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Comparative epidemiology of three tropical whitefly-transmitted geminiviruses

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Summary — Spatial and temporal patterns of spread of African cassava mosaic, okra leaf curl and tobacco leaf curl viruses in West Africa, East Africa and India share some general characteristics. By comparing the results and running new analyses on available data, it is shown that the epidemiology of these viruses is controlled by the same key variables. For instance, spatial spread is characterised by strong border effects due to accumulation of whitefly vectors *(Bemisia tabaci)* on the wind-exposed field borders under the influence of the prevailing wind. This results in pronounced environmental gradients of disease. Temporal patterns of virus spread are driven by the sinusoidal fluctuation of temperature over the year which correspond with changes of whitefly populations.

epidemiology / whitefly / geminiviruses / African cassava mosaic virus / okra leaf curl virus / tobacco leaf curl virus

Introduction

The epidemiology of African cassava mosaic geminivirus was studied in Côte d'Ivoire from 1980 to 1990 in a multidisciplinary project initiated by Prof Hirth and conducted by ORSTOM and NRI scientists involving virologists, entomologists and climatologists. The main features of the spatial and temporal patterns of spread were established. The spatial pattern was highly dependent on the wind regime and was characterised by pronounced downwind-orientated disease gradients. It results from the accumulation of whitefly vectors *(Bemisia tabaci)* carried by the prevailing south/west orientated wind on the wind exposed borders [7]. The temporal pattern of spread was highly dependent on temperature fluctuation. It showed a strong seasonality and mostly reflected the changes of whitefly populations which were also linked to the temperature fluctuation over the year ([6]; Fargette *et al*, submitted).

Here we present results on African cassava mosaic (ACMV), okra leaf curl (OLCV) and tobacco leaf curl viruses (TbLCV) in West Africa, East Africa and India obtained either by the authors themselves ([7, 15, 22]; Fargette, Fauquet, Thouvenel, unpublished) or by other scientists [17]. The results and new analyses of the data provide evidence that spatial and temporal patterns of spread of several geminiviruses in different environments share some general characteristics. In particular, these comparisons and new analyses suggest that wind and temperature regimes have similar impacts on the ecology of several whitefly borne viruses, of different crops and in diverse environments.

Materials and methods

African cassava mosaic virus at Adiopodoumé (Côte d'Ivoire)

All experiments were performed at Adiopodoumé at the ORSTOM experimental farm, 20 km of Abidjan in the forested lowland coastal region of the country at 5° north and 4° west, at 20 m altitude. In all experiments on cassava, fields of the variety CB were divided into blocks of 100 plants at 1 x 1 m spacing and disease incidence was followed weekly, fortnightly or monthly by visual inspection of each plant. Depending on the field, diseased plants were either kept and labelled or removed and disease incidence for each individual block calculated. Adult whiteflies were either trapped using yellow water traps kept at canopy level or counted on the terminal leaves of 10 plants on the diagonal of each block. The observations on okra were made in an 0.5 ha field where disease incidence and whitefly counts were recorded similarly.

African cassava mosaic virus at Kiwanda (Tanzania)

The experiment started in March 1934, on land provided by the School of the Universities Mission to Central Africa at Kiwanda near Amani, 5° north and 38° east. Kiwanda lies at an altitude of about 170 m, in a valley near the eastern foot of the East Usambara Mountains, about 8 km from Amani. Forty-eight square plots, each of nine plants, were laid out. Each plot was surrounded by a row of mosaic-diseased cassava. In each plot, one healthy cassava plant from the Mbarika variety was established at the beginning of each month for a period of 2 years. Records were taken at the beginning of each month for 2 years and all plants that developed mosaic symptoms were removed. The mean probability of the disease appearing during each month of the year in plants of all ages between 2 and 8 months was calculated.

Tobacco leaf curl virus in Bangalore (India)

Experiments were carried out at Bangalore which lies at an altitude of 930 m, 12° north and 77° east. Tobacco cv FCV special seedlings were raised in an insect-proof glasshouse and 45-day-old healthy seedlings were transplanted monthly throughout 1988 at the Agricultural University Farm, Bangalore. The spacing was 90 x 60 cm in plots of 4.5 x 7.5 m that were replicated twice. There were 60 plants per planting date. The incidence of TbLCV was recorded fortnightly up to 90 days from transplanting. Adult whiteflies were collected in monthly plantings. Plastic dishes (30 cm diameter) were painted in a bright yellow colour, filled with water and placed at ground level. Two plastic dishes were kept in each of the two replicates at a distance of 3 m apart. After 24 h the adult whiteflies trapped in each dish were counted. In each sequential sowing, whitefly populations were recorded fortnightly up to 90 days.

