Aspects of the epidemiology of okra leaf curl virus in Côte d'Ivoire

K. P. N'GUESSAN†, D. FARGETTE‡, C. FAUQUET and J-C. THOUVENEL

Laboratoire de Phytovirologie, Institut Français de Recherche pour le Développement en Coopération (ORSTOM), BP V 51 Abidjan, Côte d'Ivoire

Abstract. Transmission of okra leaf curl virus by seed or by mechanical inoculation was not demonstrated. Transmission by the whitefly vector Bemisia tabaci, Genn. from okra to okra was successful but not from okra to cassava or N. benthamiana. The disease was more prevalent in the savannah area in the north of Côte d'Ivoire than in the forest area to the south. The spatial distribution of infected plants and the whitefly vector led to gradients downwind from sources of infection.

1. Introduction

Okra leaf curl virus (OLCV) causes a serious disease of okra (Abelmoschus spp.) that limits the culture of numerous varieties in Côte d'Ivoire. The disease also occurs in Nigeria where it is reported to be most devastating during the wet season (Lana, 1976; Atiri and Fayoyin, 1989). The characteristic symptoms include curling of the leaves, vein thickening and a decrease of leaf surface area. Until now, OLCV has been the subject of few studies and little is known about its epidemiology or biochemical properties. Preliminary investigations have shown that in the field the disease is transmitted by the whitefly Bemisia tabaci, Genn. It can also be transmitted experimentally by grafting (Fauquet and Thouvenel, 1987). Seed transmission and transmission by mechanical inoculation have not been demonstrated. Geminivirus-like particles have been observed and associated with the disease symptoms, but no return inoculation has been made and no antiserum has yet been obtained (Fauquet and Thouvenel, 1987). This paper reports experiments in 1986 and 1987 on aspects of the epidemiology of the disease in Côte d'Ivoire, its transmission, prevalence in the country and development in time and space.

2. Materials and methods

2.1. Seed transmission

Over 200 seeds from infected okra, var. Clemson spineless, were sown. Because of the poor quality of seeds from infected plants, only 150 seedlings were obtained. They were maintained in an insect-free glasshouse for symptom observation.

2.2. Mechanical inoculation

Attempts were made to transmit OLCV mechanically from okra to okra var. Clemson spineless and from okra to cassava (Manihot esculenta), var. CB and to Nicotiana benthamiana The method used was that adopted by the ORSTOM virology laboratory at Adiopodoumé for transmission of many other tropical plant viruses. Extracts were prepared by grinding young infected okra leaf tissue in 0·07 M phosphate buffer (pH 7·1) containing 0·35% (w/v) cysteine and 0·25% (w/v) bentonite. Test plants were dusted with carborundum powder and inoculated with the forefinger dipped in inoculum. The experiment was repeated three times and each time 25 plants of each species were inoculated. Leaves were rinsed with tap water after inoculation and the plants were kept in an insect-free glasshouse for symptom development.

2.3. Vector transmission

Adult B. tabaci collected on infected okra or cassava in the field were placed directly onto young healthy okra var. Clemson spineless, cassava var. CB or N. benthamiana, under cylindrical cages for transmission tests. In each cage there were 20 whiteflies on a single test plant. Whiteflies were allowed to feed on the test plants for 4 days, after which they were sprayed with Dursban 4ER (chloropyrophosethyl) insecticide and the plants transferred to an insect-free glasshouse for symptom observation. The experiment was repeated three times and each time 25 plants of each species were infested. Larvae were counted on each plant after 8 days and symptoms were recorded after 15 days. The percentage of viruliferous whiteflies, P, was deduced from the formula of Gibbs and Gower (1960):

$$P = 1 - (1 - R/N)^{1/i}$$

in which R is the number of infected plants, N is the total number of plants tested and i is the number of whiteflies per test plant.

