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REVIEW

Mild strain protection of cocoa in Ghana against cocoa swollen shoot virus—a review

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CONTENTS

INTRODUCTION

Cocoa (*Theobroma cacao*) has been grown in Ghana, formerly the Gold Coast, since the nineteenth century. Cocoa, which originated from the lower Amazon of Brazil, was brought to Ghana from Fernando Po in 1879 and from São Tome in the 1880s (Legg, 1972) and the uniform population derived from these imports became known as the West African Amelonado cocoa (Thresh *et al.*, 1988a). The first recorded export of beans from Ghana was in 1891 (Legg & Owusu, 1977) and Ghana became the world's leading producer with a peak production of 557 000 tonnes in 1964–1965 (Bateman, 1974). Owing to a decline in the world market price of cocoa and economic constraints in addition to pest and disease problems, Ghana is now the world's third largest cocoa producer (295 000 tonnes in 1989–1990) and accounts for just over 12% of world production.

Several pests and diseases affect Ghanaian cocoa production. Mirids (*Distantiella theobroma* and *Salbergella singularis*) are major pests in most cocoa-growing areas while black-pod, a fungal disease caused by *Phytophthora palmivora* and *Phytophthora megakarya*, causes chronic crop losses. Cocoa swollen shoot virus (CSSV), while a particular problem in the Eastern Region, is prevalent throughout the cocoa-growing regions of Ghana.

VIRUSES OF *THEOBROMA CACAO*

Brunt & Kenten (1971) described the viruses affecting cocoa in West Africa and identified CSSV, cacao mottle leaf virus (CMLV), cacao yellow mosaic virus and cocoa necrosis virus. CSSV, a member of the badnavirus group, infects cocoa in all the major cocoa growing areas of West Africa. CMLV, found in the Volta Region and elsewhere in Ghana, and in Alaparun, Nigeria, is morphologically very similar and serologically closely related to CSSV strains and is now included within the CSSV group (Posnette, 1947). In addition to these bacilliform viruses, cocoa necrosis virus (CNV) is found in both Ghana and Nigeria and is assigned to the nepovirus group of small spherical nematodetransmitted viruses (Posnette, 1948; Kenten & Owusu, 1970). Other virus and suspected virus diseases of *T. cacao* such as cocoa yellow mosaic virus (tymovirus group; Blencowe *et al.*, 1963b), cocoa Trinidad virus and other putative virus diseases have been reported from Cameroon, Sierra Leone, Tanzania, Central and South America, West Indies, Indonesia and Malaysia (Thorold, 1975).

This review considers only the 'isolates' and 'strains' of CSSV found in Ghana. The term 'isolate' will be used to describe individual accessions of CSSV which are usually named after the area or village from which they were obtained. Many of the isolates are incompletely characterized. The word 'strain' will be used to describe pure cultures of virus, as determined by consistent symptom expression in Amelonado cocoa.

Cocoa swollen shoot disease was first recognized in 1936 (Steven, 1936) but almost certainly occurred in 1920 (Dale, 1962). The presence of the causal agent, CSSV, was later demonstrated by Posnette (1940). Brunt *et al.* (1990) have published a recent description of the virus. Several attempts have been made to calculate the impact of CSSV on Ghanaian cocoa production. Estimates of annual yield losses due to CSSV vary from about 20 000 tonnes (Phillips, 1962) to approximately 120 000 tonnes of cocoa from Eastern Region alone (Hale, 1953). Legg (1977) estimated the average annual loss between 1946 and 1974 to be worth over £3 650 000.

CSSV isolates may induce both leaf symptoms and swellings on the stems and roots. Leaf symptoms may include red vein banding (Posnette, 1941) where the red coloration is attributed to accumulation of anthocyanins (Knight & Tinsley, 1958) along the veins and veinlets. This is followed by chlorotic vein flecking or banding which may extend along larger veins giving angular flecks (Posnette, 1947). The banding may be confined to the secondary veins to form a 'fern leaf' pattern. Stem swellings may develop at the nodes, internodes or tips of shoots (Posnette, 1941, 1947). Many isolates of CSSV also induce swellings on the roots (Attafuah, 1957). The most apparent symptom exibited by pods is a change in shape, although some green mottling may occur. The pods become rounder and may at times become almost spherical (Posnette, 1943). In addition, infected pods are smaller than healthy pods and their surface is smoother. Some isolates of CSSV induce only leaf symptoms (e.g. Kpeve, Enchi), others induce stem and root symptoms with only very mild leaf symptoms (e.g. Bakukrom), while many other isolates induce both types of symptoms (e.g. Kofi Pare and New Juaben). In some cases the leaf symptoms are transient while in other cases severe symptoms recur and the infection leads to the death of the plant.

CSSV is transmitted by at least 13 species of mealybugs of the family-group Pseudococcidae within the Coccoidae (Roivainen, 1976) and at least a further four incompletely identified mealybug species may also be vectors (Thorold, 1975). Vectors include *Planococcoides njalensis*, *Pl. citri*, *Ferrisia virgata*, *Phenacoccus hargreavesi*, *Pl. kenyae*, *Pseudococcus concavocerrari*, *Ps. longispinus*, *Delococcus tafoensis* and *Paraputo anomalous* (Posnette, 1941, 1950a; Cotterell, 1943; Dale, 1957; Bigger, 1972). The mealybugs (nymphs of both sexes and adult females) spread the disease radially between adjacent trees by crawling through the canopy from infected to healthy trees or by being carried by attendant ants (*Crematogaster* and *Camponotus* spp.). Adult male mealybugs have only rudimentary non-functional mouthparts and do not feed. Occasionally, jump spread may occur when infective mealybugs are blown by the wind and infect trees some distance from the original site of infection (Strickland, 1950; Cornwell, 1960; Thresh *et al.*, 1988a).

CSSV has been isolated from several naturally occurring tree hosts including *Adansonia digitata*, *Ceiba pentandra*, *Cola chlamydantha*, *Cola gigantea* var. *glabrescens* and *Sterculia tragacantha* (Posnette, 1950b; Attafuah, 1965; Posnette, 1981). A morphologically similar virus has been isolated from the herbaceous plants *Asystesia* sp. and *Commelina* sp. in the Eastern Region of Ghana (Anonymous, 1980; H. D. Obiatey, Cocoa Research Institute of Ghana, personal communication, 1991) and can be detected using polyclonal antiserum raised against CSSV 1A.