Meteorological data

Monthly average data on different variables were used: i) temperature in degrees Celsius. Maximum temperature was selected as preliminary analysis indicated that it was more closely associated with virus spread than minimum temperature; ii) relative humidity. Minimum relative humidity was selected whereas maximum humidity was not considered as it deviates little from *ca* 95% at Adiopodoumé and from *ca* 90% at Tonga; and iii) rainfall estimated as the amount of precipitation (in mm). At Adiopodoumé and Bangalore, irrigation was necessary to permit monthly planting but was not included in the analyses.

Data gathered at Adiopodoumé and Bangalore came from the meteorological station of each of the experimental agricultural research stations [14, 22] and the monthly averages were calculated for the period of the experiments. As no exhaustive meteorological data were available for Kiwanda, the data used were from Tonga meteorological station [13], a site nearby with comparable altitude and with temperature fluctuations close to those quoted by Storey and Nichols [17]: a maximum of 33°C in February and a minimum of 20°C in July.

Statistical analyses

Statistical analyses were done using the SYSTAT 5.2 statistical software [18]. Cross-correlation function plots were made to explore relationships between disease incidence and each individual climatic variable available with different lag times. Stepwise multiple regressions were performed to investigate the relationships between the disease spread and the three climatic variables altogether. Non-linear regressions were done to model the relationship between spread and temperature with time.

Results

Spatial pattern of spread

Figure 1 presents catches in yellow water traps at canopy height in the cassava fields at Adiopodoumé in October/December 1982 and in January/February 1983. In October/December 1982, there was the usual prevailing south/west orientated wind (*alizé*) and the distribution of whiteflies was characterised by higher catches on the south and west borders than on the north and east ones. In January/February 1983, there was an exceptionally prolonged north-east orientated wind (*harmattan*) and whitefly distribution in a nearby cassava field was very different from the previous experiment as the fewest catches were observed on the west border and the maximum ones were on the east border.

The relationship of whitefly deposition to the wind direction at Adiopodoumé was also found in the distribution of adult whiteflies counted directly on the cassava plants. This was particularly apparent when assessing whitefly numbers along a south-west axis. Higher numbers were counted on the south/west borders and the numbers decreased downwind. This was also obvious in the okra field (fig 2). Such a curvilinear distribution corresponded to that of the disease (fig 2) and clear disease gradients were observed with both ACMV and OLCV. However, whitefly and disease gradients were less pronouced for OLCV than for ACMV: the ratio max/min virus incidence was 1.8 *vs* 5.4 and 1.8 *vs* 8.5 for whiteflies and disease, in the okra and cassava fields, respectively.

South/west orientated disease gradients were noticed at Adiopodoumé in all years and months of planting in several cassava fields ranging from 0.5 to 4.0 ha. They were expressed over distances from 70 to 200 m (fig 3) and they all exhibited a sharp decrease in disease incidence from the south/west border, minimum being reached at 20–50 metres. However, in figure 3 is indicated that the precise pattern of the gradients differed among fields. Actually, among cassava fields with similar overall disease incidence, the ratio max/min virus incidence ranged from 4 to 13. Gradient shapes could be further modified. For instance, there was an increase of disease incidence on the north border and also in the middle of the cassava field at either side of a 10 m large path (fig 3, bottom).

Temporal pattern of spread

Figure 4 illustrates the incidence of ACMV 2 months after planting in successive plantings over the year in

