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Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.), (Hom., Aleyrodidae)

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Abstract

Host-associated differences within populations of the whitefly *Bemisia tabaci* from cassava, okra and other host plants in Côte d'Ivoire, West Africa, were investigated by iso-enzyme electrophoresis and experimental host range studies. Two biotypes were identified. One was found only on cassava and eggplant; the other was polyphagous, but did not infest cassava. Differences in esterase patterns matched these host range restrictions exactly. The implications of these finding are discussed in relation to the role of *B. tabaci* as a virus vector.

1 Introduction

The pest status of the whitefly *Bemisia tabaci* (Genn.) in most tropical countries is related to its extreme polyphagy and its capacity as a virus vector: more than 500 plant species are listed as hosts (Greathead 1986), and it transmits at least 19 virus diseases (Brunt 1986). In Côte d'Ivoire, West Africa, more than 70 host plant species have been recorded (Burban, unpubl. results), and *B. tabaci* transmits several virus diseases of importance, notably African cassava mosaic and okra leaf curl geminiviruses (Fauquet and Thouvenel 1987). The taxonomy of whiteflies is mainly based upon characters of the "pupa", as the fourth nymphal instar is commonly called. *Bemisia tabaci* was described differently many times before it was found that many of the morphological characters used for identification were labile and that much of the variation was host-induced (Mound 1963; Mohanty and Basu 1986). Thus, conventional taxonomy is of little value at the intraspecific level. Molecular techniques are being used increasingly in systematics, including entomology (Berlocher 1984). They are especially useful at the lower taxonomic levels, and can be used for example to confirm the existence of biotypes, the presence of which may have been suspected on morphological, ecological or behavioral grounds, and to assess the degree of separation between them (Diehl and Bush 1984). For epidemiological and agro-economic purposes it is essential to understand the intimate interactions that develop between local *B. tabaci* populations and their local host plants, especially any host-related differentiation between populations.

The purpose of this study was to test for host-associated differences within sympatric *B. tabaci* populations, using isozyme electrophoresis and host range studies. Experiments concentrated on material from cassava and okra, the whitefly-borne viruses of which have been much studied in Côte d'Ivoire (Dubern 1979; Fargette 1985; Fauquet and Fargette 1990; N'Guessan et al. 1991). The work was undertaken mainly in the lowland rain-forest zone of Côte d'Ivoire, West Africa, at Adiopodoumé, 20 km west of Abidjan. This paper develops preliminary results presented elsewhere (Burban et al. 1989).

2 Materials and methods

2.1 Biological material

Insects: pupae of *B. tabaci* were collected in the field from different plant species and identified using a binocular microscope, according to MARTIN (1987). Upon emergence, some adults were transferred onto the same host plant species, maintained in a greenhouse and used to form colonies. Others were used directly or after freezing for electrophoretic study. The insect material was collected mainly from around Abidjan in southern Côte d'Ivoire. In addition, samples of field populations were also collected along a 600 km north-south transect across the country to be analysed electrophoretically. About 10 insects by site by host plant species were analysed.

Plants: for the greenhouse experiments using crop plants the following cultivars and varieties were used: cassava (Kasimbidgi Green), okra (Clemson Spineless) and cotton (ISA 205). The varieties of eggplant (*Solanum melongena*) and tomato used could not be ascertained. Wild plants were grown from seeds or cuttings collected around Abidjan.

