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J. Phytopathology 130, 289—302 (1990)

ISSN 0931-1785

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The Spread of African Cassava Mosaic Virus into and within Cassava Fields

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With 4 figures

Received December 20, 1989; accepted April 19, 1990

Abstract

Only a small proportion of *Bemisia tabaci* collected in totally infected cassava fields at a site in Côte d'Ivoire transmitted African cassava mosaic virus (ACMV) to test plants. Nevertheless, the monthly increase in disease incidence in an experimental planting was directly related to numbers of adult whiteflies counted on plants 6 weeks earlier.

In plots at different spacings, the greatest incidence of ACMV expressed as a percentage of the total stand occurred at the lowest plant density. Much spread into the spacing trial and into two other experimental plantings occurred from outside sources and followed downwind gradients. By contrast, spread from ACMV-infected sources within plantings was limited. It occurred in all directions but over distances of only a few metres. These contrasting patterns of spread are attributed to the different behaviour of *B. tabaci* above and within the crop canopy.

It is concluded that contamination of cassava fields in the coastal forest area of Côte d'Ivoire is due mainly to rapid spread from outside sources which leads to internal foci that contribute to some further, although limited, spread. These findings are discussed in relation to possible control strategies based on the release of healthy cuttings, dense planting and subsequent roguing. Such measures are unlikely to be effective in the coastal forest region of Côte d'Ivoire and adjacent countries unless varieties are grown with greater resistance to infection than those currently used.

Zusammenfassung

Die Verbreitung des African cassava mosaic Virus in und innerhalb Maniokafeldern

Festgestellt wurde, daß nur ein kleiner Teil der *Bemisia tabaci*, die in völlig befallenen Maniokafeldern an der Elfenbeinküste gesammelt wurden, African cassava mosaic Virus (ACMV) an Testpflanzen übertragen konnten. Nichtsdestotrotz war die monatliche Zunahme des Krankheitsauftretens in einer Versuchsplantage mit der Zahl der adulten weißen Fliegen, die vor 6 Wochen an den Pflanzen gezählt worden waren, unmittelbar verbunden.

In Versuchsparzellen mit unterschiedlichen Pflanzenabständen wurde das höchste ACMV-Auftreten dargestellt als Prozent des Gesamtstandes, bei der niedrigsten Pflanzendichte. Die Verbreitung in dem Pflanzenabstandsversuch und auch in zwei weiteren Versuchsbeständen wurden hauptsächlich von Quellen außerhalb des Versuchsfeldes verursacht, sie folgt auch der Windrichtung. Im Gegensatz dazu war die Verbreitung von ACMV-befallenen Pflanzen im Bestand sehr gering. Sie fand in allen Richtungen statt, allerdings nur über Entfernungen von einigen Metern. Diese gegensätzlichen Verbreitungsbilder werden dem unterschiedlichen Verhalten von *B. tabaci* über- und innerhalb eines Pflanzenbestandes zugeschrieben.

Die Schlußfolgerung dieser Beobachtungen ist, daß die Verseuchung der Maniokafelder im Küstenwaldgebiet der Elfenbeinküste hauptsächlich durch eine schnelle Verbreitung von Quellen außerhalb der Plantagen verursacht wird. Dies führt zu Herden innerhalb eines Bestandes, die für eine weitere, obwohl begrenzte Verbreitung beitragen. Diese Ergebnisse werden in Zusammenhang mit möglichen Kontrollmaßnahmen diskutiert, die auf die Verwendung von gesunden Stecklingen, hohen Pflanzendichten mit nachträglicher Ausdünnung basieren. Jedoch werden solche Maßnahmen in diesem Gebiet von der Elfenbeinküste und in benachbarten Ländern wenig Erfolg haben, es sei denn, daß Sorten mit höherer Resistenz als die, die zur Zeit gepflanzt werden, angebaut werden.

African cassava mosaic disease is a widespread and serious disease of cassava (*Manihot esculenta*) in Africa. The causal virus is transmitted either in cuttings derived from infected plants or by the whitefly *Bemisia tabaci* (STOREY and NICHOLS 1938). The virus was first isolated in East Africa (BOCK and GUTHRIE 1977) and eventually named African cassava mosaic virus (ACMV) (BOCK and WOODS 1983) and ascribed to the geminivirus group (HARRISON *et al.* 1977).

This paper reports studies on the influence of virus sources, vectors and host plant density on the spread of ACMV. Primary spread into plantings from distances of up to several kilometres has been reported previously (FARGETTE *et al.* 1985). Patterns of local spread from virus sources of different sizes within cassava fields and the importance of these sources are described here. The amount and timing of disease spread is also examined in relation to vector numbers and infectivity and to host plant density. Knowledge of these factors is of great practical importance in developing effective control strategies (THRESH 1976, 1988 a, b).

