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Some isolates of virus causing swollen-shoot disease of cacao in Nigeria and their interrelationships

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SUMMARY

Many symptomatically distinct virus isolates were made from cacao trees infected with swollen-shoot disease in Nigeria. The symptoms caused by typical viruses from six different localities were studied on seedling Amelonado cacao inoculated as beans. Two isolates caused swellings as the only permanent symptom and another caused only leaf chlorosis. The others caused both swellings and chloroses of different type and severity. Two atypical isolates were much less virulent than the others and caused only transient and very inconspicuous leaf symptoms.

Isolates from the same or from adjacent trees usually protected against each other, whereas those from dissimilar areas did not. This suggests that the isolates are not all closely related and they may be grouped according to the results of plant protection tests.

INTRODUCTION

Virus infection is widespread in West African cacao and several distinct viruses have been distinguished (Thresh & Tinsley, 1959). The cacao mottle leaf and cacao necrosis viruses are the least important and occur only locally, whereas the viruses which cause swollen shoot and form the subject of the present paper occur in many of the most important cacao-growing areas of Sierra Leone, Ivory Coast, Ghana and Nigeria.

The symptoms of swollen-shoot disease are not everywhere the same. Indeed, the characteristic stem swellings may be rare or even absent, and the leaf symptoms also differ between and within outbreaks. These differences are sometimes due to the host, but several strains of virus have been recognized and described from Ghana and Nigeria (Posnette, 1947*a*; Posnette & Todd, 1955). They differ not only in the type and severity of symptom they cause, but also in transmission by mealybugs (Posnette, 1950) and host range (Posnette, Robertson & Todd, 1950; Tinsley & Wharton, 1958). Avirulent strains usually protect plants against virulent ones from the same outbreak or locality, but rarely protect against viruses from elsewhere (Thresh & Tinsley, 1959, 1960).

The present paper describes the symptoms caused by different isolates of virus from outbreaks of swollen-shoot disease in Nigeria and shows that isolates can be grouped according to their ability to protect plants against each other.

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MATERIALS AND METHODS

Virus isolates

Viruses were isolated by grafting patches of bark from naturally infected trees to cacao seedlings (var. West African Amelonado) in the insectary. With a few exceptions, all the isolates described were from trees showing the commonest type of symptom in disease outbreaks within and around the areas of mass infection in Western Region (for detailed maps see Lister & Thresh, 1957).

The different isolates are described under the name of the village near which they were first collected. This convention differs from that used by Posnette (1947*a*, 1950), who referred all isolates to *Theobroma* virus I and distinguished between them by letters. This is no longer practicable, as more than seventy isolates were being studied in 1953 (Tinsley, 1953) and there are even more available now. The use of place-names is a convenient alternative, but does not imply that the virus described occurs only at the type locality, or that it is the only one to occur there.

Symptom observations

The isolates were maintained on Amelonado seedlings infected by grafts. Such seedlings did not produce uniform symptoms and mealybugs were used to infect plants as beans (Posnette, 1947*b*) for all the detailed experiments.

Symptoms changed rapidly as the leaves expanded and infected plants were examined twice each week to follow the full sequence of symptom development. This was done for at least 6 months, because distinct isolates sometimes caused similar symptoms at some stages of infection.

Protection tests

Plants used in tests of the ability of one isolate to protect against another were infected by grafting. Healthy Amelonado seedlings infected with the first virus were cut back when they had shown typical symptoms and then at least five were grafted with bark patches containing the second virus. Avirulent viruses and those causing only swellings or leaf mosaic were introduced first and later challenged with isolates causing more conspicuous symptoms. Reciprocal tests were not always possible and experiments with viruses causing similar symptoms usually gave inconclusive results.

RESULTS

The symptoms caused by isolates from different localities

1 (*a*). *Egbeda virulent strain.* The virus from this locality is the most virulent known to affect cacao in Nigeria and in early transmission work it killed some seedlings infected as beans. Transmissions from surviving plants selected a somewhat less virulent form which killed rarely, although it still caused very obvious symptoms.

The first leaves produced by inoculated beans usually showed a red banding caused by an accumulation of anthocyanins in the tissues along the primary, secondary and tertiary veins. This inconspicuous symptom was frequently associated with a distortion

and crinkling of the leaves, which sometimes became discoloured and fell. The red vein-banding disappeared as the surviving leaves hardened and showed an extensive interveinal chlorosis (Pl., Fig. 1). The plants were severely stunted and some died at this acute stage of infection. However, most plants recovered to produce leaves which were either symptomless or showed a transient and relatively mild red vein-banding, followed by vein clearing and chlorotic banding alongside the principal veins (Pl., Fig. 2). Some leaves were affected on only one side of the midrib and the distribution of symptoms then resembled the injection patterns obtained by introducing the appropriate mineral solution to shoots showing severe deficiency symptoms (Maskell, Evans & Murray, 1953). Conspicuous swellings usually appeared on the stem, hypocotyl and tap root.