Severe isolates of CSSV occur throughout the cocoa-growing regions of Ghana. The Eastern Region of Ghana has been the worst affected and an area of the Region was designated the Area of Mass Infection (AMI) in 1985 and surrounded by a Cordon Sanitaire (Fig. 1), an area under strict CSSV control, which is intended to reduce the risk of spread westwards. The AMI comprises over 90% of all known infected trees (Cocoa Services Division, Accra, unpublished data) but many outbreaks of CSSV have been

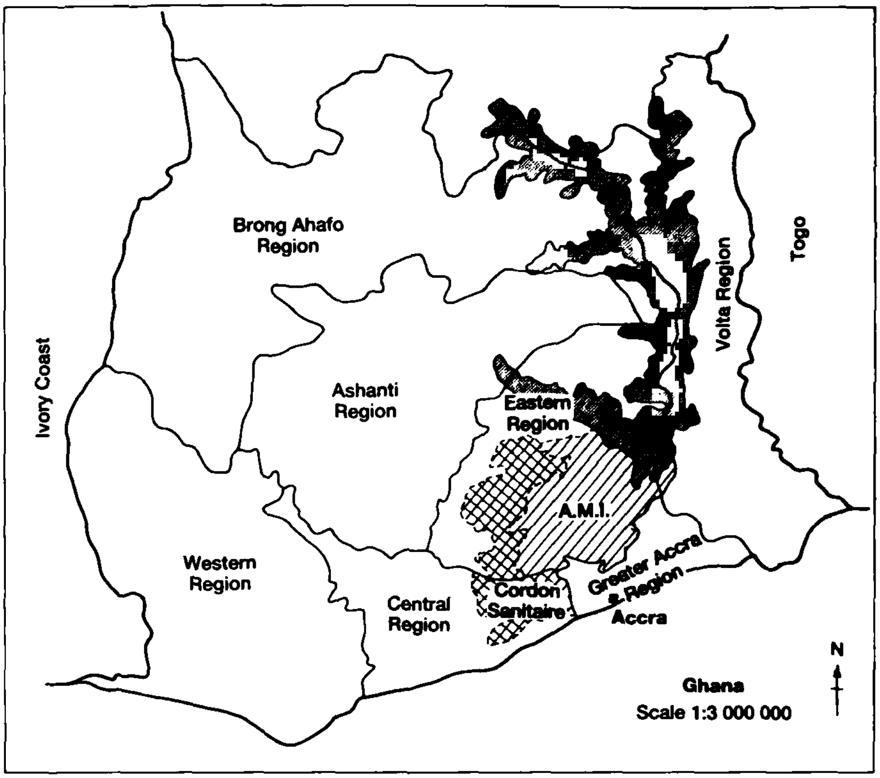

Fig. 1. Map of the cocoa-growing regions of Ghana indicating the Area of Mass Infection (AMI) of cocoa swollen shoot badnavirus (CSSV) and the Cordon Sanitaire.

identified in Brong Ahafo, Ashanti, Central, Western and Volta Regions. CSSV is also endemic in the cocoa growing areas of the neighbouring countries of Côte d'Ivoire and Togo.

Control of CSSV in Ghana is by regular inspection of the cocoa, cutting out of visibly infected trees and contact trees and subsequent reinspections of treated farms. Official statistics of trees removed by this method exist from the early 1940s. By 1988 more than 190 million trees had already been removed with an estimated 5·15 million still infected in 1989 (Ollennu *et al.*, 1989) and over 10 million in 1990 (Cocoa Services Division, Accra, unpublished data). However with the rate of discovery of new outbreaks in relation to the present rate of treatment, it is unlikely that CSSV can ever be fully controlled by this method.

In this paper research on mild strains of CSSV, their effects and ability to protect is reviewed and the potential for using mild strain protection to control CSSV in Ghana is discussed.

MILD STRAIN PROTECTION

The concept of mild strain protection with respect to plant viruses was initiated with the discovery in the late 1920s and 1930s of attenuated forms of some viruses that normally cause severe symptoms. These included tobacco mosaic tobamovirus (McKinney, 1929) and potato Y potyvirus (Salaman, 1937). Mild strains of the severe isolates may, when inoculated to susceptible plants, prevent infection by closely related severe strains. Details of the development of cross-protection against virulent plant viruses have been reviewed (Fulton, 1986). In addition to the control of citrus tristeza closterovirus in Brazil and tomato mosaic tobamovirus in the United Kingdom and elsewhere, Fulton (1986) lists other viruses for which mild strain protection has been considered: papaya ringspot potyvirus, passionfruit woodiness potyvirus, citrus concave gum sobemovirus, peach and apple mosaic ilarviruses, cauliflower mosaic caulimovirus, tomato aspermy cucumovirus and avocado sunblotch viroid. CSSV control was also included as a candidate for mild strain protection. A recent success has been achieved in the control of zucchini yellow mosaic potyvirus in cucurbit crops (Lecoq *et al.*, 1991; Walkey *et al.*, 1992).

Posnette & Todd (1955) described some conditions which they considered must be met before mild strain protection can be used:

1. the disease must be endemic and impossible to eradicate (for political or economic reasons);
2. the virus must spread rapidly enough to endanger new plantings, particularly where re-planting has taken place after destruction of the previous crop;
3. losses from the disease must be so great that some reduction in yield as a result of infection by the mild strain is an acceptable alternative;
4. there must be evidence that mild strains can protect against the severe strains without themselves causing undue harm.

Fulton (1986) lists several reasons why mild strain protection should be used with caution. There is a possibility that protection is only partial or incomplete. It is necessary to determine the relative concentration of challenge virus that will super-infect the mild strain protected plant and to determine whether this concentration is likely to be encountered naturally. It is also necessary to determine whether the protecting mild strain can be transmitted to other hosts where its effect may be more severe. The possibility of synergism cannot be discounted. If a distantly or unrelated virus spreads into the crop a more severe reaction might occur because of the presence of the mild strain. The crop may also be more susceptible to infection by other pathogens. A further reason for caution is the possibility that the mild strain could mutate into a strain that may cause severe crop losses. Finally, the logistics of inoculating a whole crop with the mild strain could be a problem and render this means of control impracticable or uneconomic.

