CHARACTERISATION OF *BEMISIA TABACI* (GENNADIUS) (HOMOPTERA: ALEYRODIDAE) BIOTYPES INFESTING SOME SELECTED CROPS IN SOUTHERN GHANA.

BY

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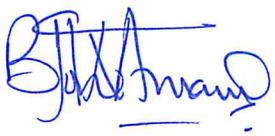
DECLARATION

I do hereby declare that, with the exception of other scholars' work which I have duly acknowledged, all the experimental work described in this thesis was carried out by me and has not been presented, in whole or part, to any other institution for the award of another degree.



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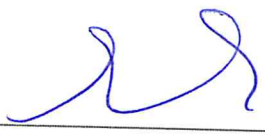

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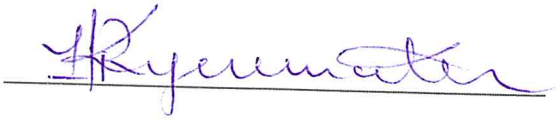

DEDICATION

To my father,

Mathias Oduor Aman Makaman, who will never read it,

and to my mother,

Paschalia Auma Oduor, who nursed a fledgling.

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LIST OF ABBREVIATIONS

µl	microlitre
µm	micro metre
µM	micromolar
ACMV	African Cassava Mosaic Virus
IGR	Insect growth regulator
AFLP	Amplified Fragment Length Polymorphism
AGMV	Asystasia Golden Mosaic Virus
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
AR	Antennal ratio
BR	Body ratio
CMD	Cassava Mosaic Disease
CRD	Completely randomised design
CYSD	Curcubit Yellow Stunting Disorder
dNTP	deoxyribonucleotide triphosphate
EC	Emulsifiable concentrate
EDTA	disodium ethylene diamine tetraacetate.$2H_2O$
EthBr	Ethidium bromide
Fw	Formula weight
GPS	Global Positioning System
H_2O	Water
HCl	Hydrochloric acid
JMV	Jatropha Mosaic Virus

KCl	Potassium chloride
LC_{50}	Median Lethal Concentration
LFL	Lower Fiducial Limit
LIYV	Lettuce Infectious Yellow Virus
M	Molar
$MgCl_2$	magnesium chloride
MOFA	Ministry of Food and Agriculture
Mw	Molecular Weight
NaOH	Sodium hydroxide
NPK	Nitrogen-Phosphorus-Potassium complete fertiliser grade
NTSYS	Numeric taxonomic Systems (software)
PAUP	Phylogenetic Analysis Using Parsimony (software)
PAGE	Polyacrilamide gel electrophoresis
PCR	Polymerase Chain Reaction
pH	Hydrogen ion exponent.
PPRSD	Plant Protection Regulatory Services Directorate
RAPD PCR	Randomly Amplified Polymorphic DNA Polymerase Chain Reaction
R.H	Relative humidity
RFLP	Restriction Fragment Length Polymorphism
rpm	revolutions per minute
RR	Resistance Ratio
$sddH_2O$	sterile double distilled water
SDS	Sodium dodecyl sulphate (also sodium lauryl sulphate)
SPCSV	Sweet potato Chlorotic Stunt Virus
TAE	Tris-Acetate EDTA

TE	Tris-EDTA
Tm	Melting temperature
ToCV	Tomato Chlorosis Virus
Tris	2-amino-2-(hydroxymethyl)-1,3 propanediol
TYLCV	Tomato Yellow Leaf Curl Virus
ToYLCV	Tomato Yellow Leaf Curl Virus
UFL	Upper Fiducial Limit
UPGMA	Unweighted pair-group method using arithmetic averages
UV	Ultra – violet