Trees infected by grafts showed an indistinct transition from the severe acute to the relatively mild chronic phase of infection. However, the first leaves produced after inoculation often showed a preliminary symptom (Posnette, 1947*a*) of limited angular chlorotic flecks along portions of the third- and fourth-order veins, which sometimes became necrotic. The young leaves of the next flush then showed particularly severe symptoms and often fell before maturing, causing leafless shoots which frequently developed large terminal swellings. The pods borne on plants infected with the Egbeda virus often showed a dark-green mottle, which was sometimes the earliest symptom recorded.

1(*b*). *Egbeda intermediate strains.* Some trees in the Egbeda area showed atypical symptoms and distinctive isolates were collected from farms around the villages of Ajia and Koroboto. Infected trees at one Koroboto farm were observed monthly for 5 years and some consistently showed conspicuous leaf symptoms and numerous swellings, whereas others produced only swellings or leaf symptoms, which were sometimes mild and restricted to a few leaves. Grafts to uniform seedlings usually reproduced the field symptoms and a range of different isolates was made from adjacent trees.

1(*c*). *Egbəda avirulent strains.* The virus from Egbeda usually caused consistent symptoms in the laboratory, but occasional plants, especially those infected by single mealybugs, showed mild symptoms. Back tests showed that some of the plants contained the usual virulent strain, but others contained viruses which had consistently mild effects on seedlings, even after several transmissions by grafts or by many mealybugs.

Several of the avirulent isolates were studied and all caused a very inconspicuous transient red vein-banding, which was followed by occasional speckled clearings in the interveinal areas of a few mature leaves (Pl., Fig. 3). Some isolates also caused small root swellings.

Plants infected with avirulent isolates gave no additional symptoms when challenged with virulent viruses from Egbeda or nearby villages. By comparison, other viruses, including those from Abaku, Olanla and Ilesha, caused their usual symptoms.

2, 3. *I.N.A. farm and Ilaro.* The isolate from the Ibadan Native Administration farm was from one of the twenty infected trees in an outbreak discovered and eradicated in 1954. The virus resembled that from Bisa in Ghana (Posnette, 1947*a*), but differed from most of those collected in Nigeria in that it caused large stem swellings,

but no conspicuous or permanent leaf symptoms. Indeed, leaves showed only a very inconspicuous transient red vein-banding, sometimes followed by a slight interveinal chlorosis.

The I.N.A. virus closely resembled that from an extensive outbreak more than 60 miles away in the Ilaro area of mass infection of Abeokuta Province. Indeed, the two isolates had such similar effects that protection tests were impracticable. In other experiments, isolates from Olanla, Abaku, Ilesha, Egbeda and elsewhere caused their usual leaf symptoms in plants already infected with viruses from I.N.A. or Ilaro.

4(*a*). *Abaku.* The outbreak found near Abaku in 1957 was in a cacao area at least 4 miles from any other known infection. Isolates were made from some of the twenty infected trees and most caused inconspicuous red vein-banding, followed by an extensive interveinal chlorosis (Pl., Fig. 4). This acute phase of infection was sometimes delayed and the first leaves were then symptomless or showed only a few flecks. The subsequent flush was severely affected and sometimes the leaves and growing point became necrotic. Surviving plants recovered by producing axillary shoots with red and chlorotic vein-banding restricted to the tissues along the main veins. These symptoms were usual on plants in the chronic phase of infection, but swellings were never found.

The conspicuous symptoms of the Abaku isolates appeared after inoculating plants already infected with viruses from elsewhere. Similarly, other viruses caused swellings on plants already infected with isolates from Abaku.

4(*b*). *Abaku avirulent strains.* A few trees at Abaku showed unusually mild symptoms restricted to a few branches. Particularly avirulent strains were transmitted by single mealybugs from two of these trees. The isolates protected against virulent isolates from Abaku but not from elsewhere.

5. *Olanla.* A typical isolate from Olanla caused conspicuous transient red banding along the principal veins. These symptoms were followed by limited clearing and chlorotic banding of the tissues along and between the third- and fourth-order veins. The red vein-banding symptom became so conspicuous in later leaves as to cause a red mottle near the leaf margins, which later became an unusually intense green. Other symptoms appeared as extensive yellow interveinal specks, with translucent patches along the secondary veins of some leaves. Leaves of subsequent flushes showed the red mottle symptom, but the clearings which developed as the leaves hardened were not extensive and tended to be restricted to the tissues along the margins of the dark-green areas. Swellings appeared on plants infected for several months and the translucent areas continued to appear in some leaves.