2.4. Disease incidence

Because of their different responses to OLCV, as noticed during preliminary trials (Hamon, unpublished results),

three okra varieties from the ORSTOM germplasm collection (ORS 520, ORS 958 and Clemson spineless) and four of their hybrids were sown in late March 1986 at the ORSTOM experimental farm at Adiopodoumé (20 km west of Abidjan). There were seven plots of each genotype arranged in a 7 × 7 Latin square, each square being 4·2 m long. The planting was fully exposed to the direction of the dominant wind from the southwest, which is one of the main factors influencing patterns of spread of whitefly-transmitted diseases in Adiopodoumé, particularly African cassava mosaic virus (ACMV) (Fargette et al., 1985). There was a 1·5 m wide unplanted strip across the middle of the field to permit irrigation as needed. The nominal number of experimental plants was 1764 (36 plants per plot), but only 1136 grew, because of poor germination and insect attack. The number of plants showing symptoms was recorded fortnightly and the number of adult whiteflies on 196 marked plants (four plants/plot) was counted weekly, beginning 1 month after planting. Disease incidence was expressed as percentage of the total stand and the vector population as the cumulative total of the average number of whiteflies counted in successive weeks.

2.5. Disease prevalence

Previous observations have shown that OLCV occurred in many regions of Côte d'Ivoire (Siemonsma, 1982; Fauquet and Thouvenel, 1987). In 1987 a survey was made of the prevalence of the disease at four sites where irrigation facilities were available (Figure 1). The four sites were the state farm of SODEFEL in Sinemantiali (northern Côte d'Ivoire in the savannah area where there is one rainy season from June to October), in Marabadiassa (central Côte d'Ivoire; savannah and forest, one rainy season from April to October), in Go-hermankono (southern Côte d'Ivoire,

forest region, two rainy seasons from March to July and September to November) and at the ORSTOM experimental farm at Adiopodoumé (as for Go-hermankono but to the southeast along the coast). Two okra varieties, Clemson spineless and ORS 520, were planted in 20 × 20 m plots at each site, in early April. Disease incidence and numbers of whitefly adults were recorded weekly.

3. Results

3.1. Transmission experiments

Symptoms of OLCV were not observed on any of the seedlings raised from infected plants. Moreover characteristic OLCV symptoms did not develop on young okra, cassava and N. benthamiana plants mechanically inoculated with extracts from infected okra leaves. However, OLCV was transmitted to 50% of the okra plants infested with the whiteflies from infected okra. The average number of larvae counted per plant was 217 and the percentage of viruliferous whiteflies, as calculated by the formula of Gibbs and Gower (1960) was c. 4%. By contrast, OLCV was not transmitted by whitefly from okra to cassava and to N. benthamiana. No larvae developed on infested cassava and N. benthamiana. These results demonstrate transmission by whitefly to the same host plant species but not mechanically and through seeds.

3.2. Field spread

Figure 2 illustrates the distribution of infected plants and whitefly vectors along a SW/NE transect of the varietal trial at Adiopodoumé 30, 40, 60, 74 and 90 days after planting (d.a.p.). Disease incidence was not uniform within the field and marked border effects occurred. Greater infection and
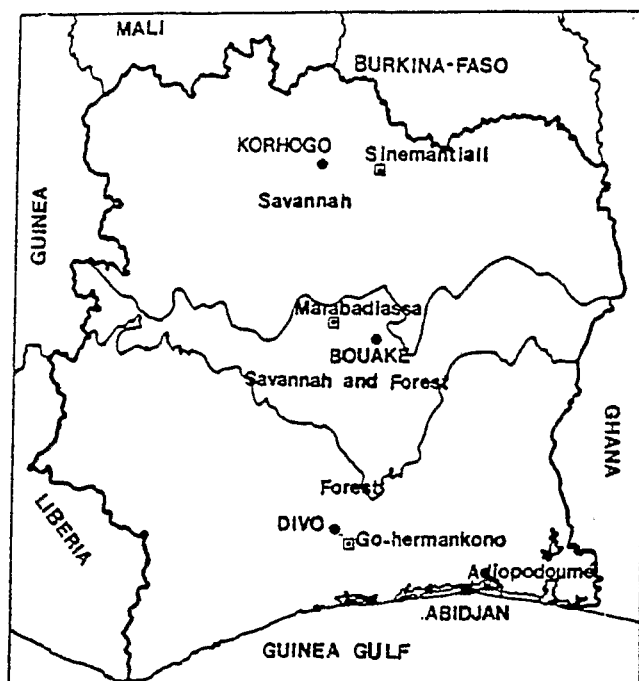