ABSTRACT

Intraspecific variation and biological heterogeneity in the whitefly *Bemisia tabaci* make it a major pest in diverse agricultural systems worldwide. The economic burden of this insect pest owes to its apparent polyphagy, vectorial capacity for viruses, and propensity for insecticide resistance development. Ecological specialisation has been observed in the species, which compounded with the variability, and adaptability pose serious impediments in its control. Effective description of local populations is, therefore, an essential basis of integrated management. Populations of *B. tabaci* infesting cassava, okra, garden egg and tomato were characterised in three locations in southern Ghana. Descriptions were based on ecological, taxonomic and agricultural parameters. Morphological and molecular characterisation were done by morphometric analysis and RAPD PCR, respectively. Host preference was assayed by two-choice and multiple-choice landing and oviposition preference assays. The host races putatively identified were compared on the rearing suitability on eight hosts and insecticide tolerance. Finally, mating compatibility between them was assessed. There were no distinct morphometric differences between the populations as adults, but nymphal classification was less overlapping. Two genetic clusters (associated with okra and cassava) with 45% similarity were revealed. Host affiliation contributed significantly to total variability, but geographical isolation did not. Whiteflies generally chose their rearing host for landing and oviposition, but clear demarcation on cassava and okra was evident. Okra populations survived on all hosts except cassava, while cassava biotype survived on cassava, tomato, garden egg, eggplant and cowpea but not on okra, pepper or cabbage. The okra populations were more tolerant to chlorpyrifos and lambda cyhalothrin than were the cassava populations. Mating and fertilisation were successful but reproductive isolation was evident in male-dominant sex ratios of progeny. Inter biotype mating produced 2.3: 1 (male: female) sex ratio compared to

0.6 in intra biotype mating, while unmated whiteflies produced only males. Female crosses were normal, and able to oviposit. The existence of two morphologically similar biotypes demarcated by cassava and okra was confirmed. Host interactions and RAPD PCR consistently identified the two biotypes and are suggested for use in their identification. Random primers OPA 02, OPB 08, OPC 05 and OPD 16 are recommended for this. The host range of the cassava biotype seems wider than earlier reported. Solanaceous plants acted as common hosts and their role as potential reservoir hosts was speculated. Toxicological reactions were attributed to different insecticide pressure at the sites. Partial reproductive isolation between the two biotypes was revealed but the stability of the crosses in nature is not clarified.

CHAPTER ONE

INTRODUCTION

Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) is a devastating pest of vegetable, ornamental and field crops worldwide. It colonises numerous crops in the field and greenhouse, as well as weeds (Mound and Halsey, 1978; Muniz, 2000). *B. tabaci* is thus variously known as tobacco, cotton, cassava or sweet potato whitefly (Brown *et al.*, 1995). The pest is now found on all the continents of the world except Antarctica (Oliviera *et al.*, 2001). In Ghana, it is a serious pest of cassava, tomato, okra, cucumber, cotton, eggplant and legumes (Gerken *et al.,* 2001).

The increasingly grave pest importance of this species stems from the multiple and heterogeneous nature of the losses it causes. *B. tabaci* inflicts direct damage by sucking sap, and causing debilitating phytotoxic disorders of unknown aetiology. It transmits at least 111 different plant viruses (Jones, 2003) and contaminates harvested produce by the production of honeydew (Naranjo and Ellsworth, 2001; Hilje *et al.*, 2001). The presence of whitefly nymphs on horticultural produce is unacceptable, increasing the cost of protection of export crops (Gerken *et al.,* 2001)

Heavy losses have been associated with whiteflies worldwide. For example, Brazil suffered accumulated losses exceeding 5 billion US dollars as a result of whitefly damage to beans, tomatoes, cotton, melons, okra and cabbage (Lima *et al.*, 2000). In the USA, up to 500 million dollars is lost yearly in produce and control efforts on cotton alone (Oliviera *et al.,* 2001).

In Africa, *B. tabaci* is an important pest of cassava, cotton, horticultural and annual field crops (Legg, 1994). Cassava Mosaic Geminiviruses, the most devastating crop diseases in Africa, are transmitted by *B. tabaci* (Legg, 1999). Infection rate of up to 100% has been observed (Fauquet and Fargette, 1990). High populations of *B. tabaci* are associated with the prevalence of ACMV in West Africa, and the more virulent pathotypes, East African Cassava Mosaic Virus (EACMV), ACMV - Uganda Variant (UgV), in eastern and central Africa (Gibson *et al.*, 1996; Pita *et al.*, 1998). In Ghana, the pressure of virus disease control and stringent quarantine regulations have led to great increase in spraying frequency on the pest on tomato and okra with attendant economic and environmental problems (Gerken *et al.,* 2001).