2.2 Electrophoresis

Four enzyme systems were studied using vertical slab polyacrylamide gel electrophoresis: Esterases (EST), Glucose phosphate isomerase (GPI), Malate dehydrogenase (MDH) and Xanthine dehydrogenase (XDH). Single adults were homogenized in 10 µl of a Trudgill buffer (TRUDGILL and CARPENTIER 1971), with 10 % Triton X 100 for EST, MDH and XDH. The homogenates were transferred in pockets of a stacking gel (2.5 % acrylamide), superposed on the resolving gel (7 % acrylamide: EST, XDH; 9 %: MDH, GPI). The reservoir contained borate buffer 0.15 M, pH 8.2 (EST, GPI, XDH) (WOOL et al. 1989) or Tris 0.06 M glycine 0.37 M buffer pH 8.3 (EST, MDH). Gels were run at 100 V for 15 min then 200 V for 90 min (EST, XDH) or 100 V then 250 V (GPI, MDH) for the same lengths of time. Bromophenol blue was used as a running marker. The staining techniques used were taken from SECOND and TROUSLOT (1980) for EST, GPI, MDH, and PASTEUR et al. (1987) for XDH.

2.3 Experimental host range studies

The host range and transfer experiments used newly-emerged adult *B. tabaci* collected from the greenhouse colonies. Three types of experiments were undertaken as described below.

1. To test the polyphagy of the local *B. tabaci* populations the following locally recorded hosts (BURBAN, unpubl. data) were placed in each of two separate insect-proof greenhouses: Compositae: *Chromolaena odorata*; Euphorbiaceae: *Euphorbia heterophylla, Manihot esculenta* (cassava); Leguminosae: *Centrosema pubescens, Crotalaria* sp., *Pueraria phaseloides*; Malvaceae: *Abelmoschus* sp. (okra), *Sida rhombifolia, Sida carpinifolia*; Solanaceae: *Lycopersicon esculentum* (tomato), *Solanum nigrum*. About one hundred okra-reared adult *B. tabaci* were introduced into one greenhouse and the same number from cassava into the other. Six weeks later, a time interval permitting the development of two generations and hence allowing for a greater opportunity for colonization of the plants, the presence or absence of *B. tabaci* nymphs on each plant species was noted.

2. To test the acceptability of particular host species in relation to the original host of the whitefly, a non choice experiment was set up. Twenty-five adults on the point of emergence from pupae taken from the chosen hosts (cassava, cotton, crotalaria, eggplant, okra or tomato) were transferred to cages containing the host to be tested: a single plant of one of these species or of *Manihot glaziovii*. The transfer was considered successful if a bisexual generation completed development on the new host (*B. tabaci* is a arrhenotokous species [LOPEZ-AVILA 1986], with unfertilized eggs giving rise exclusively to males). Transfers to the same host species were performed as controls.

3. The third set of experiment was similar to the second, but the insects tested, reared on cassava, eggplant or okra, were in this case given a choice of cassava or okra. Two weeks after the experiment was begun, plants were put into separate cages, i.e. before the emergence of the resulting generation.

For all experiments, electrophoretic analyses (EST) of adult whiteflies from both the original colonies and those reared on the host plants tested were undertaken.

3 Results

3.1 Electrophoretic studies of field populations

GPI, MDH and XDH showed little or no polymorphism, irrespective of the host species from which the whiteflies were collected (not illustrated). GPI and XDH zymograms showed one or two bands and MDH zymogram always two bands.

By contrast, the EST patterns showed variation and were comprised of three isozyme systems: EST A, EST B and EST C (figs. 1, 2). EST A was the slowest migrating band and its intensity was weak if present with EST B. EST B was composed of several bands among which two were particularly frequent and strong. EST C, present only with EST A but never with EST B, showed one or two fast migrating, weak bands.

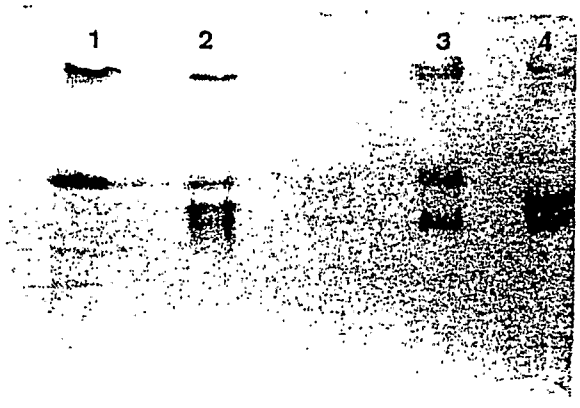

Fig. 1. Bemisia tabaci esterase patterns. 1: for insect reared on okra; 2–4: for insects reared on cassava