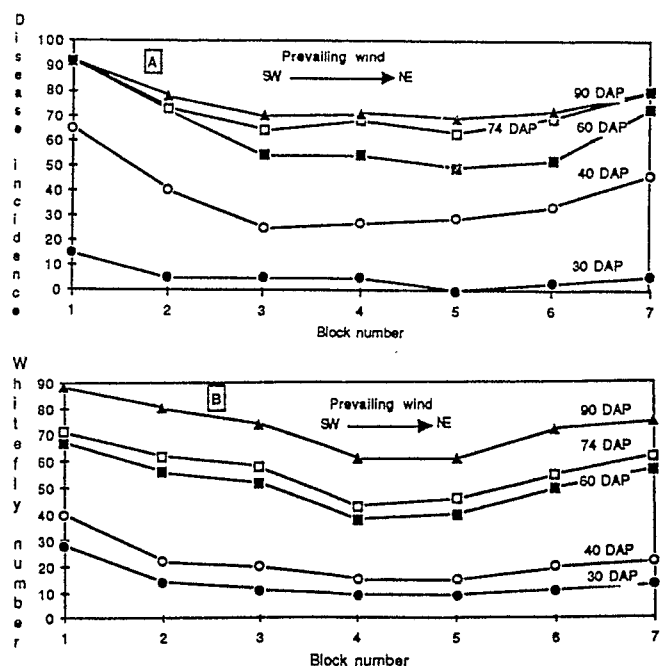Figure 1.   Map of Côte d'Ivoire showing study sites (□).

Figure 2.   Disease incidence (A) and whitefly infestations (B) along a southwest/northeast transect of the varietal trial at Adiopodoumé (each block is 4·2 m long).

Table 1.   *Virus incidence and whitefly infestations in the varietal trial at Adiopodoumé: ratio of maximum/minimum values within the trial calculated 30–90 days after the planting*

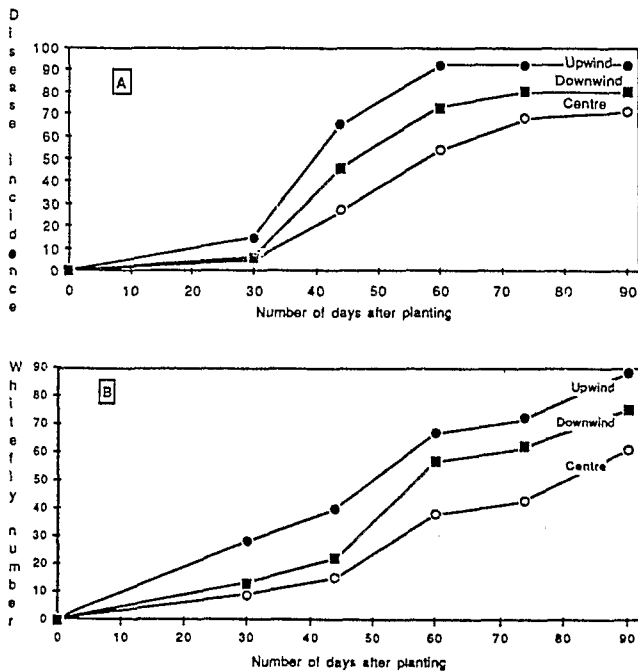|	Days after planting				
	30	44	60	74	90
Max./min. virus incidence	5·0	2·6	1·9	1·5	1·3
Max./min. whitefly number	3·1	2·7	1·7	1·7	1·4