While several possibilities have been discussed by Sequeira (1984), Sherwood (1987) and Urban *et al.* (1990) in order to explain the mechanism of viral cross-protection, the actual mechanism(s) involved are not known.

VARIATIONS WITHIN THE CSSV GROUP

Posnette (1941) considered it likely that more than one virus or strain of the virus was responsible for the different swollen shoot disease symptoms observed. The variation in symptoms that Posnette (1947) described permitted him to identify four variants of the virus as 1A, 1B, 1C and 1D but he stated that this was 'without prejudice to their possible relationship as distinct viruses or strains'. Table 1 lists the CSSV isolates which have previously been described as well as those which have been collected and maintained at the Cocoa Research Institute of Ghana (CRIG).

These isolates have been grouped at CRIG according to geographical distribution and symptom expression as follows.

Isolates from Ashanti Region:
stem/root swellings, red vein banding and conspicuous leaf symptoms.

Isolates from Brong Ahafo Region:
stem/root swellings, red vein banding and conspicuous leaf symptoms.

Isolates from Central Region:
stem/root swellings, red vein banding and conspicuous leaf symptoms.

Isolates from Easter Region:
(a) severe symptoms. These viruses generally induce severe leaf symptoms, stem/root swellings and pod distortion;
(b) mild, transient, symptoms, generally believed to be mild isolates of the severe New Juaben isolate;
(c) red vein banding and/or stem/root swellings but no conspicuous and persistent leaf symptoms;
(d) cocoa mottle leaf virus isolates from *Adansonia digitata*.

Isolates from Volta Region:
(a) cocoa mottle leaf type, mild symptoms, red vein banding and leaf mottling;
(b) stem and root swellings, occasionally red vein banding;

Isolates from Western Region:
(a) mild symptoms, red vein banding;
(b) mosaic symptoms (derived from virus infecting *Cola chlamydantha*);
(c) severe leaf symptoms and conspicuous stem/root swellings.

The mild strains which have been considered for the purposes of cross-protection are of the Eastern Region group b. The mild strains were collected within severe CSSV outbreaks from symptomless trees. In addition, a mild type of the relatively avirulent Western Region type, Enchi, has been isolated.

Sagemann *et al.* (1985) distinguished five groups (A–E) of isolates of CSSV on the basis of leaf symptoms and serological properties (by

Table 1. Ghanaian cocoa swollen shoot virus isolates which have been maintained at the Cocoa Research Institute of Ghana; the isolates from each Region are listed alphabetically with the natural host from which they were isolated, any synonyms and a brief description of their symptoms in Amelonado cocoa

Isolate	Synonyms	Source (Region)	Natural host	Symptoms I	L	S	R	P	References
AD 75	CMLV[a]	Ashanti	*Adansonia digitata*	+	+	–	–	–	8
Amakom/Bosomtwi		Ashanti	*Theobroma cacao*	+	+	+	+	–	8
Amanchia		Ashanti	*T. cacao*	+	+	+	+	–	8
Bobriso/Juaso[b]		Ashanti	*T. cacao*	+	+	+	–	–	8
Bosomtwe	1J	Ashanti	*T. cacao*	+	+	+	+	–	6, 7, 8
Koben		Ashanti	*T. cacao*	+	+	+	+	–	8
Konongo	1K	Ashanti	*T. cacao*	+	+	+	+	+	5, 8
Krofa/Juansa		Ashanti	*T. cacao*	+	+	+	+	–	8
Kwakoko/Juansa		Ashanti	*T. cacao*	+	+	+	+	–	8
Madjeda/Nkwanta		Ashanti	*T. cacao*	+	+	+	+	–	9
Morso		Ashanti	*T. cacao*	+	+	+	+	–	8
Onyimso/Agogo		Ashanti	*T. cacao*	+	+	+	+	–	8
Sedi-Nkawie		Ashanti	*T. cacao*	+	+	+	+	–	8
Bechem	F1	Brong Ahafo	*T. cacao*	+	+	+	+	–	8
Kwadwokumkrom		Brong Ahafo	*T. cacao*	+	+	+	+	–	9
Kwaku Anyan	T1	Brong Ahafo	*T. cacao*	+	+	+	+	–	8
Nkrankwanta	T1	Brong Ahafo	*T. cacao*	+	+	+	+	–	8
Nkwanta/Dorma Ahenkro		Brong Ahafo	*T. cacao*	–	+	+	+	–	9
Okerikrom		Brong Ahafo	*T. cacao*	+	+	+	+	–	8
Sankore		Brong Ahafo	*T. cacao*	+	+	+	+	–	8
Techimantia		Brong Ahafo	*T. cacao*	+	+	+	+	–	8
Nsaba		Central	*T. cacao*	+	+	+	+	–	8
Group A									
Achiasi		Eastern	*T. cacao*	–	+	+	+	–	9
Agyepomaa		Eastern	*T. cacao*	+	+	+	+	–	9
Asamankese		Eastern	*T. cacao*	–	+	+	+	–	9
Dawa[b]	1H	Eastern	*T. cacao*	+	+	+	+	+	5, 8
Dochi	1G	Eastern	*T. cacao*	+	+	–	–	–	5, 8
Kofi Pare	1A	Eastern	*T. cacao*	+	+	+	+	+	8
Mampong	1M	Eastern	*T. cacao*	+	+	+	+	+	4, 6, 7, 8
Miaso		Eastern	*T. cacao*	(+)	+	+	+	–	8
New Juaben	Tafo yellows, 1A	Eastern	*T. cacao*	+	+	+	+	+	4, 7, 8
Nkawkaw	1D	Eastern	*T. cacao*	+	+	+	+	+	4, 8
Pamen	1E	Eastern	*T. cacao*	(+)	+	+	–	–	5, 8
Tease Atomsu Aboum		Eastern	*T. cacao*	–	+	+	+	–	9
Group B									
N1[b]		Eastern	*T. cacao*	(+)	(+)	+	–	–	9
SS 70[b]	M1	Eastern	*T. cacao*	(+)	(+)	–	–	–	3, 8
SS 90[b]	M1	Eastern	*T. cacao*	(+)	(+)	–	–	–	3, 8
SS 167[b]	M1	Eastern	*T. cacao*	(+)	(+)	–	–	–	3, 8
SS 365B[b]	M1	Eastern	*T. cacao*	(+)	(+)	–	–	–	3, 8
Group C									
Bisa[b]	1B	Eastern	*T. cacao*	(+)	–	+	+	–	4, 7, 8
Donkorkrom[b]		Eastern	*T. cacao*	(+)	(+)	+	+	–	9
Tease Aduadan		Eastern	*T. cacao*	(+)	(+)	+	+	–	8
Tease Adeakyi		Eastern	*T. cacao*	(+)	(+)	+	+	–	8
Group D									
AD 7	CMLV	Eastern	*A. digitata*	+	+	–	–	–	8
AD 36	CMLV	Eastern	*A. digitata*	+	+	–	–		8
AD 49	CMLV	Eastern	*A. digitata*	–	+	(+)	–		9
AD 111	CMLV	Eastern	*A. digitata*	–	+	(+)	–		9
AD 135	CMLV	Eastern	*A. digitata*	+	+	–	–	–	8
AD 196	CMLV	Eastern	*A. digitata*	+	+	–	–		8