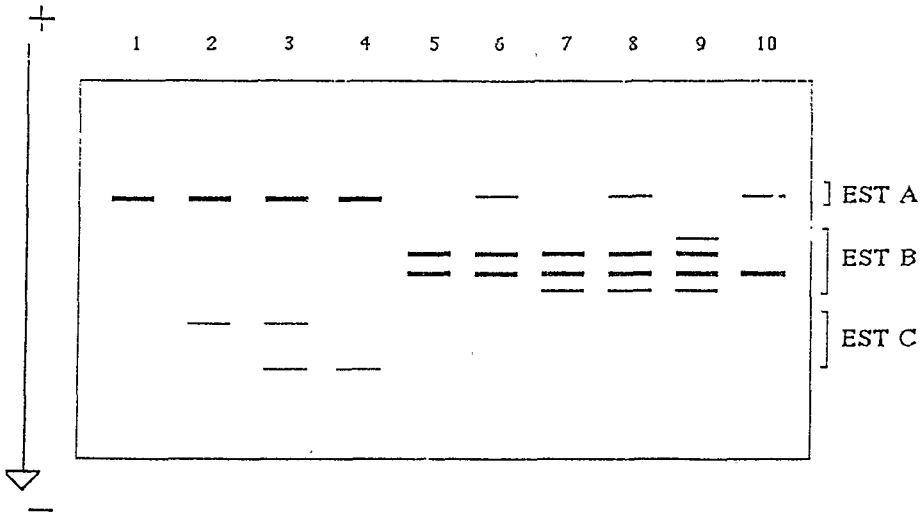

Fig. 2. Diagrammatic representation of the esterase patterns obtained from *B. tabaci*. 1–4: okra patterns; 5–10: cassava patterns

Thus, two major types of EST pattern were distinguishable which, as they were clearly host-associated (table 1), were termed the cassava pattern and the okra pattern respectively according to the presence or absence of EST B. The okra pattern was found on samples from all plants other than *Manihot* spp. while the cassava pattern was limited to specimens from *Manihot esculenta* (cassava), *M. glaziovii, Solanum melongena* (eggplant) and *S. aethiopicum* (a locally cultivated eggplant). Okra and cassava EST pattern whiteflies occurred together only on eggplant.

Polymorphism within these two types of pattern (the whole ranges of each are illustrated in fig. 2), was apparently not related to host plant. As the genetic determinism of EST isozyme is unknown, further work is needed to investigate the significance of this variation.

Bemisia tabaci samples collected throughout the north-south transect showed the same EST patterns, without any apparent geographical variation. However, at the three northernmost sites, no *B. tabaci* were found on cassava and those collected there from eggplant all showed the okra EST pattern (fig. 3). Elsewhere along the transect the situation was the same as was found around Abidjan.

Table 1. Presence or absence of isozymes B in Esterase pattern of *B. tabaci* from field populations

Number of individuals tested from different host plant species

Host plant species	Esterase isozymes of *Bemisia tabaci*	
	Absence of EST B	Presence of EST B
Abelmoschus spp. (okra)	115	0
Borreria ocymoides	5	0
Centrosema pubescens	15	0
Crotalaria spp.	20	0
Eupatorium odoratum	15	0
Gossypium hirsutum (cotton)	24	0
Ipomoea batatas (sweet potato)	10	0
Lycopersicon esculentum (tomato)	15	0
Manihot esculenta (cassava)	0	97
Manihot glaziovii	0	11
Pueraria phaseoloides	15	0
Sida spp.	20	0
Solanum spp. (eggplant)	20	12

3.2 Transfer experiments

In the polyphagy test, colonization of the plants depended upon the origin of the whiteflies: those from cassava (cassava EST pattern) colonized only cassava, whereas *B. tabaci* from okra (okra EST pattern) colonized all plant species offered (listed in section 2.3) except cassava.