Figure 3.   *Disease progress curves* (A) *and cumulative numbers of whiteflies per plant* (B) *recorded in the varietal trial at Adiopodoumé.*

whitefly numbers were recorded along the upwind and downwind borders but numbers gradually diminished towards the centre, forming an open V-shaped gradient. This trend was apparent 30 d.a.p. and persisted until the end of the experiment, although it tended to become less obvious with time, as indicated by a progressive decrease in the ratio of maximum to minimum values of disease incidence (Table 1). The spread of OLCV with time, and the dynamics of vector populations, for the upwind, downwind and central areas of the trial, are presented in Figure 3. The similarity between disease incidence in time and whitefly populations is notable. The first whitefly vectors were recorded in the field

22 d.a.p. whereas infection was recorded 1 week later (30 d.a.p.). Thereafter both disease incidence and whitefly populations increased rapidly for a time. Little further increase in disease incidence occurred after about 70 d.a.p., whereas whitefly numbers continued to increase.

3.3.   Disease prevalence at field sites.

There were marked differences in whitefly numbers and disease incidence at the four sites surveyed (Table 2). Greater infection and whitefly populations were recorded in the north and central sites than in the two southern ones. However, whiteflies at the Adiopodoumé site in the south were considerably more viruliferous than those found elsewhere, as shown by the ratios between infection and whitefly numbers. The course of infection also differed between sites (Figure 4a). Infection occurred 30 d.a.p. at one of the southern sites but not until later elsewhere. All the infected plants that were recorded occurred before 50 d.a.p. at the Adiopodoumé and Go-hermankono sites. Infection occurred over a longer period and mainly after 60 d.a.p. at the other two sites. Whitefly populations were much greater in the north and central sites · than in the south. At Adiopodoumé and Go-hermankono whitefly populations were static from 60 d.a.p., whereas they went on rising elsewhere.

4.   Discussion

Our inability to demonstrate seed and mechanical inoculation of OLCV confirms earlier reports (Lana, 1976; Fauquet and Thouvenel, 1987) and suggests that these modes of transmission are not important in the natural spread of the virus. Hence infection in the field seems to depend only on vectors. This conclusion is supported by the good relation found in our experiments between disease incidence and vector populations. Similar relationships have been reported with ACMV and seem to be typical for many persistent and semi-persistent viruses (Thresh, 1976; Dubern, 1979; Fargette *et al.*, 1985).

The strong border effects noted in the 1986 varietal trial at Adiopodoumé were similar to those reported earlier with ACMV (Fargette *et al.*, 1985) and with many other diseases with aerial vectors. This reflects the influence of wind speed and direction on vector movement (Thresh, 1976; Harrison, 1981). Turbulence and other modifications of wind characteristics and accumulation of airborne vectors near barriers have been reported often (Lewis, 1969; Lewis and Dibley

Table 2.   *Disease incidence and whitefly populations recorded at the four sites surveyed*

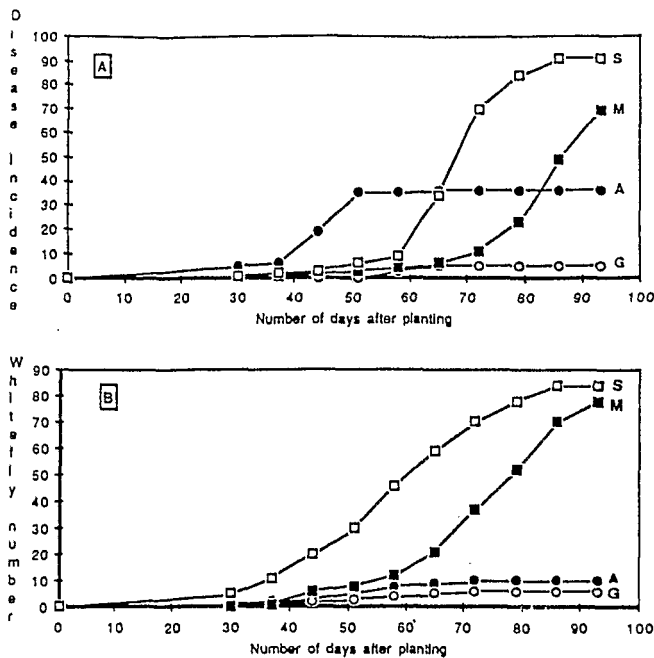|	Adiopodoumé (South)	Go-hermankono (South)	Marabadiassa (Centre)	Sinemantiali (North)
Ecological zone	Coastal forest	Forest	Savannah	Savannah
Rainfall (mm)	2000	2000	1500	1200
Percentage infected plants	36	5	69	91
Average number of whiteflies	10	6	78	84
Ratio percentage infection, no. of whitefly	3·6	0·8	0·9	1·1