The cotton whitefly is the most destructive pest of cotton in Sudan, which earns up to 65% of the country's foreign exchange, and caused near total failure in 1980 and 1981 (Abdeldaffie *et al.*, 1987). On cotton *B. tabaci* causes feeding and phytotoxic disorders, transmits cotton mosaic disease (CMD) and causes boll stickiness as a result of honeydew. *B tabaci* is a serious pest of irrigated vegetables such as tomatoes, peppers, garden eggs and okra in many parts of Africa, transmitting Tomato Yellow Leaf Curl Virus (TYLV) and Okra Green Stunting Virus (OGSV) (Burban *et al.,* 1992; Oliviera *et al.*, 2001). Leaf deformity and honeydew moulds on ornamentals reduce product aesthetic quality. Thus, the economic thresholds are very low exerting great control pressure on the pest populations.

The recent aggravation of the pest status of whiteflies was associated with ecological changes caused by intensive agriculture, and insecticide use. These have increased host suitability and insecticide tolerance (Ahmed *et al.*, 1987). Trade in ornamentals has

afforded rapid dispersal hence the worldwide proliferation especially of the highly pestilent B biotype (Naranjo and Ellsworth, 2001). Efforts to control *B. tabaci* have not been very successful especially in Africa. Chemical control has been the conventional approach but is no longer plausible owing to the development of resistance, economic and ecological implications (Ahmed *et al.*, 1987; Oliviera *et al.*, 2001; Kranthi *et al.*, 2002).

Despite the dogma of polyphagy and multiple virus transmission, various whitefly host races, populations or biotypes have been described with varying host preference, bionomics and ability to transmit specific viruses, yet are not readily morphologically distinguishable (Brown *et al.*, 1995). For instance, whiteflies are important vectors of cassava mosaic geminiviruses in Africa but not in South America. Similarly, they are serious pests of cotton in America, India and Sudan but not in Egypt. Whitefly ecological races also vary in their response to agro-ecological changes and control measures (Gadelseed, 2000). Consequently, these populations have been described based on molecular genetic markers, behaviour, host affiliations, biology, insecticide resistance, pest status and mating compatibility (Burban *et al.*, 1992; Gawel and Bartlett, 1993; Bellows *et al.*, 1994; De Barro and Driver, 1997; De Barro *et al.*, 1998; De Barro and Hart, 2000; Moya *et al.*, 2001).

Integrated whitefly management needs to be based on the understanding of the local populations and their interaction with the agro-ecosystem. Sustainable control, therefore, requires a multi-dimensional approach and may not succeed without clear identification of local whitefly populations based on behaviour, biology, pest status, potential for insecticide resistance and the role of the agro-ecosystem on the pest population.

The choice of oviposition site is an important determinant of whitefly infestation, because the nymphs are sessile (Simmons, 2002). Dispersal occurs by oviposition on new hosts, local feeding movement and migration by adults (Byrne, 1999). Rearing suitability of a host determines its effect on pest bionomics and perhaps eventual behaviour, but the factors governing host plant adaptation and those restricting host plant plasticity are not well understood (Brown *et al.*, 1995). Behavioural and host associated characteristics are essential in suppressing pest populations by ecological manipulation. The effect of available crop communities on the population dynamics is, therefore, of great importance in such approaches.

In West Africa, Burban *et al.* (1992) described the cassava biotype (found on cassava and garden eggs) and the more ploy phagous okra biotype based on host preference and esterase isozyme electrophoresis assays. Gadelseed (2000), using RAPD PCR markers, grouped the populations into cassava and non-cassava biotypes, and proposed cassava and tomatoes as determinant hosts in separating the two biotypes. But the phylogenetic relationship between the two biotypes is still unclear (Burban *et al.*, 1992; Brown *et al.*, 1995). Behavioural determinants of infestation such as oviposition acceptance and rearing preference on the natural hosts and the possibility of gene flow between them have not been studied.