Table 1. (continued)

Isolate	Synonyms	Source (Region)	Natural host	Symptoms I	L	S	R	P	References
Group A									
Goviepe Todzi		Volta	*T. cacao*	+	+	–	–	–	8
Kpeve	1C	Volta	*T. cacao*	+	+	–	–	–	4, 7, 8
Wusuta		Volta	*T. cacao*	+	+	–	–	–	9
Group B									
Djinji		Volta	*T. cacao*	(+)	+	+	+	–	8
Peki[b]		Volta	*T. cacao*	–	–	+	+	–	8
Peki Tsame[b]		Volta	*T. cacao*	–	–	+	+	–	8
Worawora[b]	1W	Volta	*T. cacao*	(+)	–	+	+	–	1, 2, 8
Group A									
Aduakaa/Enchi		Western	*T. cacao*	+	–	–	–	–	9
Enchi[b]		Western	*T. cacao*	+	–	–	–	–	9
Group B									
Aboboya		Western	*Cola chlamydantha*	(+)	+	+	+	–	8
Achechere		Western	*C. chlamydantha*	(+)	(+)	+	+	–	8
Adiembra		Western	*C. chlamydantha*	(+)	+	+	+	–	8
Aiyim		Western	*C. chlamydantha*	(+)	+	+	+	–	8
Anibil		Western	*C. chlamydantha*	+	+	+	+	–	8
CC644		Western	*C. chlamydantha*	–	+	+	+	–	9
Group C									
Amafie	1F	Western	*T. cacao*	–	+	+	+	+	8
Bakukrom		Western	*T. cacao*	+	+	+	+	–	8
Bosomuoso	1F	Western	*T. cacao*	–	+	+	+	+	7, 8
Datano		Western	*T. cacao*	–	+	+	+	+	8
Jamesi		Western	*T. cacao*	+	+	+	+	+	8
Punekrom	1F	Western	*T. cacao*	+	+	+	+	+	8
Surawno	1F	Western	*T. cacao*	+	+	+	+	+	8
Suhuma[b]	1F	Western	*T. cacao*	+	–	+	+	–	9
Wiawso	1F	Western	*T. cacao*	–	+	+	+	+	6, 8
Yiboso		Western	*T. cacao*	–	+	+	+	–	9

[a] Cocoa mottle leaf virus.
[b] Mild isolates.
Key: I, Symptoms on immature leaves, typically red vein banding; L, symptoms on mature leaves, typically mosaic, chlorotic vein clearing and flecking; S, Stem (fan/chupon) swellings; R, root swellings; P, pod symptoms.
+, Symptoms present; –, symptoms absent; ( ), symptoms rare or very mild.
References: 1, Attafuah & Dale (1951); 2, Glendinning *et al.* (1966); 3, Ollennu & Owusu (1989), 4, Posnette (1947); 5, Thorold (1975); 6, Thresh & Tinsley (1959); 7, Tinsley & Wharton (1958); 8, Owusu & Bonney (in preparation); 9, L. A. A. Ollennu (unpublished).

enzyme-linked immunosorbent assay (ELISA) and immunosorbent electron microscopy) using antiserum raised against CSSV 1A. These groupings are compared in Table 2 with those based on areas of collection and symptom expression.

Most of the samples that Sagemann *et al.* (1985) assigned to Group A are those isolates with both leaf symptoms and stem swellings, although examples from this group also exhibit only leaf symptoms or only stem swellings. The Group D isolates are those exhibiting leaf symptoms only, while Group E exhibit only stem swellings. Geographical location has no apparent relationship with the serological groups.

VIRUS COMPLEXES

There have been few published reports of cocoa swollen shoot virus complexes occurring in individual plants. Artificially produced CSSV complexes were made by Crowdy & Posnette (1947) in cross-immunity experiments with CSSV 1A, Bisa and Kpeve isolates. Evidence of infection of trees by any isolate was the

Table 2. Isolates of cocoa swollen shoot virus listed according to symptom expression and geographical location; these are compared with the five serological groups to which Sagemann *et al.* (1985) assigned the isolates on the basis of their symptoms and the results of serological tests such as enzyme-linked immunosorbent assays and immunosorbent electron microscopy

Region	Isolates exhibiting leaf symptoms only		Isolates with stem swelling only		Isolates with both leaf symptoms and stem swellings	
Ashanti	AD75 (CMLV[a])	C[b]			Bobriso/Juaso	AB
					Bosontwi	A
					Koben	A
					Morso	A
					Onyimso/Agogo	AB
Brong Ahafo					Bechen	A
					Kwadwokumkrom	A
					Nkrankwanta	A
					Sankore	A
					Techimantia	A
Eastern	AD[c] isolates (CMLV)	CD	Bisa	BE	Agyepomaa	A
	SS[d] isolates	A	Donkorkrom	AB	Kofi Pare	A
					Kampong	AC
					New Juaben	A
					Nkawkaw	A
Volta	Kpeve (CMLV)	D	Peki	E	Djinji	A
	Wusuta (CMLV)	C	Peki Tsame	A		
			Worawora	A		
					Amafie	A
Western					Anibil	A