Figure 4. Disease progress curves (A) and cumulative numbers of whiteflies per plant (B) recorded in 1987 at Adiopodoumé (A), Go-hermankono (G), Marabadiassa (M) and Sinemantiali (S).

1970; Yao et al., 1986). In the Adiopodoumé experiment, whiteflies alighted preferentially on the peripheral okra plants. This behaviour of the vector could explain the greater number of whiteflies and consequently the higher disease incidence at the upwind and downwind edges of the field compared with the central part. This gradient of infection was apparent at 30 d.a.p. and tended to flatten with time. Among the factors that contribute to this tendency, Thresh (1976) emphasized the size and arrangement of infected source plants, the number and/or activity of viruliferous vectors, the progressive increase in the extent of multiple infection and the probability that plants infected early will become infectious and so act as a secondary focus for further spread. Studies on OLCV to date have not been sufficiently detailed to show which of these factors are involved.

It has been shown that the rate at which a virus spreads between plants varies widely according to the type of virus, crop, environment and mode of transmission (Thresh, 1974). The four sites involved in our investigation were located principally in two major ecological zones; those in the north were in the savannah and those in the south in the forest. In general, the savannah area is drier, less humid and hotter than the forest area. Weed species, crops, growing seasons and agricultural practices also differ in various ways between the two zones. There are regional differences in okra production in Côte d'Ivoire. It is more widely grown in the savannah in the north and in the centre of the country than in the forest zone in the south. In addition, whereas in the south it is usually grown as a monoculture, elsewhere it is more often intercropped with other vegetables, such as tomatoes, aubergines and peppers. It has also been shown that there is a higher diversity of malvaceous plants in the savannah zone than further south. All these factors are likely to cause differences in disease incidence and vectors

populations between the savannah and the forest, and therefore between sites in the north and those in the south. However, because of the limited number of sites involved in our study, these results must be considered as provisional and subject to further study.

The characteristics of the epidemiology of OLCV as reported from this study share some similarities to that of ACMV. The temporal pattern of spread observed at Adiopodoumé also shows some similarities to that of ACMV (Fargette, 1985) with a period of rapid spread at the beginning of the rainy season (March–May) and less during the relatively cool dry season (August–September) (N'Guessan, unpublished results). These characteristics are likely to reflect the biology of their common vector B. tabaci.

The rapid infestations of okra recorded in the field, together with the low percentage of transmission obtained with whiteflies collected on infected okra and the few infected okra fields within a few kilometres of each site, suggest the existence of crops or weeds hosts other than okra. Weeds have been reported in many instances as reservoirs of both viruses and vectors (Duffus, 1971) and this could be the case with OLCV and B. tabaci. However, the failure of the whiteflies transferred from okra to adapt to a change of plant species suggests the existence of either biotypes specific to certain host species or biochemical differences between whiteflies from okra and those from cassava. Further work on the relationship between virus, vector and host plants, and also on the nature and prevalence of vector and virus reservoirs, is needed for a better understanding of the epidemiology of OLCV. However, the pathogenic agent must be identified and characterized, and a specific means of detection of the virus must be developed before much progress can be made. Progress in this direction has been made recently (N'Guessan et al., 1990).

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