The results of the non choice experiment (table 2) confirm this. It is apparent that whiteflies from cassava bred only on *Manihot* spp. and eggplant; those from eggplant bred on cassava and eggplant as well as okra and *B. tabaci* from other hosts could establish themselves on all hosts tested except *Manihot* spp. So, the differences observed in the polyphagy test do not simply indicate variation in the preferences of the insects, but major differences in their ability to colonize particular host plants. It was found that their EST patterns corresponded exactly to these host range restrictions.

Data obtained from the choice experiment (table 3) confirm this correspondance: it is noteable that insects of either EST pattern, which breed together on eggplant, will separate when given a choice of okra and cassava.

4 Discussion

There have been few studies of whitefly genetics. Two have demonstrated the use of isozyme electrophoresis in species identification: differences have been found between whiteflies species for EST and PGM, but nor for MDH (PRABHAKER et al. 1987), and EST and α-GPH (WOOL et al. 1989). Geographical variations in the EST of *B. tabaci* populations have also been found, on a continental scale (MCGRATH and HARRISON 1990), and regionally, in different valleys in Colombia (WOOL 1990). Our study is the first to show host-associated variation.

The exact match of the EST pattern with host range implies the existence of two *B. tabaci* biotypes, sympatric in southern and central Côte d'Ivoire. One, called the okra biotype, is polyphagous but excludes cassava from its host range; the other, the cassava biotype is found only on *Manihot* spp. (including cassava), *Solanum aethiopicum* and *S.*