The isolate was also studied in Ghana, where it was referred to as $1\,O_2$ (Posnette, 1950). It caused similar but more severe symptoms than the first isolate from this locality, which did not cause translucent lesions. Plants already infected with $1\,O_1$ showed no additional symptoms when inoculated with $1\,O_2$ and both isolates protected against a third from an isolated outbreak 60 miles away at Ilesha. The Olanla isolates did not protect plants against other viruses and all three isolates caused a red mottle in infected plants not already showing this symptom.

6. *Offa-Igbo.* The isolate from Offa-Igbo caused transient red vein-banding followed by yellow vein-banding and stem swellings. There was no clear distinction

into acute and chronic phases, although the vein banding in later leaves became restricted to the principal veins.

In experiments in Ghana the isolate used in these investigations protected plants from the effects of subsequent inoculation with a similar isolate from the same locality, but there was no evidence of relationship with isolates from elsewhere.

Isolates from other localities. The numerous isolates collected from other localities have been little studied. Some of them, including those from Balogun caused leaf symptoms with only occasional swellings, often restricted to the roots. Other isolates caused swellings with few obvious leaf symptoms. However, most of the isolates including those from Ife, Araromi and Ikire resembled those from Offa-Igbo and caused both swellings and leaf symptoms of various types and severities. The few protection tests gave equivocal results, but isolates from different localities seemed not to protect against each other, suggesting that they were not closely related.

Table 1. *The symptoms caused by different isolates of cacao swollen-shoot virus from various localities in Nigeria*

Isolate*	Red vein-banding	Red mottle	Inter-veinal chlorosis	Vein clearing and chlorotic banding	Trans-lucent patches on leaves	Stem swellings	Marked acute and chronic phases	Leaf crinkling and abscission	Marked stunting
Egbeda	+	−	+ +	+ +	−	+ +	+ +	+ +	+ −
Mild Egbeda	+	−	−	+	−	±	−	−	±
Koroboto	+	−	+	+ +	−	+	−	−	+
Ajia	+	−	+	+ +	−	+	±	−	+
Abaku	+	−	+ +	+ +	−	−	+ +	+ +	+ +
Mild Abaku	+	−	−	+	−	−	−	−	±
Offa-Igbo I	+	−	+	+ +	−	+	+	−	+
Offa-Igbo II	+	−	+	+ +	−	+	+	−	+
Olanla I	− +	+ +	−	+ +	−	+	−	−	+
Olanla II	+ +	+ −	−	+ +	+	+	−	−	+
Ilesha	+ +	+	−	+ +	−	+	−	−	+
I.N.A.	+	−	−	+	−	+	−	−	±
Ilaro	+	−	−	±	−	±	−	−	±
Balogun	+	−	−	+	−	±	−	−	±

* The number of plus signs indicates the severity of each symptom. The sign ± indicates a mild symptom not always produced.

The relationships between the different isolates

The symptoms of swollen-shoot disease are not the same throughout Nigeria and considerable differences occur between and within outbreaks, resembling the situation in Ghana (Posnette & Todd, 1955). In each country many symptomatically distinct isolates have been obtained from different localities, outbreaks, trees and branches (Table 1).

Attempts to produce antisera have failed and plant protection tests have given results of critical importance. They enabled the different isolates to be ascribed to groups, within which there is good protection, but between which there is not. The isolates in each group are assumed to be closely related strains, they usually caused the same type of symptom and came from the same area. By comparison, the isolates in different groups often came from dissimilar areas and caused symptoms which differed in type and severity.

One interpretation of these results is that swollen-shoot disease is caused by many

unrelated viruses, each of which occurs in related strains. However, this assumption is unwarranted, because experience with viruses of other crops has shown that serologically related strains of the same virus do not always protect plants against each other (Bawden & Kassanis, 1951; Harrison, 1958).

Reference to distinct viruses with separate names is also inconvenient and gives a false impression of heterogeneity amongst isolates which have several features in common, have a similar host range, cause the same type of disease and are unusual in having mealybug vectors. For these reasons it is suggested that the different groups are major subdivisions of a single virus or virus complex. In the same way, curly-top disease of sugar beet is caused by viruses which do not interprotect; similarly with the viruses causing mosaic diseases of cassava (Storey & Nichols, 1938). Tobacco necrosis is another example of a disease which is caused by viruses that are not all closely related and some are serologically distinct, although they are not given separate names (Bawden, 1941).

