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Occurrence of whitefly-transmitted geminiviruses in crops in Burkina Faso, and their serological detection and differentiation

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Summary

Whitefly-transmitted geminiviruses were found to be associated with four diseases of crop plants in Burkina Faso: cassava mosaic, okra leaf curl, tobacco leaf curl and tomato yellow leaf curl. Tomato yellow leaf curl is an economically serious disease, reaching a high incidence in March, following a peak population of the vector whitefly, *Bemisia tabaci*, in December. Okra leaf curl is also a problem in the small area of okra grown in the dry season but is not important in the main period of okra production in the rainy season. The geminiviruses causing these four diseases, African cassava mosaic (ACMV), okra leaf curl (OLCV), tobacco leaf curl (TobLCV) and tomato yellow leaf curl (TYLCV) viruses, were each detected in field–collected samples by triple antibody sandwich-ELISA with cross-reacting monoclonal antibodies (MAbs) to ACMV. Epitope profiles obtained by testing each virus isolate with panels of MAbs to ACMV, OLCV and Indian cassava mosaic virus enabled four viruses to be distinguished. ACMV and OLCV had similar but distinguishable profiles. The epitope profile of TobLCV was the same as that of one form of TYLCV (which may be the same virus) and was close to the profile of TYLCV from Sardinia. The other form of TYLCV reacted with several additional MAbs and had an epitope profile close to that of TYLCV from Senegal. Only minor variations within each of these four types of epitope profile were found among geminivirus isolates from Burkina Faso. *Sida acuta* is a wild host of OLCV.

Key words: *Bemisia tabaci*, cassava, geminivirus, monoclonal antibody, okra, tobacco, tomato

Introduction

Geminiviruses transmitted by the whitefly, *Bemisia tabaci*, are widespread in tropical and sub-tropical regions of the world, where they cause numerous diseases in dicotyledonous

plants including cassava, pulses, vegetables, tobacco and cotton (Muniyappa & Veeresh, 1984; Harrison, 1985; Brown & Bird, 1992). Several of these diseases can cause major yield losses, depending on the crop and virus, environmental conditions, prevalence of *B. tabaci* and other factors. For example, mosaic is estimated to cut the overall yield of cassava in Africa by about a third (Thresh, Fishpool, Otim-Nape & Fargette, 1994) and (yellow) leaf curl diseases decrease yields by more than 80% in many tomato crops in the Middle East (Makkouk, Shehab & Majdelani, 1979) and India (Saikia & Muniyappa, 1989).

All whitefly-transmitted geminiviruses (WTGs) studied in enough detail have proved to be serologically related, and this interrelationship has facilitated their detection by ELISA (Sequeira & Harrison, 1982; Cohen *et al.*, 1983) or immunosorbent electron microscopy (Sequeira & Harrison, 1982; Roberts, Robinson & Harrison, 1984) with either polyclonal or cross-reacting monoclonal (Thomas, Massalski & Harrison, 1986) antibodies. However, these tests do not differentiate WTGs from one another. In earlier years, time-consuming biological tests were used to distinguish them. More recently, many WTGs have been distinguished by determining the reactivity in ELISA of individual virus isolates with panels of monoclonal antibodies (MAbs), raised against purified particles of selected WTGs and having ranges of cross-reactivity with heterologous WTGs (Thomas *et al.*, 1986; Harrison *et al.*, 1991; Swanson & Harrison, 1993).

Burkina Faso is one of the main producers of vegetables and cotton in West Africa but there is no report of the occurrence of any WTG in the country. The aim of the work described here was to find out whether diseases associated with WTGs occur in a range of important crops in Burkina Faso and, if so, whether the causal viruses can be detected and distinguished serologically.

Materials and Methods

Crops examined

The crops studied were cassava (*Manihot esculenta*), cotton (*Gossypium hirsutum*), okra (*Abelmoschus esculentus*), tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*). During the dry season (November to May), cassava, okra and tomato crops were examined in the five main regions where okra and tomato crops are produced under irrigation: Bobo-Dioulasso (Vallée du Kou), Bourzanga, Fada N'Gourma, Guièdougou and Ouagadougou (Fig. 1). During the rainy season, crops were examined in the south and south-west parts of the country where cotton and tobacco production is centred. Leaves, cuttings and scions were collected from plants showing green or yellow mosaic, leaf curling and/or leaf distortion. Some intact plants were also collected. The samples were kept in a refrigerated box until they were tested or were frozen for testing later. When possible, whole plants were potted and kept in a screenhouse.