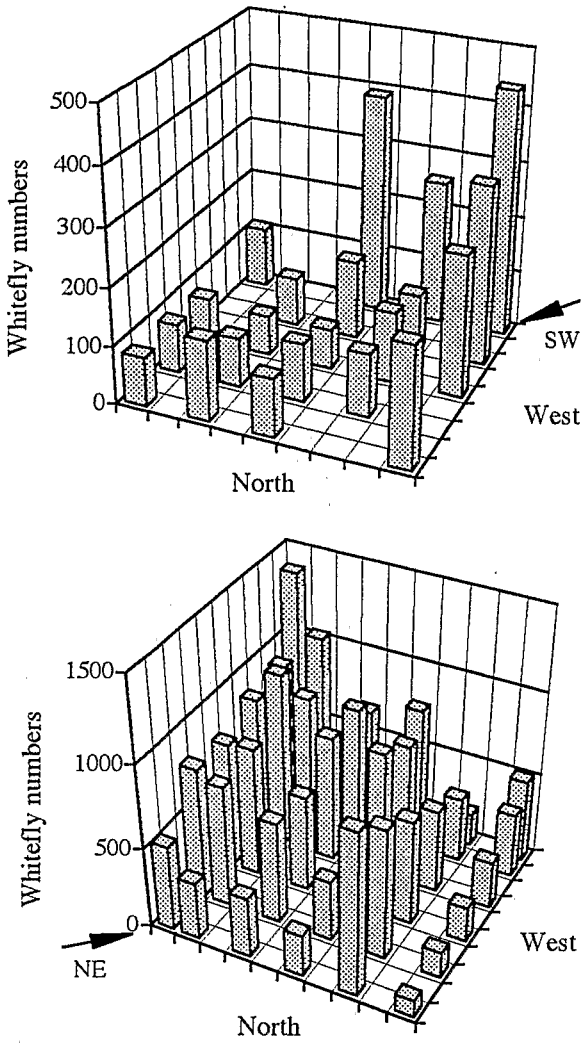

Fig 1. Catches of adult whiteflies at Adiopodoumé in Côte d'Ivoire in yellow water traps in 10 x 10 m sections of an 0.9 ha cassava field in October/November 1982 (top) and in an 1.0 ha cassava field in January/February 1983 (bottom). The traps were kept at canopy height. Arrows indicate the direction of the prevailing wind during the experiment.

Côte d'Ivoire and in Tanzania and for TbLCV in India. There were big differences in disease spread between months of the year. The extent of the variation was different between sites: 6–60% (1/10) for ACMV in Côte d'Ivoire, 0.6–8 (1/25) for ACMV in Tanzania and from 7–94% (1/13) for TbLCV. Although the patterns of spread differed between sites, they all showed a pronounced seasonal component with maximum spread between February and April for ACMV in Côte d'Ivoire, between February and May for ACMV in Tanzania and between March and July for TbLCV in India.

Statistical analyses indicate that disease spread is closely related to monthly maximum temperature, minimum relative humidity being only marginally significant at one occasion and rainfall always non-significant whatever the lag time chosen (table I). Figure 5 and table II indicate that the fluctuation of spread is close to the variation of temperature of the

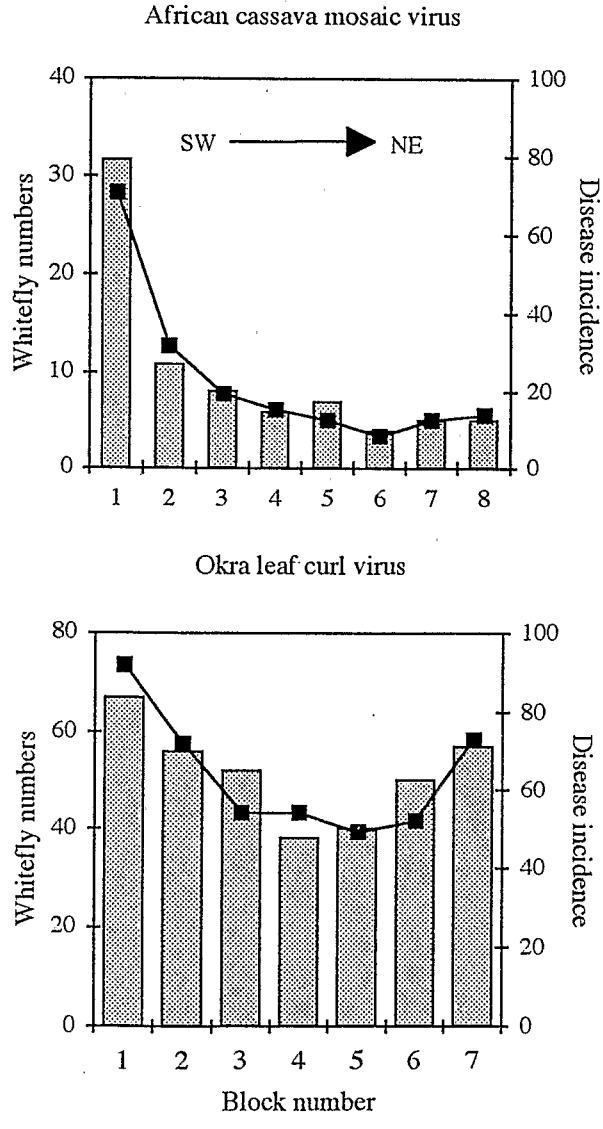

Fig 2. Whitefly numbers (histograms) and disease incidence (square points) at Adiopodoumé in Côte d'Ivoire along a SW/NE axis in a cassava (top) and in an okra field (bottom).