Materials and Methods

Sources of cuttings and location of the trials

All healthy cuttings originated from cassava fields of cv. CB at Toumodi Experimental Station in the savanna region, 200 km north of Abidjan in Côte d'Ivoire. Trial Fields 1, 2 and 3 were planted in July 1983, October 1984 and October 1982, respectively. Periodic weed control was done manually.

The three trials were located in the Experimental Station of ORSTOM at Adiopodoumé in the lowland rainforest region, 20 km west of Abidjan. This is an important area of cassava production

where virtually all farm plantings are totally infected with ACMV. Consequently, the trials were subject to infection by whiteflies from extensive outside sources, including some that were only a few hundred metres away.

Field 1: design and recording

Field 1 was a square of 0.49 ha comprising a 7 × 7 array of plots, each of 100 plants arranged 10 × 10 at 1 × 1 m spacing. Each plot comprised four sub-plots of 5 × 5 plants. One large and eight smaller groups of infected cuttings were established within the field to act as internal sources of infection (Fig. 1a). The large group of 50 plants occupied two contiguous sub-plots in the eastern section of the field to provide the main internal source. The eight other groups, each of four infected plants, were centrally situated within plots along the south-west, windward side of the planting. In the western half of the site, plants that became infected during the trial were allowed to remain in the field (Fig. 1a). In the eastern part, they were removed (rogued) as soon as they were seen and recorded during weekly inspections (Fig. 1a). This systematic arrangement of sources and treatments was adopted to decrease interference between plots. The western and eastern portions of the field were separated by a row of 'buffer' plots in which diseased plants were rogued (Fig. 1a). All the plants in each plot were examined weekly and the infected ones were recorded.

Field 2: design and recording

Field 2, of 4 ha, was divided into four 1 ha plots separated from each another by access paths 3 m wide. In the south-east plot three primary sources of 9 (3 × 3), 25 (5 × 5) and 100 (10 × 10) mosaic-infected cuttings were planted at 25 m intervals along the SE-NW diagonal of the field, at right angles to the direction of the prevailing SW wind. Plants were spaced at 1 × 1 m. The distribution of infected plants was noted 4 months after planting. These secondarily infected plants were retained and not rogued.

Field 3: design and recording

Field 3, of 0.9 ha, was square and sub-divided into a 9 × 9 array of 81 plots, each of 10 × 10 m. Within the field, the central 5 × 5 array of 25 plots was planted at five different spacings using a latin square design: 1.3 × 1.3 m (≈ 5,917 plants/ha), 1.2 × 1.2 m (≈ 6,944 plants/ha), 1.1 × 1.1 m (≈ 8,264 plants/ha), 1.0 × 1.0 m (≈ 10,000 plants/ha), 0.9 × 0.9 m (≈ 12,345 plants/ha). Plots surrounding the spacing trial were at a uniform 1 m × 1 m spacing. All the plants in the trial were examined weekly and the infected ones were recorded.

Whitefly surveys

Vector populations were estimated in Field 1 by weekly counts of adult whiteflies on ten plants along one diagonal of each plot. The infectivity of vectors from different locations was assessed several times by collecting adults from the field between August 1982 and August 1983. They were placed in groups of 40 on young cassava test plants, cv. CB, in an insect-proof glasshouse (SEIFF 1981). The samples were collected from cassava fields in which virtually all plants were virus-infected. The proportion of infective whiteflies was estimated by the formula of GIBBS and GOWER (1960):

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

where N is the number of test plants used, i is the number of vectors tested on each plant, R is the number of test plants which become infected, p is the maximum likelihood estimate of the proportion of infective vectors in the population.

Statistical analysis

The statistical analyses were done using the Statistical Analysis System (SAS) software. For all tests, except the comparison of spread from the large internal source in Field 1, the data analyzed are the percentages of infected plants per plot. These percentages are transformed by the angular function to ensure the additivity of effects (SNEDECOR and COCHRAN 1967). The means are expressed as percentages in the text after back transformations by the reverse angular function. Normality is checked by the Shapiro-Wilk statistic (SHAPIRO and WILK 1965).

