees, has continued to this day (Ameyaw et al. 2015).

The Future of CSSV Research in Ghana

Plant virus research in Ghana started at a time when plant virology had received little attention in Africa. Using the technology available at the time, the Central Cocoa Research Station (which later became the West African Cacao Research Institute, then the Cocoa Research Institute of Ghana) pioneered the research into the CSSV, documenting its progress in annual reports starting from 1938. These reports are available at the library of the Cocoa Research Institute of Ghana for public access. However, the pace of research has not kept up with recent advances in plant virology. Perhaps it is time now to accelerate development in the field of African plant virology.

How fast CSSV is evolving and if it is evolving at all over the years since its discovery are not known, although a recent publication pointed to evidence of recombination events occurring among coinfecting CSSV strains in a shared cacao host (Ramos-Sobrinho et al. 2020). Are the emergent recombinant virus strains still transmissible by the mealybug vectors? Could they have gained additional vectors or perhaps additional modes of transmission? These are fertile fields for future research.

Masking of symptoms in rather infectious hosts is quite common among CSSV-infected cacao plants. What are the biotic and abiotic factors that promote masking of symptoms in infectious cacao plants? Are the characteristic red vein banding symptoms seen in newly infected cacao plants a manifestation of a defense response elicited against the offloading of the virions from the leaf vasculature into the leaf tissues? Does gene silencing have any role to play in the tolerance of cacao plants to CSSV infection? Which secondary metabolites or phytohormones are critical to CSSV defense signaling? Has the maintenance of the virus strains in cacao seedlings by frequently passing them through susceptible cacao seedlings using

mealybugs over these past decades altered the virus strains we work with?

By establishing a state-of-the-art research center such as, perhaps, an African Center for Plant Virology and Bioinformatics Research, all of these research questions could be answered here in Africa. In addition, breeding for CSSV resistance could be accelerated. Such a modern research facility would not only broaden the field of knowledge on CSSV to equip us with good information on what the effective management of the disease may entail but also could be a center for researching the virus diseases of other important African crops that are equally battered by virus diseases. Establishing such a center in Africa could also entice young African talents into pursuing plant virology as a career pathway. The Government of Ghana, the Cocoa Research Institute of Ghana, and the public universities in Ghana will be more than excited to assist in setting up such a research facility on plant virology research in Africa.

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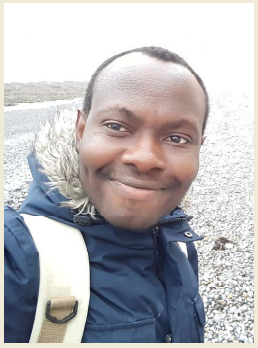

Ebenezer Antwi Gyamera

Having been brought up in rural Ghana where the predominant occupation was subsistence farming, I grew up with a natural inclination toward agriculture. Therefore, it is probably not surprising that I studied Agricultural Science at Amaniampong Senior High School from 2002 to 2005, Agriculture Science (B.Sc.) at the University of Cape Coast from 2006 to 2010, and Nuclear Agriculture (Mutation Plant Breeding and Plant Biotechnology, M.Phil.) at the Graduate School of Nuclear and Allied Sciences, University of Ghana from 2011 to 2013. My curiosity in plant virology blossomed at the University of Ghana after taking a course on plant viruses. I was happy to pursue a career as a virologist and so decided to write my M.Phil. thesis on detection of cucurbit viruses in Ghana after it was proposed by the Head of Department, Prof. Harry Mensah Amoatey. In 2015, I gained admission into the University of Cambridge, United Kingdom to further explore my passion for the exciting world of plant viruses. I completed my doctoral research on induced resistance to aphids (*Myzus persicae*) and viruses in *Arabidopsis thaliana* and *Capsicum annuum* under the supervision of Prof. John Carr at the Department of Plant Sciences in 2019. I returned to Ghana in 2020 and, in 2021, I started working as a plant virologist at the Cocoa Research Institute of Ghana. Currently, I work on virus diseases of cacao plants but, in 2023, this will be expanded to include research on coffee viruses as well.

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