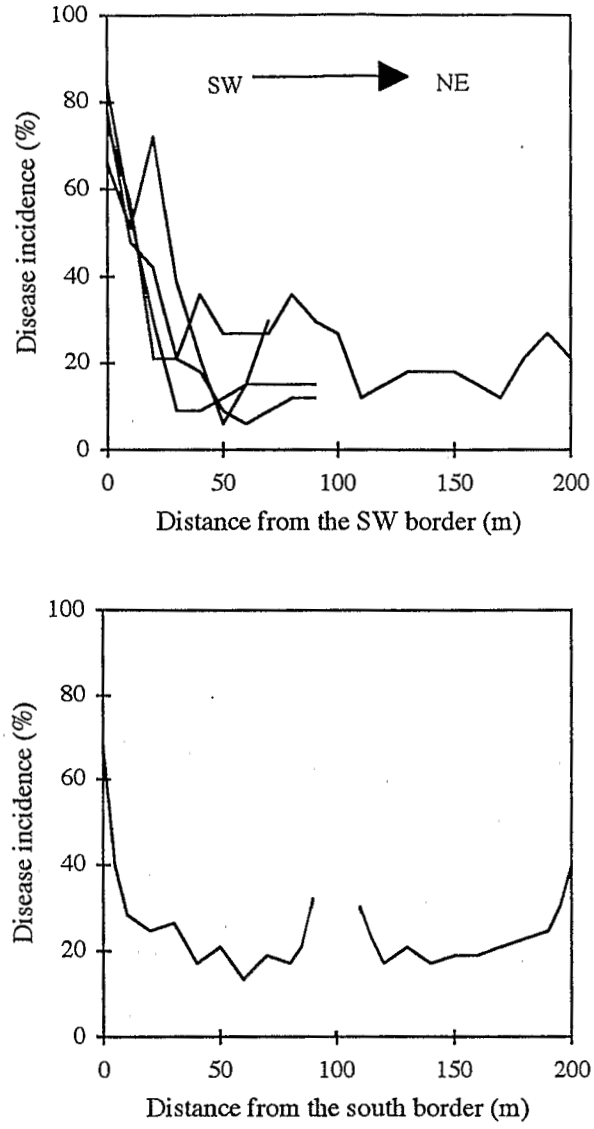

Fig 3. Disease gradients at Adiopodoumé in Côte d'Ivoire along the SW/NE axis in several cassava fields ranging from 0.5 to 4.0 ha (top). Disease gradient along a south/north axis in a field with a 10 m path in the middle (bottom).

first month of growth with ACMV at Adiopodoumé and TbLCV in Bangalore. There was a 1–2 month lag for ACMV in Kiwanda, possibly because meteorological data were used from a local station and not from the experimental site. There is a great fluctuation in amount of spread despite a limited variation of temperature and both variables over the year could be approximated by sinusoidal functions (table II). The