Results

The pattern and sequence of spread

When considering the amount, pattern and sequence of infection in the three field trials, it is convenient to distinguish between the four possible types of spread:

(a) from outside sources (Fields 1—3),
(b) from the large sources planted within Field 1 (50 plants) and Field 2 (100 plants),
(c) from the smaller sources planted within Field 1 (groups of 4 plants) and Field 2 (9 and 25 plants),

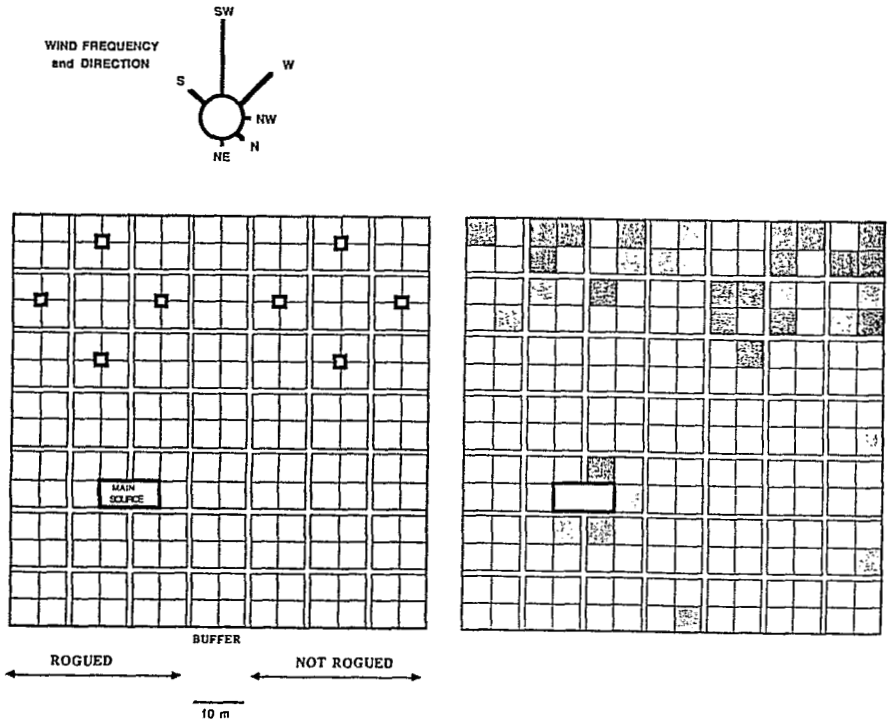

Fig. 1 a (left). Design of Field 1: 49 plots of 100 plants each arranged in four sub-plots of 25 plants at 1 × 1 m spacing. Eight small sources of four infected cassava cuttings (□) and a large one of 50 cuttings were set at planting (▭). The direction symbol indicates the wind frequency in each direction. Plants that became infected during the trial were retained in the western portion of the field (not rogued) and removed (rogued) in the eastern part as soon as symptoms were detected

Fig. 1 b (right). ACMV incidence in each 25-plant sub-plot of Field 1, 6.5 months after planting: 0—10 % (▫), 10—20 % (▫); 20—30 % (▫); 30—40 % (▫); 40—50 % (▫); 50—60 % (▫); > 60 % (▫)

(d) from the plants which became infected after establishment (these plants were retained in Fields 2 and 3 and in the unrogued part of Field 1).

The comparison between areas was performed by protected pairwise T-tests: Firstly, the F-test of the general analysis of variance was calculated, and if there was a significant "area" effect, the areas were compared one by one using the T test; the residual was that of the analysis of variance (MILLER 1966).

(a) Spread from outside sources

Spread from outside sources was most clearly evident in Field 3, where no primary source of infection was planted within the plots and where the nearest infected cassava fields were several hundred metres away. Nevertheless, ACMV appeared early and spread rapidly in the trial with a characteristic pattern in relation to the prevailing SW wind. Three months after planting, the incidence of infection was significantly greater ($p < 0.05$) along the exposed south and west margins (51.5 % and 47.4 %, respectively) than along the north and east borders (22.9 % and 18.1 %) or in the centre of the field (25.8 %).

There was a similar situation in Field 2 where the incidence of infection was significantly greater ($p < 0.05$) along the south and west borders (51.1 % and 54.2 %, respectively) than in any other parts of the field (39.3 %, 28.6 % and 25.4 % for north, east and centre plots, respectively), except immediately alongside the planted sources of infection and along the internal paths where disease incidence was also high.

In Field 1, the general pattern of spread was also characterized by a pronounced wind-oriented border effect along the south-west windward margin of the field (Fig. 1b). Observations 6.5 months after planting revealed that the incidence of ACMV was greatest in the upwind sub-plots up to 25 m from the S/W margin (51.0 %) ($p < 0.05$). Disease incidence elsewhere was usually less, except in the sub-plots neighbouring the largest (50-plant) source of infection. Spread from outside sources was most clearly evident in the rogued plots that were without planted foci of infection and where there was little opportunity for secondary spread.