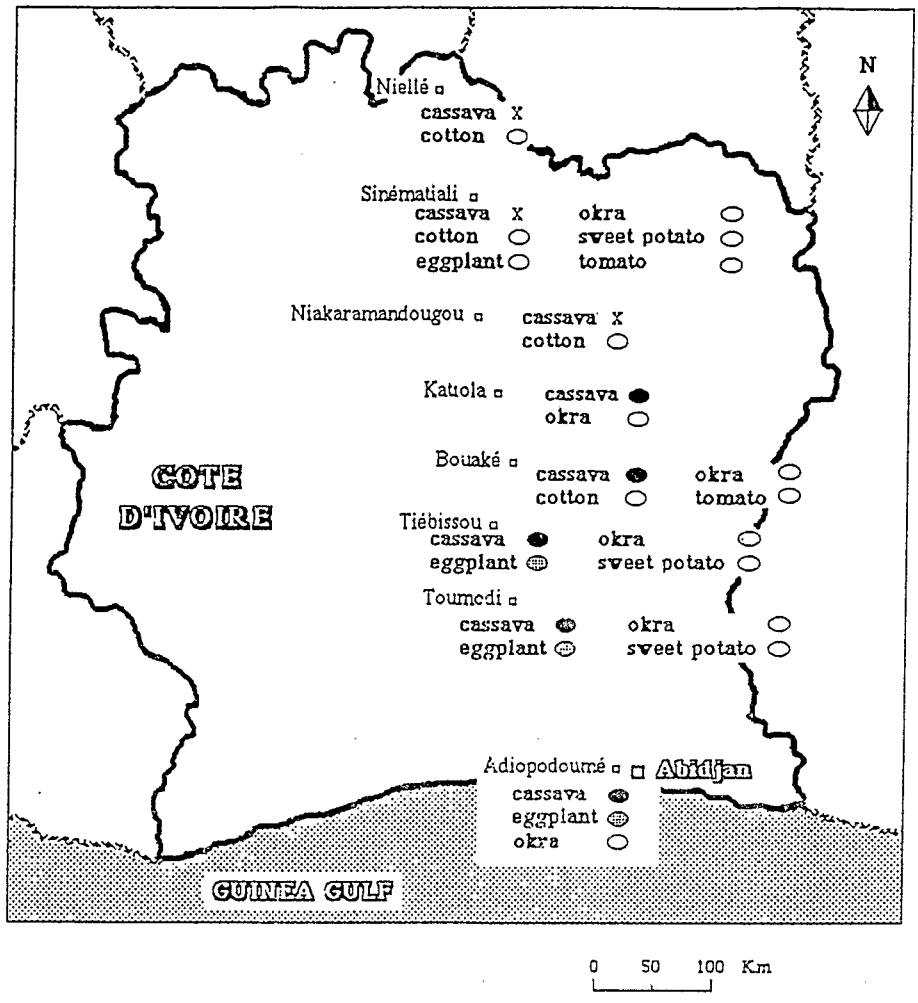

Fig. 3. Esterase patterns of *B. tabaci* from various crops along a north-south transect across Côte d'Ivoire. □: site of study; X: no insect on the crop; ●: cassava pattern; ○: okra pattern; ◍: two patterns mixed

melongena; the latter two species the only hosts shared by the two biotypes. The differences in the EST patterns of the two biotypes match these host range restrictions exactly. It should be stressed that the isozymes are considered only to be markers. It is not suggested that there is a causal relationship between EST pattern and host range. Thus, EST patterns were conserved between generations when insects were transferred to new host species; each type of pattern was relevant to insects from several host plant species and eggplant supported insects of both pattern. No differences were noted for the others isozymes tested, GPI, MDH and XDH.

Crossing experiments are necessary to establish the degree of separation between these two biotypes. Preliminary trials between insects reared on okra and cassava were attempted but were unsuccessful. This result is however not conclusive, because too few insects were tested. Eggplant should be used as host for further crossing experiments, as it supports both biotypes.

There is much published data on the reproductive performance of *B. tabaci* on different host plants. The results presented here can help in the interpretation of some of these, particularly those involving cassava. The failure of *B. tabaci* to breed on cassava in South America was used to infer that *B. tabaci* from New World and Old World are distinct races (COSTA and RUSSELL 1975); from this study, it can be argued that the biotype breeding on cassava in Africa is probably absent from South America. In Northern Nigeria and Sudan *B. tabaci* was found to be common on cotton but rarely infested cassava, whereas the opposite is true in Southern Nigeria; in these countries, transfer experiments gave mixed results, depending upon the original host plant of the whiteflies, and where they were performed (MOUND 1981, 1983). This may reflect a situation comparable to that in Côte d'Ivoire. *Bemisia tabaci* may be expected to be widespread on cassava in areas where cassava is widely cultivated, since it offers favourable habitat to the cassava biotype of *B. tabaci*. In other places such as northern Côte d'Ivoire, where there is less intense cultivation of cassava and of alternative known hosts, there is less likelihood of the cassava biotype colonising these areas. An analogy can perhaps be made here to the situation that occurs in parts of Asia where in India *B. tabaci* is a well known pest of cassava (NARESH and NENE 1980), but NACHAPONG and MABBET (1979) indicate that in Thailand cassava is not attacked by *B. tabaci*, even when grown in proximity to other plants infested by the pest.

Host-associated biotypes are frequent in phytophagous insects, but usually in monophagous or oligophagous species (DIEHL and BUSH 1984). West African *B. tabaci* populations are notable for having one biotype that is polyphagous while a second is oligophagous. A similar situation has been described in Puerto Rico where two races of *B. tabaci* are distinguished according to their host range and vector capacity (BIRD 1957): *B. tabaci* race *sidae* is polyphagous but does not breed on *Jatropha* spp., whereas *B. tabaci* race *jatropha* is limited to *Jatropha* spp. Factors favouring the evolution of host specialization of a particular *B. tabaci* biotype on cassava include preference for oviposition on the original host (BURBAN, unpubl. data), mating on the host, as well as the perenniality and high cropping density of cassava and its low acceptability and suitability to the polyphagous biotype of *B. tabaci*.