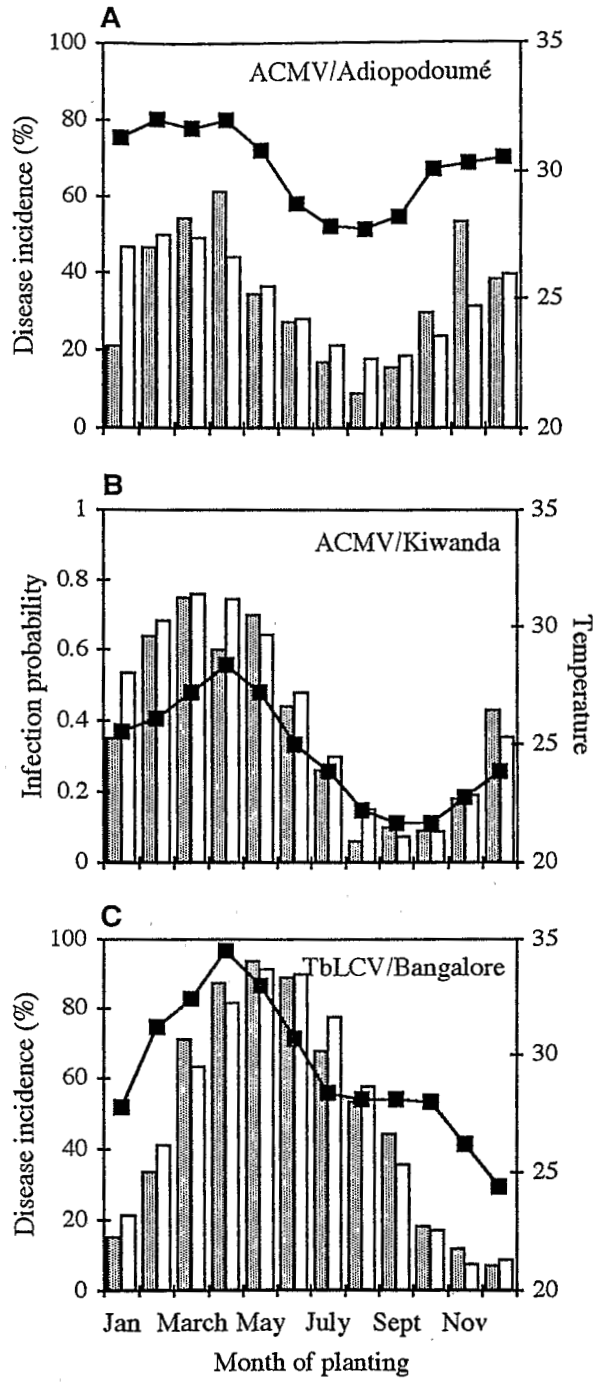

Fig 4. Observed (grey histograms) and calculated (through sinusoidal regression with time; white histograms) virus incidence and mean monthly temperature (square points) for (**a**) ACMV in Côte d'Ivoire, (**b**) ACMV in Tanzania (data taken from [17]) and (**c**) TbLCV in Bangalore. Incidence is expressed as percentage of infection in (**a**) and (**c**) and as probability of infection in (**b**).

Table I. Adjusted multiple squared R, parameter values, standard errors and level of significance of parameters of stepwise regression between disease incidence and temperature, relative humidity and rainfall.

		ACMV Adiopodoumé	*ACMV Kiwanda*	*TbLCV Bangalore*
Temperature	Coefficient	14.6	0.17	9.0
	Std error	3.3	0.02	1.5
	Probability	0.002	$P < 0.001$	$P < 0.001$
Relative humidity	Coefficient	NS	NS	1.8
	Std error			0.6
	Probability			0.01
Rainfall		NS	NS	NS
Constant	Coefficient	–515	–4.5	–287
	Std error	143	0.5	51
Adj square multiple R		0.71	0.90	0.79

Table II. Correlation R squared, parameter values and standard errors of coefficients of the non-linear regression: disease incidence = $a + b \sin(\text{time} + c)$.

	ACMV Adiopodoumé	*ACMV Kiwanda*	*TbLCV Bangalore*
Temperature			
Coefficient a	30.1	29.6	29.4
Std error	0.2	0.1	0.4
b	2.04	–2.02	3.55
	0.25	0.16	0.63
c	0.47	10.27	–0.74
	0.12	0.08	0.18
Cor R squared	0.88	0.94	0.78
Spread			
Coefficient a	34.0	0.42	49.4
Std error	3.9	0.03	1.9
b	16.3	0.35	42.8
	5.5	0.04	2.7
c	0.37	–0.18	–1.24
	0.34	0.10	0.06
Cor R squared	0.50	0.91	0.96

fit was very close for ACMV/Kiwanda and TbLCV and looser, although still highly significant, for ACMV/Adiopodoumé.

Figure 5 illustrates the relationships between whitefly populations and virus spread for ACMV and TbLCV. Low spread was consistently associated with low whitefly numbers and high spread with higher numbers. However, the relationship was not strictly linear, some unexplained variation remained (adjusted R^2 was 0.54 with ACMV and 0.70 with TbLCV) and high residues were apparent with high whitefly numbers. Actually, with high numbers of whiteflies (above 10 for ACMV and 15 for TbLCV), subsequent increases were not proportionally translated into higher spread, possibly because of the limited number of healthy plants available to infect.