(b) Spread from large internal sources

In Field 1, there was little virus spread for the first 5.5 months after planting. Then, there was a sudden increase in whitefly populations (see later section). This was followed by rapid virus spread which occurred in all directions from the source (Fig. 1b) but was not apparent over distances exceeding a few metres (Fig. 2). Numbers of infected plants were significantly greater upwind than downwind both at 6.0 and 6.5 months after planting ($p < 0.05$, χ^2 test) and occurred over greater distances.

Various models, including linear ($y = a\,x + b$), power [$\text{Log}\,(y) = \text{Log}\,(a) - b\,\text{Log}\,(x)$] and logarithmic [$\text{Log}\,(y) = \text{Log}\,(c) - d\,x$], have been proposed to relate disease incidence with distance from the source (MINOGUE 1986). In each model y is the incidence of disease, x the distance from the source, a and b are constants. The validity of the regression was assessed through the correlation coefficients between observed and calculated data and through the examination of

the residuals. Tests were done both on untransformed data (y = percentage of plants showing symptoms per row) and on data transformed to allow for multiple infection (y_t = percentage of diseased plants transformed to multiple infection units) (GREGORY 1948, THRESH 1976).

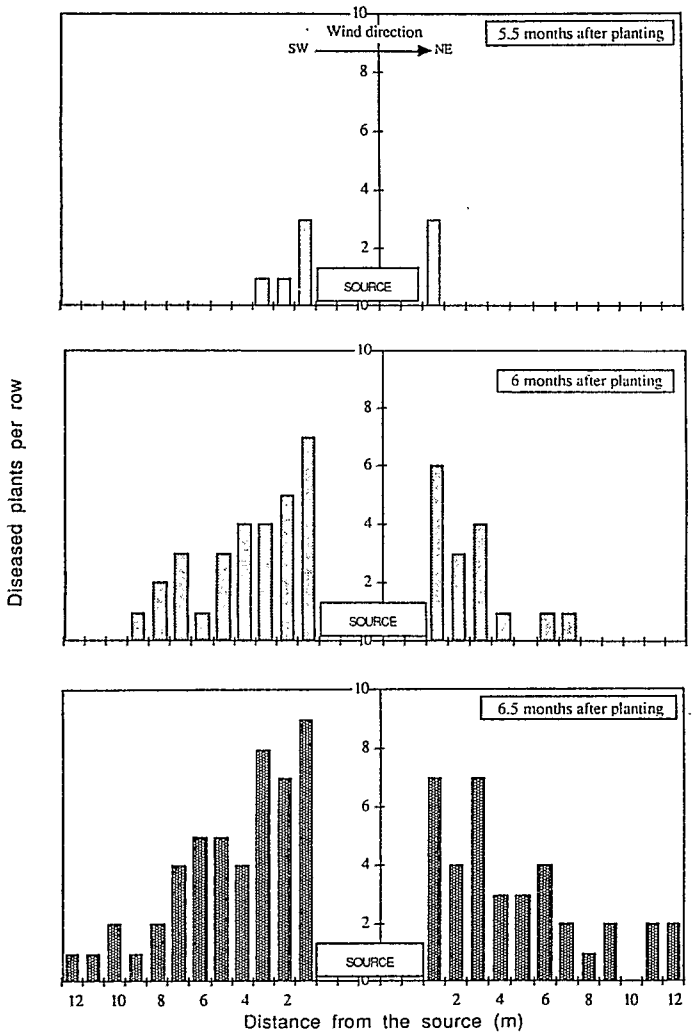

Fig. 2. Number of plants showing symptoms in each row of 10 plants at increasing distances downwind (right) and upwind (left) from the 50-plant source in Field 1, 5.5, 6.0 and 6.5 months after planting (top, middle and bottom figures, respectively)

A good fit was observed with the linear model on untransformed data:

Upwind: $y = 0.88 - 0.073 x$ ($r = 0.94$)
Downwind: $y = 0.62 - 0.047 x$ ($r = 0.79$)

Exponential and linear models were also tested. However, to deal with the nill value 10 m downwind from the source, the transformation Log (y + k) was used where k is a constant. Systematic values of k ranging from 0.01 to 100 were tested. Analysis of residuals of the regressions indicate that k values should be between 0.15 and 0.45. A good fit was obtained for the logarithmic model with k = 0.25, but the adjustment between observed and calculated values was not significantly better than that observed with the linear model. Equations are:

Upwind: $\text{Log}(y_t + 0.25) = 0.860 - 0.175 x$ ($r = 0.93$)
Downwind: $\text{Log}(y_t + 0.25) = 0.216 - 0.113 x$ ($r = 0.78$)

Local spread around the large internal 100-plant source also occurred in Field 2: Disease incidence was 44%, 31% and 33% in rows 1, 2 and 3 m from the source. Further away, disease incidence was not significantly greater than the background level of 18%.