From an applied perspective, the occurrence of host-limited biotypes in *B. tabaci* is of particular interest in relation to its role as a virus vector.

Table 2. Non-choice host transfer trials of *B. tabaci*: ratios indicate the number of successful transfers/number of transfers attempted
Esterase patterns of original greenhouse population and of the resulting generation are indicated: (O) okra pattern; (C) cassava pattern. The symbol "–" indicates that the transfer was not attempted

Original host plant (EST pattern)	Insects transferred to							
	Cassava	Okra	Cotton	Tomato	Eggplant	Crotalaria	*Manihot glaziovii*
Cassava (C)	20/20 (C)	0/40	0/10	0/10	10/10 (C)	0/5	5/5 (C)
Okra (O)	0/40	20/20 (O)	10/10 (O)	10/10 (O)	10/10 (O)	5/5 (O)	0/5
Cotton (O)	0/10	20/20 (O)	20/20 (O)	5/5 (O)	–	–	–
Tomato (O)	0/10	10/10 (O)	–	10/10 (O)	–	–	–
Eggplant (mixed O & C)	10/10 (C)	10/10 (O)	–	–	10/10 (O&C)	–	–
Crotalaria (O)	0/10	10/10 (O)	–	–	–	10/10 (O)	–

Epidemiological studies of African cassava mosaic virus (ACMV) in the south of Côte d'Ivoire indicate that cassava is the main reservoir of ACMV (FARGETTE et al. 1985). This may well be related to the fact that its whitefly vector has developed a particular cassava biotype, with a very restricted host range. Thus, cassava would not only be the main reservoir of the virus but also of the vector, which may explain the fact that the spread of the disease is positively correlated with the intensity of cassava cultivation (FAUQUET and FARGETTE 1990). This clearly has important implications for control and sanitation measures against ACMV.

Table 3. Transfer trials of *B. tabaci* where the insects tested were given a choice of cassava and okra

Plant of the two species were separated before the emergence of the new generation. Ratios, esterase patterns: see table 2

Original host plant (EST pattern)	Insects transferred to mixed okra and cassava	
	Cassava	Okra
Cassava (C)	10/10 (C)	0/10
Okra (O)	0/10	10/10 (O)
Eggplant (mixed O & C)	10/10 (C)	10/10 (O)

By contrast, epidemiological studies of okra leaf curl virus (OLCV) suggest the existence of virus hosts other than okra (N'GUESSAN et al. 1991), which, given that the *B. tabaci* biotype breeding on okra is polyphagous, is possible. Control strategies against OLCV may therefore have to involve the vector's alternative hosts.

Indeed, all studies of the ecology of *B. tabaci*, whether of disease epidemiology, alternative hosts, population dynamics, parasitism and predation, or resistance to insecticide, must take into account the existence of biotypes. Knowledge however, of the intraspecific variation within *B. tabaci* is still very incomplete and further genetic studies world-wide are required for a full understanding of *B. tabaci* and its host plant relationships.

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Zusammenfassung

Wirtspflanzenbedingte Biotypen einer westafrikanischen Population von Bemisia tabaci (Genn. Hom., Aleyrodidae,

Mittels der Isoenzymanalyse und experimenteilen Versuchen zum Wirtspflanzenspektrum wurden wirtspflanzenbedingte Unterschiede von *Bemisia tabaci*-Populationen, die von *Manihot esculenta*, *Abelmoschus* sp. und anderen Wirtspflanzen an der Côte d'Ivoire, Westafrika, stammten, erforscht. Es konnten zwei Biotypen ermittelt werden. Eine kam nur auf *Manihot esculenta* und Aubergine vor, die andere war polyphag, beide aber nicht *Manihot esculenta*. Die Unterschiede im Esterasemuster zeigten das Fraßpflanzenspektrum exakt an. Diese Ergebnisse werden in Hinblick auf die Bedeutung von *B. tabaci* als Virusvektor diskutiert.

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