Field observations

The incidence of symptoms in crops was recorded at roughly monthly intervals in the three main agro-ecological zones: (a) the north guinean zone in the south and south-west parts of the country, with 900 to 1200 mm rainfall per year, (b) the savannah–sudanese zone in the central and eastern parts, with 600 to 900 mm rainfall, and (c) the sahelian zone in the north, with less than 600 mm rainfall. Populations of *B. tabaci* were assessed by counting adults on okra and tomato leaves in the field.
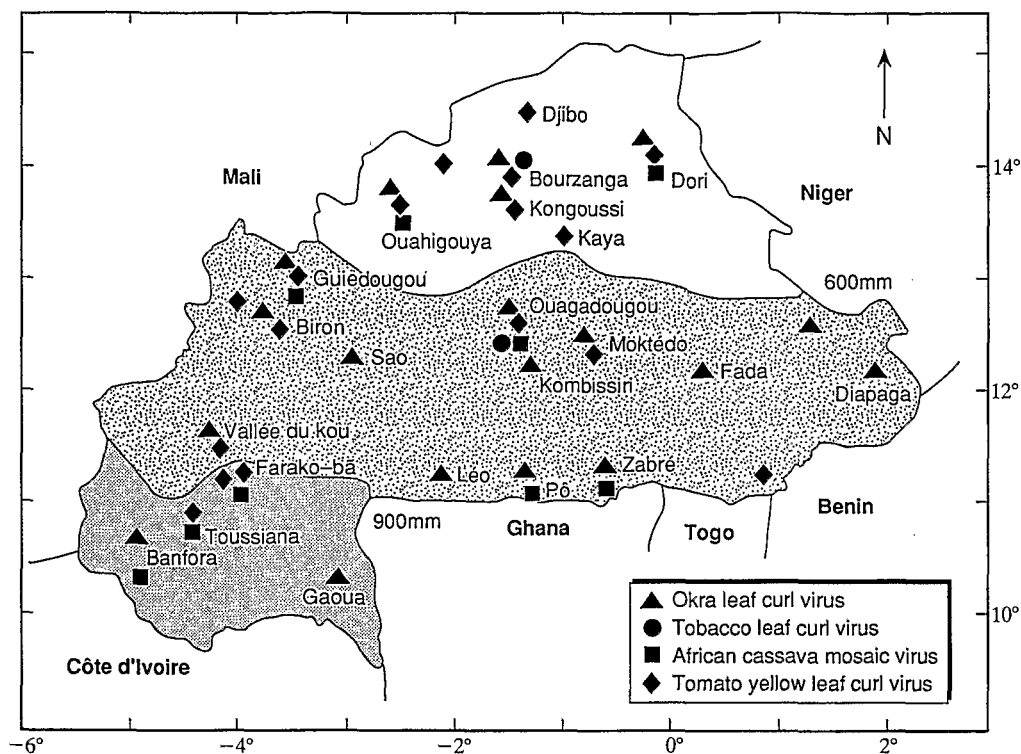
Fig. 1. Location of sites in Burkina Faso where plants infected with whitefly-transmitted geminiviruses were found. The differently shaded zones are separated by the 600 mm and 900 mm isohyets for annual rainfall, respectively.

Serological tests

Triple antibody sandwich enzyme–linked immunosorbent assay (TAS–ELISA) used MAbs raised against particles of African cassava mosaic virus (ACMV*; Thomas et al., 1986), Indian cassava mosaic virus (ICMV; Aiton & Harrison, 1989) or okra leaf curl virus (OLCV; Swanson & Harrison, 1993). Polyclonal antiserum to ACMV (Sequeira & Harrison, 1982) at 1:10000 was used to coat wells in microtitre plates, leaf sap diluted 1:10 to 1:20 was used as the antigen, MAbs in tissue culture supernatant fluids diluted 1:3 were used as the detecting antibodies, and bound MAbs were detected with rabbit or goat anti-mouse globulin/alkaline phosphatase conjugate (Sigma). Absorbances ($A_{405\ nm}$) were recorded after incubation with p-nitrophenyl phosphate for 1 to 2 h at room temperature and again after overnight incubation at 4°C. Unless otherwise stated, the final reading is quoted. Leaf sap was extracted in 0.05 M Tris–HCl, 0.06 M sodium sulphite, pH 8.5 (tomato samples) or 0.05 M Tris–HCl, 0.005 M EDTA, 20 g/litre polyvinylpyrrolidone (M.W. 44000), 5 ml/litre Tween 20, pH 8.0 (other samples). Other details were essentially as described by Thomas et al. (1986).