(c) Spread from small internal sources

Three factors were considered in the statistical analysis: (1) The presence of internal foci of four infected plants inside some plots (factor of interest); (2) The degree of exposure to the prevailing SW wind (three line blocks); (3) The impact of roguing.

The regular disposition of the small infected foci is unlikely to have caused problems in the analysis as no previous experiment had used the same pattern in this field. However, the disposition creates unbalance and so an unbalanced three-way crossed classification was performed. Interactions between treatments were shown to be statistically non-significant which indicate that interference due to spread between plots was avoided and show that the experimental layout of Field 1 was indeed appropriate. The effects were estimated by the least square means (GRAYBILL 1976).

Fig. 3a illustrates the progress of disease incidence in plots with and without small primary sources, summing the data for the rogued and unrogued sections of Trial 1. Disease incidence was consistently greater in the plots with 4-plant sources than in those without. The differences between the treatments became significant 6 months after planting, when disease incidence was still less than 25%. Differences in disease incidence between the treatments persisted throughout the study, although the absolute differences never exceeded 12%.

Spread from the small sources of 9 and 25 infected plants in Field 2 was restricted and apparent only on the first row of plants around each source. These results indicate that although the small groups of infected cassava cuttings inside the plots contributed to virus spread, their effect was limited both in magnitude and in extent compared with spread from outside sources.

(d) Spread from plants infected after establishment

Fig. 3b illustrates disease incidence in plots of Field 1 where infected plants were rogued and in plots where they were retained, summing the data for plots with internal sources and those without. As roguing was done systematically and

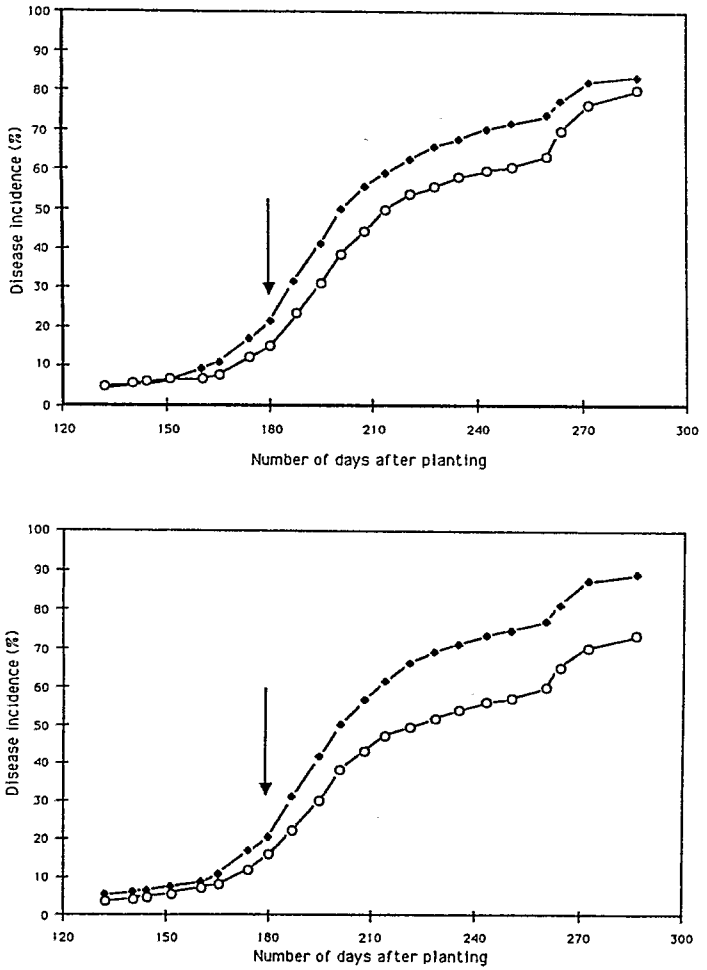

Fig. 3. Disease progress curves in plots of Field 1:
(3 a; top) with an initial 4 % source of inoculum (●) and without an initial source (○); (combined data for rogued and unrogued plots). The arrow indicates the data when the differences of disease incidence between the treatments becomes statistically significant.
(3 b; bottom) where plants that became infected during trial were allowed to remain (●) and in plots where they were rogued (○); (combined data for plots with and without primary foci)

not in randomized plots to avoid interference (see Materials and Methods), differences must be interpreted cautiously because positional effects cannot be excluded. Nevertheless, the rate of virus spread and final incidence of infection were consistently less in rogued than in the equivalent unrogued plots and differences in disease incidence reached up to 18%. These results suggest that roguing was only partially effective in decreasing spread and the final incidence of infection in rogued plots was still great. This provides a further indication of the limited importance of spread from internal foci within plantings that are subject to much inoculum from outside sources. It also indicates the relative inefficiency of roguing as a control measure with cultivars of the CB type that do not have substantial resistance to ACMV infection.