Discussion and conclusion

Spatial patterns of spread of ACMV and OLCV are characterised by strong south/west orientated border effects and disease gradients. These gradients are linked to wind characteristics and are referred as 'environmental' gradients [9] as they are not associated with specific virus sources (referred as 'dispersal' gradients). The information on the wind regimes, whitefly numbers and virus incidence considered together, suggests the following scenario. In Côte d'Ivoire, wind is blowing mainly from the south-west *(alizé)* as the intertropical front (ITF) is north of the coastal region of Côte d'Ivoire for most of the year. The prevailing wind direction is reversed for some days or weeks in January/February *(harmattan)* when the ITF is at its southernmost position. Above the crop canopy, the whiteflies are unable to control their flight and are carried predominantly downwind, possibly over distances of several kilometres. Indeed, healthy cassava fields isolated from virus sources by *ca* 10 km were quickly contaminated by incoming viruliferous whiteflies [6]. This is consistent with results from experiments in Israel which showed that marked whiteflies were trapped up to 7 km from their origin [5].

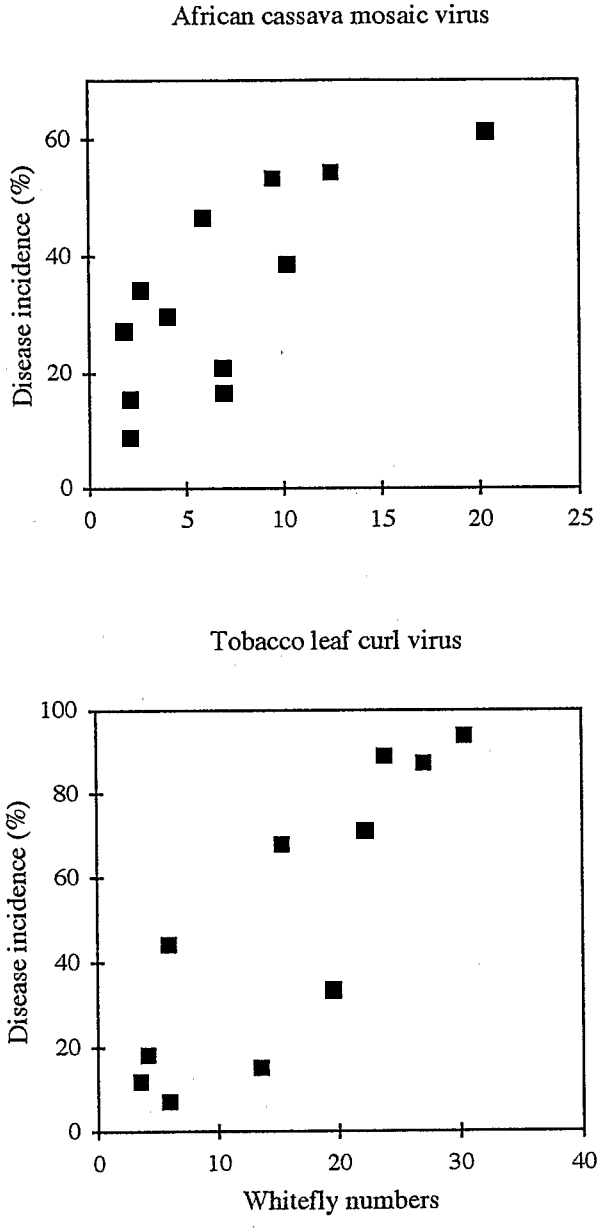

Fig 5. ACMV and TbLCV incidence two months after planting and average adult whitefly numbers recorded in successive monthly plantings of cassava in Côte d'Ivoire (top) and tobacco at Bangalore, India (bottom).

Whiteflies tend to accumulate on the wind-exposed borders of the crops they encounter, which are the south and west ones most of the year. This is likely to result from a reduction of wind speed (N'Guettia, Fishpool and Fargette, unpublished results) and turbulences and eddies which occur on the wind-exposed borders above the canopy which would bring whiteflies into the still boundary layer within which vectors can control their flight and alighting behaviour [16].

Further turbulences and eddies may also result in accumulation of whiteflies and subsequent increase virus incidence such as that observed along paths (fig 3, bottom). It was shown that smoke markers tend to penetrate deeply into fields preferentially through internal paths or alleys [11], which suggests that insects as small as whiteflies could be carried similarly along the paths into the field. It is possible also that increased disease incidence along the paths results from a modification of the alighting behavior due to the influence of bare ground.