For double antibody sandwich ELISA (DAS–ELISA), the same procedure was followed except that alkaline phosphatase-conjugated polyclonal antibody to either ACMV or OLCV

*In this paper, the name ACMV is used for isolates of the type previously referred to as group A; group B isolates are considered to represent a separate geminivirus, now called East African cassava mosaic virus (Hong, Robinson & Harrison, 1993)

was used as the detecting antibody (Sequeira & Harrison, 1982; Swanson & Harrison, 1993). WTG-free leaf extracts gave readings ($A_{405 \text{ nm}}$) of <0.15 both in DAS–ELISA and in TAS–ELISA.

Results

Occurrence and incidence of diseases

Cassava mosaic

Two types of mosaic were observed in cassava. One was a distorting mosaic, erratically distributed among the leaves on an affected plant, and typical of that caused by cassava geminiviruses in other countries. It was recorded at several sites in the western and central parts of the country (Fig. 1), but not in the far east, where little cassava is grown. In individual plots, the proportion of plants affected always exceeded 20% but nowhere did it reach 100%. The second type of symptom, although within the range induced by cassava geminiviruses, was not typical. It consisted of a chlorotic mosaic without distortion and affected many of the leaves on a plant. It was recorded only in a few plants at one site at Biron, near Guièdougou.

Cotton mosaic

Two kinds of symptom were observed on cotton, a green mosaic and a yellow mosaic without distortion. Affected plants occurred in patches in a few crops grown in the west-central part of the country during the rainy period (from June to October). However, no WTG was detected serologically in affected plants of either type (see below).

Okra leaf curl

Plants bearing leaves with strong upward curling, and thickening and distortion of leaf veins, were observed in many crops. Strong downward curling of leaves occurred on some plants. These symptoms, which are typical of those caused by OLCV (Fauquet & Thouvenel, 1987), occurred in most parts of the country (Fig. 1). Some okra crops are planted in September and grown under irrigation in the dry season. The incidence of okra leaf curl in these crops increased greatly in November–December, reaching 60% or more in many crops and remaining at these values until March. Over a 3-year period, okra leaf curl incidence in this season had mean values of 51% (sahelian zone), 36% (savannah–sudanese zone) and 39% (north guinean zone). No okra is grown in April–May and leaf curl incidence was low in the many crops grown during the hot and rainy season (June to September).

Evidence was obtained that OLCV occurs naturally in wild hosts. Vein thickening symptoms were observed in the leaves of four malvaceous species: *Sida acuta, S. alba, S. stipulata* and *Hermania* sp. Moreover, leaf curl symptoms developed in okra plants exposed to *Bemisia tabaci* which were raised on virus-free okra and then allowed to feed on symptom-bearing *S. acuta* before transfer to okra test plants. Extracts of diseased *S. acuta* and of the okra test plants reacted with the same monoclonal antibodies that reacted with OLCV (see below). It is therefore clear that *S. acuta*, which shows vein thickening all the year round, is an alternative host of OLCV that may act as an important reservoir of the virus during the okra-free period of the year. This would fit with the view of N'Guessan, Fargette, Fauquet & Thouvenel (1992), who considered that the epidemiology of okra leaf curl in the Ivory Coast was suggestive of the existence of naturally occurring OLCV hosts other than okra.

Tobacco leaf curl

A few tobacco plants were found with curling and thickening of their leaves, and thickening and distortion of leaf veins. The symptoms closely resemble those caused by tobacco leaf curl virus (TobLCV) in other countries. Diseased plants occurred sporadically at sites in the sahelian (Bourzanga) and savannah–sudanese (Ouagadougou) zones, where tobacco was growing in the dry season in mixed plantings of market garden crops, which included yellow leaf curl-affected tomato. They were not found in the rainy season in the main area for tobacco production in the north guinean zone in the west of the country.