[a] Cocoa mottle leaf virus.
[b] Serological groupings according to Sagemann *et al.* (1985).
[c] Isolates from the alternative host of CSSV, *Adansonia digitata*.
[d] Mild strain isolates.

production of visible symptoms. In trees inoculated with Bisa (producing only stem swellings) and Kpeve (producing only leaf mottle), additional symptoms of mosaic were produced after inoculation with 1A, indicating super-infection. There is some evidence that two isolates can remain in the same tree for a considerable time. The authors found that there was some partial protection by the Bisa isolate against 1A on the basis of crop yield. This protection continued in one of the experiments for 2 years, after which the effect disappeared. Presumably, after this time the severe isolate became dominant. The authors did not determine whether the Bisa isolate could be recovered from the trees after the partial protection had broken down.

Posnette & Todd (1951) found both mild and severe isolates of CSSV 1A in inoculated Amelonado seedlings. In part of their experiment they used a mild strain of 1A to protect seedlings from super-infection by the severe 1A isolate. After graft inoculation of the mild strain-protected seedlings with the severe strain, both mild and severe symptoms were produced. While the authors were primarily able to recover mild strains from plants with mild symptoms and severe strains from those with severe symptoms, they were also able to recover mild strains from plants with severe symptoms and severe strains from plants exhibiting mild symptoms.

A naturally-occurring virus complex was described by Posnette & Todd (1955). They investigated a CSSV-infected farm on which the trees had been coppiced but some of the infected stumps had regenerated. Some of the regenerated trees had developed symptoms characteristic of the severe isolate of CSSV while some exhibited only mild 'fern leaf' symptoms, which was described by Posnette (1947) as characteristic of attenuated CSSV 1A. The farm had, after felling, been replanted with cocoa and the authors found that the young trees had either mild or severe symptoms. Uniform Amelonado seedlings were used for testing the replanted and regenerated trees. Test plants inoculated from replanted trees with severe symptoms uniformly developed severe symptoms. One of the seedlings inoculated from a regenerated tree with conspicuous symptoms only developed moderate symptoms while the other test seedlings developed severe symptoms. However, a more convincing indication of mixed infection was shown when a seedling showing only 'slight' symptoms induced both mild and intermediate reactions on different test plants. From these results and observations the authors concluded that more than one variant of CSSV was present in the original CSSV outbreak and that some of the trees were infected with both mild and severe ('virulent') types.

The same authors described the results of patch grafting from naturally infected trees showing slight symptoms. They took samples from different parts of one of the trees and found that when samples from the lateral roots of these trees were grafted to a uniform series of test seedlings both mild and intermediate isolates were found and one of the test seedlings produced symptoms indicative of the severe isolate. The other parts of the tree produced mild symptoms in the test plants with some of the sites (basal leaf-bearing chupons (shoots) and distal leaf-bearing twigs in the canopy) producing intermediate symptoms. Severe, intermediate and mild types were isolated from a naturally infected tree showing severe symptoms and both mild and intermediate types from a tree with severe defoliation. In further experiments the authors found no selective effect of roots and stems on the mild, intermediate and severe types isolated independently of the site from which they came. Further studies indicated that a complex of virus types occurs in most cocoa trees naturally infected with CSSV 1A and that serial transmission tends to segregate the strains in test seedlings. The separation of the strains was, at times, found to require up to three sequential transmissions by grafting to test seedlings and the authors suggest, as the strains can eventually be separated by grafting, that the different strains arise not primarily from a high virus mutation rate but from mixed infections.

Posnette & Todd (1955) were further able to inoculate simultaneously mild and severe ('virulent') isolates of CSSV to young trees. They found some of the trees exhibiting mild symptoms, some with severe symptoms and others with intermediate symptoms. After cutting back, some of the trees with severe symptoms later developed only mild symptoms and one of these trees later developed severe symptoms again. These observations would indicate virus complexes existing in the doubly inoculated trees, with different strains dominating, depending on the quantity of virus in the initial inoculum and the physical condition of the tree.

Some cocoa plants, in which mild strains of CSSV (SS70, SS90, SS167E and SS365B) were maintained for many years in a collection, were reported by Ollennu & Owusu (1989) to have become infected with severe isolates. Instead of only transient red vein banding symptoms, plants infected with a mixture of nominally mild strains developed fine vein flecking, vein banding, chlorosis and stem swellings. By serial mealybug transfer, the complexes of strains could be separated and apparently uncontaminated mild strains were retrieved.

At present mild strain protection is being considered for controlling CSSV in the AMI (Eastern Region) and Central Region where the severe 1A isolate predominates. Many other isolates from this area have been identified as serologically closely related to several 1A isolates (Hughes & Ollennu, unpublished data). Most complexes of more distantly related CSSV isolates have been produced artificially. It is likely that if virus complexes were to be a problem in the implementation of mild strain protection, it would only be where areas of cocoa infected with serologically distinct isolates have adjoining boundaries, and this is unlikely to occur in the AMI.

EXPERIMENTAL TRANSMISSION OF MILD AND SEVERE STRAINS AND ISOLATES

There are three methods of experimental transmission of CSSV: budding or grafting, mealybug transmission and manual inoculation.

Posnette (1940, 1941) first transmitted the agent causing swollen shoot disease by budding from infected trees in the field to healthy cocoa seedlings. Many other researchers continued to use budding and grafting, both for laboratory studies with seedlings (Posnette & Todd, 1951, 1955) and field trials (Crowdy & Posnette, 1947; Glendinning *et al.*, 1966; Legg & Kenten, 1971;

Brunt, 1975) using severe isolates of CSSV. Graft transmission of mild isolates and strains of CSSV has also been achieved (Posnette & Todd, 1951, 1955; Ollennu & Owusu, 1989).