Whitefly identity and infectivity

The identification of whiteflies is based on the form of the pupal cases (MOUND 1965). The great majority of those observed in cassava fields at the Adiopodoumé Experimental Station were of *B. tabaci*, although some *B. hancocki* sometimes occur in small numbers (FISHPOOL, pers. comm.). Table 1 indicates percentage transmission of ACMV to cassava by adult whiteflies collected on different dates from various cassava fields at Adiopodoumé. Transmission as estimated per individual was always very low, the mean was 0.37% with 95% confidence limits of 0.18% and 0.67% (MONNESTIER and LABONNE 1981). Such very low levels of transmission occurred even when the very susceptible cv. H58 was used as the test variety.

Table 1
Percentage*) of *Bemisia tabaci* which transmitted ACMV

	Trial number							
	1	2	3	4	5	6	7	Average
Infected plants/ total number tested	2/10	5/10	1/10	1/10	1/10	2/20	0/20	12/90
% of transmission	0.56	1.70	0.26	0.26	0.26	0.26	0	0.37
Lower confidence limit	0.06	0.52	0.01	0.01	0.01	0.03	0	0.18
Upper confidence limit	2.0	4.10	1.40	1.40	1.40	0.95	0.46	0.67

*) Percentage estimated from the number of plants showing symptoms after groups of 40 whiteflies had been placed on young cassava test plants.

Whitefly populations and disease incidence

Fig. 4 indicates how changes in the mean numbers of adult whiteflies per plant (means of 40 plants) as estimated weekly in Field 1 were followed by changes in disease increments. The best correlation was obtained between whitefly populations and disease increment recorded 6 weeks later (angular transformation, $r = 0.70$; $df = 27$; $p < 0.001$). The large number of whiteflies in

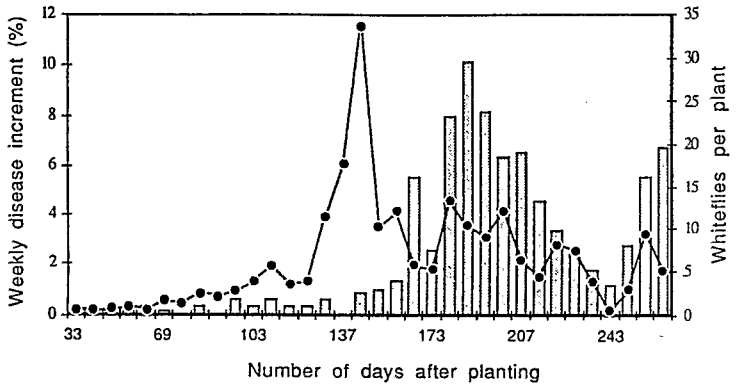

Fig. 4. Number of adult whiteflies per plant of Field 1 estimated each week (●) and weekly disease increment ( )

relation to the comparatively small disease increment suggests that the percentage of adult *B. tabaci* which actually transmit the disease in the field is low, which is consistent with the results of the transmission experiments presented in the previous section.

Crop density and ACMV spread

Table 2 presents, for the last three assessments dates, disease incidence expressed as percentages of total stand for the plots planted at different densities in Field 3. As the number of plants per plot does not vary more than twofold, a classical unweighted variance analysis was performed. Disease incidence increased from c. 50% to c. 80% between these surveys and there were significant differences between spacing treatments ($p < 0.05$); on each occasion disease incidence was greatest at the lowest plant density and least at the greatest plant density. There was a similar trend on earlier assessments but the differences were not significant. This suggests that the experimental trial was not powerful enough (in relation to the residual variation) to detect significant effects and indeed the

Table 2
The influence of plant density on the incidence of ACMV

Date	Density D1	D2	D3	D4	D5
21/4/83	61.4 (a)	53.1 (ab)	46.2 (b)	43.0 (b)	41.9 (b)
10/5/83	76.8 (a)	75.8 (a)	66.9 (ab)	58.2 (b)	57.9 (b)
8/6/83	95.3 (a)	95.3 (a)	85.5 (b)	83.2 (b)	81.0 (b)

Percentage of plants showing symptoms in plots planted at different densities: D1: 5,917 plants/ha; D2, 6,944 plants/ha; D3, 8,264 plants/ha; D4, 10,000 plants/ha; D5, 12,345 plants/ha.
Means within lines followed by the same letter are not significantly different ($p = 0.05$) according to Duncan's multiple range test.

power of the test for the observed difference between treatments (densities) was less than 80% (PEARSON and HARTLEY 1966).