Tomato leaf curl and yellow leaf curl

A range of symptoms was found in tomato. Leaf curling predominated in some plants, leaf yellowing and stunting in others, and some plants expressed both types of symptom. Affected plants occurred in many parts of the country (Fig. 1) in crops grown during the dry season. There was no obvious association between symptom type and agro-ecological zone. The incidence of disease varied greatly from year to year and from one season to another. For example, little disease was observed in the 1991/92 dry season, but much in that of 1992/93. Within the dry season (the main period for tomato production), the incidence of disease became high in many crops. In September to December, it remained below 10%, but then rose to 60% by April. The disease was not observed in the few tomato crops grown in the rainy season.

Relation of disease incidence to whitefly population

In tomato crops, the population of *B. tabaci* typically peaked in November–December (about 10 to 40 adult insects/plant), decreasing afterwards to less than five per plant in March. Hence there was a greater delay between the occurrence of maximum whitefly numbers and maximum amount of disease spread than could be accounted for by the incubation period of the virus in the plants. In okra crops grown from September to March, the *B. tabaci* populations ranged up to 10 per plant but did not peak at a specific period. In June to August, few adult *B. tabaci* were found on okra.

Serological detection of geminiviruses

Three MAbs raised against ACMV particles and known to react with the particles of many other WTGs (Harrison *et al.*, 1991; Muniyappa, Swanson, Duncan & Harrison, 1991; Swanson, Brown, Poulos & Harrison, 1992*a*; Swanson, Varma, Muniyappa & Harrison, 1992*b*; Macintosh, Robinson & Harrison, 1992; Swanson & Harrison, 1993), *viz.* SCR 18, 20 and 23, were used in TAS–ELISA in Burkina Faso to test a range of symptom-bearing plants suspected of being infected with WTGs. Table 1 shows examples of the results obtained. All three MAbs detected WTGs in cassava with mosaic (50 samples), okra (> 200 samples) and tobacco (20 samples) with leaf curl, and in tomato with yellowing or leaf curl, or both (> 150 samples). In contrast, WTGs were not detected in seven samples of cassava with chlorotic mosaic from one location, or in cotton with green or yellow mosaic. Table 1 gives results recorded after overnight incubation with substrate, but in many instances a positive reaction was already evident, especially with SCR 23, after the initial 1 h incubation at room temperature with substrate.

Epitope profiles and virus differentiation

Tables 2 and 3 show the results of tests at Dundee by TAS–ELISA on leaf extracts from a selection of samples of four WTG-infected species collected in Burkina Faso. The samples

Table 1. *Detection by TAS–ELISA of geminiviruses in leaf extracts from diseased plants in crops in Burkina Faso*

Plant	Symptom	Reaction with monoclonal antibody		
		SCR 18	SCR 20	SCR 23
Cassava	Mosaic	4*	4	4
Cassava	Chlorotic mosaic	0	0	0
Cotton	Yellow mosaic	0	0	0
Cotton	Green mosaic	0	0	0
Okra	Leaf curl	4	4	4
Tobacco	Leaf curl	4	4	4
Tomato	Yellowing	1	3	3
Tomato	Leaf curl	2	2	4
Tomato	Yellowing and leaf curl	4	4	4

* Scores represent intensity of reaction: 4 ($A_{405nm} > 1.8$), 3 (1.21–1.8), 2 (0.31–1.2), 1 (0.15–0.3) and 0 (<0.15).

were tested for reactivity with 33 MAbs (16 raised against ACMV, 10 against ICMV and seven against OLCV). OLCV-containing okra extracts reacted with all the OLCV MAbs, some of the ICMV MAbs and most of the ACMV MAbs, although not with SCR 17 or, with one exception, SCR 22. Results were similar for samples from different parts of Burkina Faso (Fig. 1). Data for Kongoussi (north, near Bourzanga), Guièdougou (west central) and Vallée du Kou (south-west, near Bobo-Dioulasso) are given in Tables 2 and 3. Most of the apparent differences among samples in strengths of reaction with the same MAb can be attributed to differences in antigen concentration. However, differences in reactivity with MAbs SCR 21, 25, 27, 32, 58 and 101 probably represent correlated

Table 2. *Reactions in TAS–ELISA of geminiviruses from Burkina Faso with monoclonal antibodies to African cassava mosaic virus*