Vector transmission of CSSV using viruliferous mealybugs was described by Posnette & Strickland (1948) and has been widely used for CSSV transmission to cocoa beans, seedlings and trees (Attafuah *et al.*, 1963; Attafuah & Glendinning, 1965; Owusu, 1969; Adomako & Owusu, 1974; Legg & Lockwood, 1977; Adomako *et al.*, 1983; Sagemann *et al.*, 1983; Adomako, 1989). A low rate of transmission of mild strains with mealybugs to both Amelonado and hybrid cocoa was found by Ollennu & Owusu (1989). They found a mean overall infection rate of 41·7% using patch grafts and only 11·1% for mealybug transfers to seedlings. However, Ollennu & Owusu (1989) obtained mild strain transmission rates of 68–70% by mealybug inoculation to cocoa beans.

Manual inoculation using sap to cocoa beans was developed by Brunt & Kenten (1960, 1962). A poor rate of transmission was achieved until centrifuged cocoa extracts were used by Brunt (1963). The technique was further developed by Legg & Kenten (1969) to assess resistance to infection, and by Adomako & Owusu (1974).

Graft transmission remains a useful way of detecting CSSV in individual trees and for infecting trees with mild strains and severe isolates in the field. Mealybug transmission is now favoured for the transmission of virus from single source plants to numerous seedlings by bean inoculation, while manual inoculation requires purified virus and is generally used for assessing resistance/tolerance or for assessing the infectivity of purified preparations.

CROSS-PROTECTION OBSERVATIONS AND EXPERIMENTS

Protection by an 'attenuated' isolate of CSSV was described by Posnette (1945) who reported results of cross-immunity experiments involving a mild strain ('attenuated' strain A) of CSSV 1A discovered in apparently healthy trees surviving in cocoa farms that had been devastated by swollen shoot (Posnette, 1947). Leaf symptoms induced by the attenuated strain were inconspicuous, swellings were rarely induced and pod symptoms were absent. Bark grafts from these trees to susceptible seedlings conferred resistance to the severe isolate. The authors did not report whether the severe isolate could be isolated from the protected seedlings after challenge inoculation.

Posnette (1945) also reported the ability of the Bisa isolate to protect seedlings against infection by 1A. However, this protection is not reliable and the Bisa isolate itself induces conspicuous stem and root swellings. Posnette also noted that the Kpeve isolate failed to protect against 1A. Further cross-immunity experiments on isolates 1A, Bisa and Kpeve were described by Crowdy & Posnette (1947). Cocoa trees were first inoculated with the protecting isolate by bark grafting. Subsequent challenge by 1A was either by grafting or natural mealybug transmission. When challenged by grafting there was partial antagonism by the Bisa isolate but no protection by the Kpeve isolate. However, when the challenge was by natural transmission there was strong evidence of a protective effect by the Bisa isolate. The lower reliability of cross-protection when the challenge virus is graft inoculated was also shown by Bawden & Sheffield (1944) with potato viruses. Posnette & Todd (1955) found that if sufficient challenge strain is introduced, such as by three super-infecting grafts performed simultaneously, the normally effective cross-protection can be overcome. Challenge of cross-protection to mimic field conditions is most appropriate using vector transmission, rather than an artificial method such as grafting which can introduce a disproportionately large amount of inoculum and over a prolonged period.

Crowdy & Posnette (1947) observed 67·1% natural infection with the severe 1A isolate of CSSV in unprotected trees, 53% infection by 1A in trees protected by the Bisa isolate and 76·4% infection in those protected by the Kpeve isolate. The 14·1% reduction in severe symptom expression seen in the Bisa isolate, while statistically significant, is of little practical importance as more than 50% of the trees were still infected with the severe strain. This relatively low cross-protection occurrence is likely to be because Bisa is not very closely related to CSSV 1A.

In continued work (Posnette & Todd, 1955) it was observed that plants can be protected with mild strains against the effects of severe isolates which often remain latent. When the trees are subsequently coppiced, the severe symptoms are often exhibited. Over a 5-year period of study 76% of unprotected trees developed severe symptoms as a result of natural spread from infected guard rows compared with only 14% of mild strain protected trees.

For efficient cross-protection to occur, a very close relationship between the protecting and challenge viruses is required (McKinney, 1941; Bawden & Sheffield, 1944). This observation was subsequently made by Posnette & Todd (1955) who state that with cocoa viruses, in general, protection is rarely achieved but if a mild strain of a severe isolate is used, cross-protection can be achieved against that severe isolate. Sagemann *et al.* (1985) assigns CSSV 1A to group A while the different Bisa isolates are assigned to groups B and E. This serological difference between the two isolates has also been found in recent studies (Hughes & Ollennu, unpublished data) where Bisa is only distantly related to the 1A group.

A further example of possible incomplete cross-protection was described by Ollennu & Owusu (1989), who challenged seedlings protected with mild strains (SS isolates of the severe 1A type) by graft inoculating with the severe New Juaben (1A) isolate. They used the mild strains SS70, SS90, SS167E and SS365B to protect Amelonado, Upper Amazon × Upper Amazon and Amelonado × Upper Amazon hybrids. Isolate SS70 conferred the greatest protection against the severe isolate, with about 30% of the trees exhibiting severe symptoms after challenge. The other mild strains were less efficient, with up to 70% of the trees showing severe symptoms. Based on a limited amount of experimental material, the authors achieved complete mild strain protection of the hybrid T85/799 × IMC76 using SS70 but the rest of their experimental results indicate only partial cross-protection. An average of only just over 50% cross-protection was achieved.

Although the available information on mild strain protection of cocoa is limited it appears that, with careful selection of mild strains with respect to the new hybrids now available to farmers (and those being produced as a result of current breeding programmes), it may be possible to achieve protection of cocoa trees against severe isolates of CSSV. Further studies are still required, particularly with regard to protection under field conditions, but these early results are encouraging.

DISTRIBUTION OF CSSV STRAINS AND ISOLATES WITHIN TREES

Samples taken from all parts of CSSV-infected trees have been found to contain virus. CSSV was transmitted by mealybugs from immature ('flush') leaves as well as green shoots and shoot apices (Posnette & Strickland, 1948) and was detected in both immature and mature ('hardened') leaves by ELISA (Sagemann *et al.*, 1983). CSSV has also been detected in the bark of the stem and pods by mealybug transmission (Posnette & Strickland, 1948) and in roots by root grafting and ELISA (Posnette, 1947; Posnette & Todd, 1955; Sagemann *et al.*, 1983).