Although the *percentage* of infected plants is indeed lower at greater density, the *total number* of infected plants per surface unit would still be higher: For instance, given the percentage of infected plants 61.4%, 53.1%, 46.2%, 43.0% and 41.9% (Table 2), there would be 3,633, 3,687, 3,818, 4,300 and 5,172 infected plants in one hectare when planted at densities D1 (5,917 plants/ha), D2 (6,944 plants/ha), D3 (8,264 plants/ha), D4 (10,000 plants/ha) and D5 (12,345 plants/ha), respectively.

Discussion

When considering infection gradients, GREGORY (1968) distinguished between *environmental gradients* due to the physical effects of vegetation or microclimate and *dispersal gradients* around sources of infection. Environmental gradients of ACMV that were orientated along the direction of the prevailing south-west wind and over distances of several dozen metres have been reported previously in the forest area of Côte d'Ivoire (FARGETTE *et al.* 1985). They were again apparent in the present trials and in similar circumstances as the highest disease incidence was consistently observed on the wind exposed borders of plantings.

The extent and orientation of the dispersal gradients recorded around the internal sources within Fields 1 and 2 differed markedly from the environmental gradients. The grouping of infected plants around the large central sources of 50 plants in Field 1 and 100 plants in Field 2 clearly indicated spread from these foci. However, the gradients were steep and local spread could not be distinguished from 'background' infection over distances greater than a few metres. In contrast to the environmental gradients, the dispersal gradients were *not* only orientated downwind as spread occurred in all directions from the sources and was consistently greater upwind than downwind. There are indications that the dissimilar characteristics of dispersal and environmental gradients are related to different types of vector movement. Within the crop canopy where windspeeds are low, adult whiteflies mainly fly over short distances. They can move in any direction but evidence suggests that there is more movement upwind than downwind indicating controlled flight in the still conditions encountered within the crop. Above the canopy where there is greater air movement, flight is mainly uncontrolled, downwind and over large distances (FISHPOOL *et al.* 1987, YAO *et al.* 1987). Other features of the distribution of ACMV such as the greater incidence of infection noted along the 3 m wide path within Field 2 could be due to the effects of changes in plant architecture and canopy on wind characteristics and thus on the distribution of the vectors as reported with *Aphis fabae* in crops of broad bean (JOHNSON 1950).

These and earlier trials at Adiopodoumé demonstrate rapid primary spread from outside sources. This leads to internal foci that then initiate secondary spread within plantings. The successive increments in incidence of ACMV in the rogued plots of Field 1 that were without initial sources were variable which

suggests that the rate of primary spread is influenced by seasonal and other factors. Rapid and extensive spread from outside sources is not only characteristic of the Adiopodoumé location but has also been recorded elsewhere in the lowland coastal forest area of Côte d'Ivoire and at Tontonou in the Savanna region (FAUQUET *et al.* 1988). Small sources of infection, such as the groups of 4 infected cuttings or the plants infected after planting, did contribute to the spread. However, they were of limited importance compared to primary spread from outside sources. The large groups of 50 and 100 diseased cassava plants made a greater contribution to secondary spread which indicates that infected cassava fields are a serious source of contamination. Indeed, previous studies on ACMV have indicated the importance of cassava fields as the main source of virus and vector (FAUQUET *et al.* 1988).

ACMV spread depends not only on virus sources but also on whitefly populations and their infectivity. The percentage of individual adult *B. tabaci* which transmitted ACMV was usually very low, as established by infectivity tests. This is consistent with the large numbers of adult *B. tabaci* recorded on plants in relation to the spread of ACMV in the field as noted in these and earlier trials (FARGETTE 1985). It is not clear, however, if *B. tabaci* is an inherently inefficient vector of ACMV or if the poor rates of transmission are due to the low concentration and limited distribution of ACMV in cassava leaves. Within each field site, factors such as vector activity, percentage of viruliferous *B. tabaci* and plant susceptibility could have changed with season and stage of crop growth. Nevertheless, there was, as in Nigeria (LEUSCHNER 1977), good agreement between vector numbers and the subsequent increase in disease incidence. Large variations in whitefly populations were followed by equally marked changes in disease increment. The 6 week time lag is presumably a 'field estimate' of the latent period in cassava from inoculation to symptom expression. Shorter values of 3—4 weeks have been obtained in transmission experiments in glasshouse experiments with young plants but the discrepancy can be explained by differences in the pattern of growth between field and glasshouse plants. The long latent periods encountered in field trial 1 may also be because most spread occurred at a stage when plants are less susceptible and develop symptoms later than young plants.