In both Nigeria and Ghana each group of swollen-shoot isolates seems to be restricted to certain localities, e.g. Egbeda, Abaku and Offa-Igbo. The origin of the many different groups is obscure, but their existence has considerable practical implications. For example, an avirulent strain found to be suitable for protecting trees in one locality (Posnette & Todd, 1955) may be ineffective elsewhere. Furthermore, varieties selected from their resistance or tolerance to infection with some virus isolates may succumb to others.

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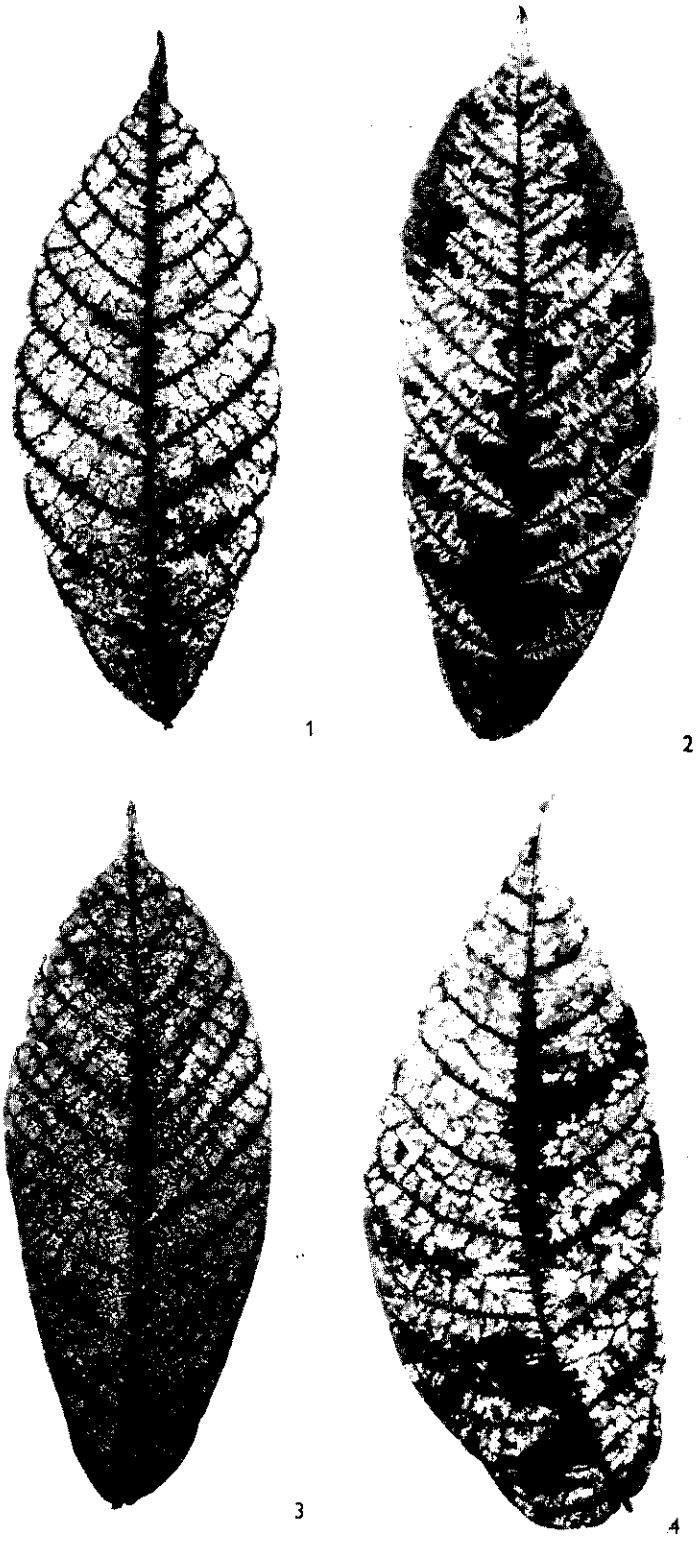
EXPLANATION OF PLATE

Fig. 1. Severe interveinal chlorosis caused by a virulent isolate from Egbeda at the acute stage of infection.

Fig. 2. Chlorotic vein banding caused by a virulent isolate from Egbeda at the chronic stage of infection.

Fig. 3. Chlorotic specks caused by an avirulent isolate from Egbeda.

Fig. 4. Severe interveinal chlorosis caused by a virulent isolate from Abaku.



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