Virus	Source plant	Source location	Monoclonal antibody (SCR No.)*																ACMV polyclonal antibody†
			11	12	13	14	16	17	18	20	21	22	23	25	27	29	32	33	
OLCV	Okra	Guièdougou-6	1‡	4	4	4	4	0	4	4	4	0	4	3	4	4	4	4	4
	Okra	Guièdougou-11	2	4	4	4	4	0	4	4	4	0	4	3	4	4	4	4	4
	Okra	Kongoussi-2	0	4	4	4	4	0	4	4	4	0	4	2	4	4	4	4	4
	Okra	Kongoussi-5	0	4	4	4	4	0	4	4	2	2	4	1	4	4	2	3	3
	Okra	Vallée du Kou-25	0	4	4	4	4	0	4	4	3	0	4	1	2	4	2	4	4
	Okra	Vallée du Kou-26	0	4	4	4	4	0	4	4	2	0	4	0	1	4	2	3	4
TobLCV	Tobacco	Bourzanga-1	0	0	0	3	0	4	4	4	0	0	4	0	0	0	0	0	4
	Tobacco	Bourzanga-2	0	0	0	3	0	4	4	4	0	0	4	0	0	0	0	0	4
TYLCV	Tomato	Bourzanga-14	0	0	0	2	0	4	4	4	0	0	4	0	0	0	0	0	—
	Tomato	Vallée du Kou-25T	0	4	4	4	4	4	4	4	0	0	4	0	1	4	2	3	—
	Tomato	Senegal	0	4	4	2	4	4	2	4	0	0	4	0	0	0	3	0	—
	Tomato	Sardinia	0	0	0	0	0	2	4	4	0	0	4	0	0	0	0	0	—
ACMV	Cassava	Ouagadougou	3	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	—

* ACMV polyclonal antibody was used as the coating antibody in all tests.
† Double antibody sandwich ELISA was used for these tests.
‡ Absorbance ($A_{405\ nm}$) after overnight incubation at 4°C with substrate was scored as follows: 4 (>1.8), 3 (1.21–1.8), 2 (0.61–1.2), 1 (0.3–0.6), 0 (<0.3), — (not tested).

differences in antigenic constitution. Tests on a further 20 okra samples gave data similar to those in Tables 2 and 3. An extract of symptom-bearing *S. acuta* leaves, like extracts of OLCV-infected okra, reacted with all seven OLCV MAbs.

TobLCV-containing tobacco extracts were distinguished from extracts containing ACMV or OLCV by their failure to react with SCR 12, 13, 16, 21, 53 and 104. Also, unlike OLCV but like ACMV, they reacted with SCR 17. Their relative reactivity with different MAbs (epitope profile) was identical to that given by some samples of (yellow) leaf curl-affected tomato, such as Bourzanga-14 (Tables 2 and 3). Indeed, the fact that the diseased tobacco plants were growing close to diseased crops of tomato suggests that the same virus may have infected both species. Other samples of (yellow) leaf curl-affected tomato gave a different epitope profile, which included strong reactions with SCR 12, 13, 16 and 29, and moderate reactions with SCR 32 and 33 (sample Vallée du Kou-25T; Table 2). Among 32 samples from tomato, more than half were of this second type. They differed from OLCV and ACMV in failing to react with SCR 21 or 53. In some instances, the two antigenic variants of tomato (yellow) leaf curl virus (TYLCV) occurred in the same plot.

Only two geminivirus-containing cassava extracts were tested in detail. The epitope profiles were typical for ACMV: reactions were obtained with all 16 ACMV MAbs but with only one ICMV MAb (Tables 2 and 3). In further tests in Burkina Faso, 50 additional samples, including some from the centre, west and south of the country, each reacted (as does ACMV) with all 11 MAbs used (SCR 11, 12, 14, 16, 18, 20, 23, 25, 29, 32 and 53). None of the samples was found to contain East African cassava mosaic virus, which does not react with SCR 11, 29 or 53.

Table 3. *Reactions in TAS–ELISA of geminiviruses occurring in Burkina Faso with mono-clonal antibodies to Indian cassava mosaic and okra leaf curl viruses*