The techniques used for determining the presence or absence of CSSV vary in their sensitivity. Symptom expression (including visual inspection, grafting, mealybug transmission and coppicing) is commonly used. Of the serological detection methods, direct ELISA is about as effective as symptom expression at detecting CSSV infection, while the indirect ELISA is more sensitive; the virobacterial agglutination (VBA) test is the most sensitive method presently available for detecting CSSV (Hughes & Ollennu, 1993). ELISA generally only detects virus in samples from trees that are exhibiting symptoms, while VBA can be used to detect CSSV in seedlings or trees with symptomless infection.

While CSSV has been detected throughout infected trees the virus is often incompletely systemic. Posnette & Robertson (1950) failed to transmit CSSV from some petiole and stem samples using mealybugs. Detailed studies on the distribution of virus in infected leaves using ELISA showed the highest concentration of virus in the lamina and the lowest in the petiole, with an intermediate amount in the midribs (Sagemann *et al.*, 1983). Further studies showed that chlorotic areas of infected leaves contained virus, while in symptomless areas CSSV could not be detected by ELISA (Sagemann *et al.*, 1985).

The absence of symptoms may indicate the virus is absent. Posnette (1947) found that neither the Kpeve nor 1A isolates could be transmitted by grafting from symptomless parts of infected trees. However, the absence of symptoms may also indicate latent infection. Symptom expression can often be induced in trees with latent CSSV infection by coppicing (Posnette, 1951; Legg *et al.*, 1984). Trees that are coppiced are cut 30–45 cm above ground level and regenerating growth often exhibits symptoms that were absent on the uncoppiced tree. Moreover, the virobacterial agglutination test has been used to detect symptomless or latent infection in trees surrounding CSSV outbreaks (Hughes & Ollennu, 1993).

Posnette & Todd (1955) studied the distribution of severe and mild isolates in trees with mixed infection. Virus was transmitted to test plants by patch grafting from leaf-bearing twigs in the canopy, older canopy branches, basal chupons and lateral roots. Two trees exhibiting mild symptoms and two exhibiting severe defoliation were investigated. From one of the trees exhibiting mild symptoms a mild virus strain was isolated from all four sites, an intermediate strain from the twigs, chupons and roots, and a severe type only from the roots. Conversely, the severe and intermediate types were isolated from the leaf-bearing twigs and roots of the other tree exhibiting mild symptoms. Similarly, one of the trees exhibiting severe defoliation was infected with a severe type at all four sites (with mild and intermediate virus types in the twigs and roots), while the mild type was isolated from the other defoliated tree. These studies show the wide range of strain virulence in trees with mixed infection.

The same authors also attempted to determine whether there were strain-selective influences in the various parts of the tree, particularly in the leaf-bearing twigs and roots. Even in trees that had been infected for more than 3 years it was found that there was no consistent distribution of the mild and severe virus types in these trees.

CSSV isolates and strains appear to be incompletely systemic but can infect any part of the tree. In addition, in a mixed infection the individual strains or isolates can be incompletely systemic and can occur in the same part of the tree or individually in different parts. Some parts of a tree with mixed infection can also be apparently healthy. However, now the more sensitive VBA test has been developed, further studies are needed to confirm these findings.

The incompletely systemic distribution of CSSV within the trees has implications for the control of the virus by mild strain protection. If a mild strain is introduced, it is important to determine whether it spreads effectively into the new branches and leaves as the tree grows. In addition, it is important to ascertain whether an incomplete distribution of the mild strain can adequately protect a tree against infection by a severe isolate and whether that degree of protection is maintained throughout the life of the tree.

EFFECT OF MILD STRAIN PROTECTION ON GROWTH AND YIELD

The effects of severe isolates of CSSV on the growth of cocoa trees are well documented. Posnette (1947) observed that most Amelonado trees infected with CSSV 1A die within 2 years and Brunt (1975) noted that the leaf canopies begin to deteriorate 6–9 months after infection, with most trees dead or moribund by the end of the third year. The growth rate of infected trees is significantly lower (Asomaning & Lockard, 1964; Legg & Lockwood, 1981), with reduced leaf areas, reduced stem diameter increments (Legg *et al.*, 1980) and a total plant dry weight that may be only 37% that of comparable healthy trees (Asomaning & Lockard, 1964). The severe 1A isolate caused a 74% reduction in stem girth compared with healthy trees after 17 months (Glendinning *et al.*, 1966). The milder isolates Bisa, Worawora and Kpeve, while also having a deleterious effect on the canopy, caused 18%, 22% and 26% reductions in girth, respectively.

Although some cocoa trees infected with severe CSSV sustain a high yield over a long period, the infected trees usually have a reduced yield (Crowdy & Posnette, 1947; Blencowe & Brunt, 1962; Brunt, 1975). Amelonado trees are particularly badly affected, with pod yields falling by 42% within 2 years (Legg *et al.*, 1980), a reduced yield of wet beans per pod (Legg & Kenten, 1968) and often with no yield at all by the third year. The yield of Upper Amazon cocoa is also reduced after infection with the severe isolates of CSSV, generally declining to 50–60% of healthy Upper Amazon trees by the third year (Blencowe & Brunt, 1962; Brunt, 1975). It has also been noted that apparently milder isolates, such as Kpeve, can have as severe an effect on yield as the 1A isolate (Glendinning *et al.*, 1966), although more usually a 50% reduction in yield can be expected (Crowdy & Posnette, 1947).

Trees inoculated with avirulent isolates tend to show an initial slight increase in yield (Blencowe *et al.*, 1963a). Glendinning *et al.* (1966) also found that inoculation with the Bisa isolate gave an initial increase in yield. Trees protected by a mild strain of 1A initially yielded more pods than the healthy trees (Posnette & Todd, 1955); however, unlike the healthy trees, their yield failed to increase towards the end of the experiment.