The Adiopodoumé experimental station is located along the seaside and is affected by the land/sea breeze whose effect on virus dispersal is unknown. It is tempting to assume, but difficult to prove, that the 'reverse' gradients which occur often on the north borders of cassava or okra field (figs 2,3) are due to the north-orientated land breezes. Actually, as whiteflies disperse mostly in the day time (Van Halder, Van Helden, Fauquet and Fargette, unpublished results), it is likely that the main south-orientated sea breeze will reinforce the usual SW trade winds and that the 'reverse' gradients are linked to eddies on the leeward borders of the fields.

This wind-orientated distribution of whiteflies results in pronounced and characteristic disease gradients, which are observed regardless of the size of the field and the type of crop. It might be expected that crop size and vigour are important features that

influence turbulence and indeed the less pronounced gradients with OLCV compared to ACMV may be due to the smaller size of the okra crop. Within the same crop however, the gradients still tend to flatten with increasing crop age [7, 15], probably because of increasing multiple infection and/or higher resistance in the older plants [19].

These results were observed with only two geminiviruses in the coastal part of Côte d'Ivoire. However, the evidence obtained on the wind and vector mechanisms responsible for the disease gradients suggests that such a phenomenon may be common among other whitefly-transmitted viruses. Indeed, there are indications from published and unpublished observations that wind also plays a key role in the spatial pattern of spread of several other whitefly-borne viruses and under other environments such as cotton leaf curl in Sudan [8] and Lima bean golden mosaic in Nigeria [23].

Temporal pattern of spread over the year shows variation with a strong seasonal component linked to temperature fluctuation. This was found with data collected under three contrasting environments, with two geminiviruses and with different experimental layouts. Our results suggest the following scenario. There is evidence that temperature plays a key role in the ecology of whiteflies [2, 4]. For instance, within the 20–30°C range, higher temperatures are generally associated with shorter developmental periods, greater fecundity and greater whitefly populations. Furthermore, temperature-driven models for *B tabaci* in cotton predict adequately population growth [1]. Then, fluctuation of the temperature over the year would be reflected by parallel changes in whitefly numbers and disease spread.

Temperature fluctuation is known to be restituted by sinusoidal functions [3]. Our results suggest that ACMV and TbLCV spread in different environments could also be modelled adequately with sinusoidal functions. Therefore, it is tempting to assume that the following model is valid to describe the relationship between temperature, whitefly and virus incidence:

Temperature (θ) $\rightarrow$ whitefly numbers (w)
$\rightarrow$ disease incidence (y)

$\theta = a + b \sin (t + c)$, t being time;

$w = \phi (\theta)$, ϕ remained to be established for *B tabaci* on cassava and on tobacco;

$y = e + f w$, within a range excluding high whitefly numbers;

$y = a' + b' \sin (t + c')$.

Although it is somewhat surprising that 'minor' differences in temperature have such a profound effect on virus spread and vector populations, these results are consistent with data from experiments in controlled conditions which showed that differences of *ca* 5°C have profound effect on whitefly biology [2]. There may be also a 'gearing' effect on the actual insect body temperature and leaf temperature that magnifies the differences and increases their significance [16]. It is possible that temperature/global radiation determines further the level of spread by affecting whitefly activity [12], by influencing geminivirus multiplication and therefore virus reservoirs and/or plant growth and susceptibility to infection.

Rainfall was not found to be significantly linked to spread in our experiments. Actually, under Adiopodoumé and Bangalore conditions, rainfall and soil moisture were not limiting throughout the year, possibly because of irrigation applied during the short dry season. Then, it cannot be excluded – although this remains to be proven – that when rainfall is a limiting factor for crop growth, the epidemiology of some whitefly-borne viruses is also influenced by rainfall pattern [21].

Epidemiology involves many complex and integrated interactions between various factors [10]. This does not preclude simple 'laws' if one factor overrides the others or acts through different but correlated paths [20]. With whitefly-transmitted viruses, the results presented here suggest that wind direction is the key variable influencing spatial spread and temperature the driving variable for temporal spread. These principles will provide guidance to investigate other whitefly-transmitted viruses either to set up experiments, to interpret the results or to model the epidemics. The wide application of the results obtained at Adiopodoumé underlines the value of the decision of Prof Hirth to initiate one of the very few in-depth epidemiological programs in Africa.

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