| | | | Monoclonal antibody (SCR No.)* | | | | | | | | | | | | OLCV polyclonal antibody† |
| | Source plant | Source location | To ICMV | | | | | To OLCV | | | | | | | |
Virus			53	55	58	66	68	100	101	102	103	104	105	106	
OLCV	Okra	Guièdougou-6	4‡	0	3	0	0	4	4	4	4	4	4	4	4
	Okra	Guièdougou-11	4	0	4	0	0	4	4	4	4	4	4	4	4
	Okra	Kongoussi-1	4	0	3	1	1	4	4	4	4	4	4	4	4
	Okra	Kongoussi-5	4	0	1	0	0	3	2	4	4	4	4	4	4
	Okra	Vallée du Kou-25	3	0	0	1	0	4	3	4	4	4	4	4	2
	Okra	Vallée du Kou-26	4	0	1	0	0	4	3	4	4	4	4	4	4
TobLCV	Tobacco	Bourzanga-1	0	1	0	0	0	4	3	4	0	0	4	4	2
	Tobacco	Bourzanga-2	0	0	0	0	0	3	2	4	0	0	4	4	2
TYLCV	Tomato	Bourzanga-14	0	0	0	0	0	—	—	—	—	—	—	—	—
	Tomato	Vallée du Kou-25T	0	0	0	0	0	—	—	—	—	—	—	—	—
	Tomato	Senegal	0	0	0	0	0	0	0	0	0	0	2	0	1
	Tomato	Sardinia	0	0	0	0	0	—	—	—	—	—	—	—	—
ACMV	Cassava	Ouagadougou	2	0	0	0	0	0	0	0	0	0	4	—	—

* ACMV polyclonal antibody was used as the coating antibody in all tests. MAbs 52, 54, 56, 60 and 62 (all to ICMV) did not react with any of the viruses.
† OLCV polyclonal antibody was used as the detecting antibody in double antibody sandwich ELISA.
‡ Reactions were scored as in Table 2.

Discussion

The results of this study provide the first definitive evidence for the occurrence of WTGs in Burkina Faso: in cassava, okra, tobacco, tomato and *Sida acuta*. In contrast, no evidence was obtained that either an aberrant chlorotic mosaic in cassava or mosaic in cotton is caused by a WTG. Further work is needed to ascertain whether this symptom in cassava is caused by a form of ACMV that is defective for particle production, and similar to that studied by Robinson, Harrison, Sequeira & Duncan (1984), or by some other agent. We did not find the whitefly-transmitted agent reported to cause cotton leaf curl in Burkina Faso in earlier years (Fauquet & Thouvenel, 1987).

All the WTGs were detected in TAS–ELISA by cross-reacting MAbs such as SCR 23. They had four types of epitope profile which were characteristic for ACMV, OLCV, TYLCV-Bourzanga-14/TobLCV and TYLCV-Vallée du Kou-25T, respectively. The OLCV isolates closely resembled ACMV in epitope profile but did not react with SCR 17. Also, the ACMV isolates did not react with SCR 104, which detected all the OLCV isolates from West Africa tested by Swanson & Harrison (1993). However, isolates of OLCV from Chad and the Middle East did not react with SCR 104, and some isolates from Chad, Ivory Coast, Nigeria and Oman reacted with SCR 17, showing that OLCV and ACMV have epitope profiles which are not reliably distinguished without using a large panel of MAbs (Swanson & Harrison, 1993). The other two types of profile, for TYLCV-Vallée du Kou-25T and TYLCV-Bourzanga-14/TobLCV, are distinguished from each other and from those of ACMV and OLCV, by the reactions of SCR 16 (or SCR 12 or 13) and SCR 21 (or SCR 53). The profile for TYLCV-Vallée du Kou-25T is close to that for TYLCV from Senegal, whereas the TYLCV-Bourzanga-14 profile is more like that for TYLCV from Sardinia (Table 2).

Our results show that peak populations of *B. tabaci* occur in tomato crops in Burkina Faso at about the same time of year as in Senegal (Defrancq D'Hondt & Russo, 1985). However, TYLCV incidence seems more variable from year to year, and slower to build up, in Burkina Faso than in Senegal. Also, OLCV was not a problem in okra in the rainy season and became prevalent only in irrigated crops grown during the less important period for okra production in the dry season. These observations suggest that a paucity of virus sources limits the spread of the viruses when winged *B. tabaci* first become numerous and that measures designed to enhance this asynchrony of occurrence of vectors and virus sources would be worth testing as an approach to minimising virus spread.

We conclude that TYLCV has become prevalent in recent years and causes an economically important disease in tomato, and that OLCV likewise causes a problem in irrigated crops grown in the dry season. Cassava is not a key crop in the Burkina Faso economy, and ACMV, although widely distributed, does not present such a serious problem. Tobacco leaf curl, which was recorded in only a few places, is not a problem in the main tobacco-growing area, where the crop is grown in the rainy season.

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