The spread of many diseases is influenced by host plant distribution and our experiments indicate that ACMV incidence is greater at low than at high plant density. This is a common feature of insect-borne viruses, especially those with aphid vectors (THRESH 1982). A possible explanation, in at least some instances, is that similar numbers of vectors enter the different stands per unit area. Thus number of immigrants per plant and consequently the proportion of plants infected are greatest in sparse stands, although similar numbers of plants are infected. There is as yet no evidence that the influx of incoming whiteflies is influenced by plant density or plant conformation. However, at least some types of vector alight preferentially on plants at wide spacing that stand out against a background of bare soil (THRESH 1982). This is unlikely to be a factor with ACMV because the main spread in the spacing trial occurred after a continuous canopy of foliage had developed. Moreover, the number of plants that became

infected was greater at high than at low density. These findings suggest that wide spacing increased the vulnerability of plants to infection and this could be due to an effect of the plant size and the degree of branching as it is known that only young tissues are susceptible and that infection occurs at or near the shoot apices (STOREY and NICHOLS 1938).

The results of these and previous epidemiological studies in Côte d'Ivoire and elsewhere can be used to indicate the feasibility of control measures. In coastal Kenya, spread to cassava by *B. tabaci* is usually low (less than 2 % per year) and there is no evidence of directional spread into fields or from sources established within plantings. In these circumstances the release of healthy material is sufficient to avoid the disease, especially when roguing is practised (BOCK 1983). In the lowland forest area of Côte d'Ivoire, rapid primary spread from outside sources occurs throughout the year. Planting healthy cassava cuttings at high density followed by roguing restrict spread but these practices are unlikely to provide an effective or practicable means of control with cultivars of the type now used. Moreover, the rapidity and extent of spread from diseased cassava fields implies the need for great isolation to maintain plantings virus-free. This is not easily achieved in areas where cassava is grown intensively in numerous small plots of diverse age and with limited separation between them (THRESH 1988b). Thus, the available knowledge on the epidemiology of ACMV suggests that a strategy based on releasing healthy material is unlikely to be successful in the coastal forest area unless cultivars are used with greater resistance to infection than those currently available. This situation is not typical of the entire Côte d'Ivoire. In areas such as Toumodi (200 km north of Abidjan), cassava is not widely grown, little primary spread occurs and adequate cultural practices, including roguing of diseased cassava and some isolation from cassava plantings upwind enable healthy stocks to be maintained successfully for years (FAUQUET *et al.* 1988).

This work was supported in part by grants from the Commission of the European Communities TSD-102 and TS2A-0137-C (CD).

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J. Phytopathology 130, 303—311 (1990)

ISSN 0931-1785

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Purification and Partial Characterization of Isometric Virus-like Particles in *Kalanchoe* Species

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With 4 figures

Received April 19, 1990; accepted April 24, 1990

Abstract

Isometric virus-like particles (IVLP) were detected in crude sap from *Kalanchoe pinnata*, *K. daigremontiana* and *K. tubiflora* plants showing a mild mosaic on the leaves. These particles of 35 nm in diameter were transmitted mechanically to several test plants but not to healthy *Kalanchoe*. Air temperatures above 30°C hindered the infection process. The IVLP were purified from systematically infected *Nicotiana benthamiana* using Triton X-100 as clarifying agent followed by PEG precipitation. IVLP were degraded by organic solvents and formed aggregates in the presence of 2 mmol/l $CaCl_2$. The particles occurred in relatively low concentration in plant sap and lost infectivity in leaves frozen at −70°C for one week and in purified preparations kept at 4°C. In buffer crude sap of *N. benthamiana* IVLP had a thermal inactivation point between 45 and 50°C on a longevity *in vitro* of 20 h at 25°C. Particles contained one nucleoprotein component with a molecular weight of 46,000 daltons and a ssRNA species which, when denatured, had a molecular weight of 1.2×10^6. IVLP purified preparations exhibited a typical nucleoprotein absorption spectrum with a maximum at 254—260 nm and a minimum at 240—243 nm and a A 260/280 ratio of 1.56. The buoyant density of the IVLP was 1.32 g/ml calculated by isopycnic centrifugation on CsCl.

Ultrastructural studies in infected leaves of *K. pinnata* indicated that IVLP caused an increase in chloroplast volume, distortion of the grana and reduced the number of thylakoids per grana. IVLP infection also impaired the diurnal pattern of synthesis and hydrolysis of starch, characteristic of CAM plants. The non-serological reaction of the IVLP with antisera specific to members of 7 different groups of spherical viruses as well as the combination of physicochemical properties and host range exhibited by these particles impeded their taxonomic location. In nature, young *Kalanchoe* plantlets acquire the IVLP through their physical connections with the infected mature leaves.

Subjected for publication on September 1989.

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