In preliminary cross-protection studies using Bisa to protect against 1A, Crowdy & Posnette (1947) found that while the wet weight of beans per pod was effectively the same in protected and unprotected trees, the total plot yield indicated that there was some partial protection against the effects of 1A by the Bisa isolate.

Posnette & Todd (1955) found, by the fourth year of an experiment using mild strains to protect against 1A, that mild strain protected plots yielded more than twice that of unprotected plots. The yield of protected plots was about 78% of the peak yield of the healthy plots.

Observations on the effects of the mild strains SS365B and N1 on yield suggest that yield losses owing to mild strain protection are likely to be small (Ollennu, unpublished data).

APPROPRIATENESS OF MILD STRAIN PROTECTION IN THE CONTROL OF CSSV

Cocoa swollen shoot disease is relatively slow-spreading and is regarded as 'an archetypal crowd disease' (Thresh *et al.*, 1988b). The vectors cannot move over large distances unless blown by wind ('jump spread', considered to be uncommon), the crop is not propagated vegetatively, nor is the virus seed-borne. This would imply that the disease should be relatively easy to control. The main method of control that has been advocated is the cutting-out of infected trees but owing to logistic and socio-economic reasons (Ollennu *et al.*, 1989) this method of control has been ineffective.

Of the several options for controlling the CSSV problem in Ghana, the potential for mild strain protection and isolation by use of cocoa-free cordons and barrier crops is only now being investigated (Owusu, 1983; Ollennu & Hughes, 1991). Other means of control: breeding for CSSV resistance, use of chemicals and biological control of the vectors have been attempted but with limited success (Hannah & Heatherington, 1955; Decker, 1955; Donald, 1956; Thresh *et al.*, 1988a). Other means of control must also be investigated, including the production of transgenic plants carrying a CSSV coat protein gene which may confer resistance against infection by CSSV.

The results of the original studies on mild strain protection in cocoa (Posnette, 1945; Crowdy & Posnette, 1947; Posnette, 1947; Posnette & Todd, 1955) although promising, could not be adopted as the deliberate dissemination of mild strains was incompatible with the government policy at that time for the control of CSSV by eradication; however, the situation has changed. Despite the efforts to control the disease by cutting out infected and more recently contact trees (200×10^6 trees removed since 1946) the disease is now more widespread than ever, particularly in the AMI. As a result of a recent review of the eradication measures for CSSV, control in the AMI has virtually been abandoned. Farmers are consequently migrating elsewhere in the country to grow cocoa, even though the Eastern Region has the best soil for cocoa cultivation in Ghana (M. R. Appiah, CRIG, personal communication, 1991).

The conditions for mild strain protection outlined by Posnette & Todd (1955) now apply to the AMI. The disease is endemic and appears impossible to eradicate; it is spreading rapidly; losses from the disease are so great that some reduction in yield over a long period resulting from infection with a mild strain is a preferable alternative and there is evidence that the mild strains protect efficiently without causing undue harm.

It is unlikely, however, that mild strain protection would be appropriate for use throughout Ghana. An overall, small reduction in yield could be justified in the AMI, where the risk of total yield loss is great. However, in areas of Ashanti, Western and Brong Ahafo Regions where the risk of infection is relatively small there can be little justification in reducing the yield through widespread dissemination of mild strains.

However, there are significant problems associated with the introduction of mild strain protection against CSSV, even in the AMI. These problems must be solved if mild strain protection is to be a satisfactory method of controlling CSSV.

The means of inoculating the mild strains to the beans or seedlings for new plantings or to existing healthy mature trees must be considered. The protection would be useful at present if it could be conferred on the many farms of healthy, mature cocoa in the AMI. This would require a labour-intensive programme of field inoculation by grafting or, less efficiently and with even greater logistic problems, by mealybug transmission. However, it is more likely that the mild strain inoculation would be to cocoa beans (by mealybug transmission from infected source plants), which would then be germinated and sold to the farmers as young seedlings. A further possibility is the propagation of mild strain-infected material by vegetative means such as budding or tissue culture, which now appears to be feasible, although further investigations are needed (Adu-Ampomah *et al.*, 1991). The feasibility, economics and cost effectiveness of these possibilities require investigation. After initial inoculation, although natural spread would slowly distribute the protecting strains over a wider area, repeated inoculations in the field and distribution of mild strain-infected planting material would have to be made.

Another problem that may hinder the introduction of mild strain protection is the public's fear of possible mutation of the mild strains to severe strains, which could cause substantial crop losses. Although mutation of the mild tomato mosaic tobamovirus to a severe strain has been observed under certain environmental conditions (Brunt, 1986) there is no evidence that such mutation of mild strains of CSSV takes place. Even if a mutation of the mild strains should occur it is very unlikely that the new strain could be more devastating than the existing CSSV 1A isolate. The potential for the breakdown of the cross-protection will also have to be considered. There is evidence for the breakdown of protection against citrus tristeza closterovirus. Possible causes include the failure of the mild strains to protect against the severe challenge strains as well as the presence of severe strains in the rootstocks to which the protected material was grafted. Breakdown of protection against papaya ringspot potyvirus was found to be caused by high inoculum pressure from nearby sources (Fulton, 1986). This aspect will need careful evaluation with respect to the control of CSSV. Fears of synergistic effects, even if groundless, would have to be overcome and convincing arguments put forward in support of mild strain protection.

The spread of the mild strains, the interactions with the severe strains, the amount of crop loss caused by protection and any other possible environmental effects will have to be closely monitored over time. Some of these factors are already being investigated in field trials (G. K. Owusu, CRIG, personal communication, 1992). Education of the farmers and the extension staff would also have to include this new aspect.

CONCLUSION

While much research has still to be done in order to exploit the potential for mild strain protection of cocoa, much progress has already been made. It will be necessary to determine the appropriate combinations of mild strains and new hybrids for distribution to the farmers, ensuring a high yield of good quality cocoa as well as adequate protection against infection by severe isolates of CSSV. Additionally, methods for inoculating the mild strains to cocoa beans, seedlings or trees will have to be developed, or a technique for multiplying mild strain-infected planting material vegetatively will have to be perfected. This could enable mild strain protection to be developed, as a part of an integrated control programme, to protect the Ghanaian cocoa industry against the devastating effects of cocoa swollen shoot bednavirus.

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