Although morphological variation between biotypes is not obvious, some have been detected between several biotypes (Bellows *et al.,* 1994; Perring, 2001). The most informative isozyme, the non-specific esterases are the present basis of biotype naming but are inducible and variable (Perring, 2001) or may not differ much between distinct biotypes (Burban *et al.,* 1992; Costa *et al.,* 1993). Therefore, molecular methods are more

reliable in revealing variation within populations (Gawel and Bartlett 1993; De Barro and Driver, 1997; Gadelseed, 2000).

RAPD PCR technique allows the analysis of genetic variability without prior knowledge of the genetic make up of the population. Also, it is technically simpler and relatively cheaper compared to other DNA-based techniques (Lima *et al.,* 2000). Because several markers are generated, an arithmetic model of the population genetic structure can be derived (Excoffier *et al.,* 1992; Lynch and Milligan, 1994).

Reliable description of *B. tabaci* populations needs to incorporate several approaches to consistently and comprehensively describe populations. Accordingly, this study sought to characterise *B. tabaci* populations naturally infesting cassava, garden egg, tomato and okra in southern Ghana based on genetic, morphological and biological attributes.

The specific objectives of this study, therefore, were to:

1. study the genetic diversity of *B. tabaci* populations naturally infesting cassava, tomatoes, garden eggs and okra in Southern Ghana.
2. investigate morphological variation among the four host populations.
3. quantitatively determine the oviposition preference and rearing suitability of *B. tabaci* host-populations between various hosts.
4. investigate the relationship between insecticide susceptibility and putative biotypes under various pesticide application regimes.
5. investigate mating interactions between populations naturally demarcated by hosts.

CHAPTER TWO

LITERATURE REVIEW

2.0.1 Introduction

Whiteflies are a group of Aleyrodid insects (including *B. tabaci*) of the order Homoptera. They are variable in size, and few species are of agricultural importance. Aleyrodids are generally about 1 to 3 mm long, moth-like insects covered with a white powdery substance on the wings. *B. tabaci* is among the smallest members of Aleyrodidae family, adults being about a millimetre long or less (Byrne and Bellows, 1991). The wings are held tent-like above the body and a little apart such that the abdomen is partially visible from the top. However, whiteflies are not true flies (Order Diptera) but are more closely related to aphids (Order: Homoptera). Because the adult emerges from a dissimilar quiescent larval exoskeleton, the term 'puparium' has been applied to the fourth instar nymph, and exuvium called the 'pupal case' (Martin, 1987). The larval stages other than the first are scale-like and quiescent.

2.1 Taxonomy

Whitefly taxonomy poses unique impediments among insects. Few taxonomic characters have been described in the adults owing partly to the difficulty in making good slide mounts. The fourth larval instar has been used instead because some of the morphological characters are available even on empty pupal cases. However, some of the morphological characteristics are labile depending on the environmental conditions and morphology of the rearing host (Martin, 1987). Thus, some of the earlier described species were mere host related variants. This is further complicated by great intraspecific variability in biology, ecology and behaviour.

2.1.1 Systematic history

The systematic history of *B. tabaci* and great host-linked variability between populations has led to the hypothesis that it is a species complex with two extant cryptic species: *B. tabaci* and *B. argentifolii* (Perring *et al.*, 1993; Bellows *et al.,* 1994; Perring, 2001). The species was first described as *Aleyrodes tabaci* in 1889 by Gennadius from whiteflies collected on tobacco in Greece (Perring, 2001). Later, other whiteflies were described on different host plants. Takahashi (1936) placed *A. tabaci* in the genus *Bemisia.* A series of synonimisations occurred to bring 23 species described from Europe, Americas, Asia and Africa and from 15 host plants under the single species *Bemisia tabaci.* These included *B. emiliae, B. signata, B. rhodesiaensis, B. vayssierei* and *Neobemisia rhodesiaensis* from tobacco*; B. gossypiperda, B. goldingi* from cotton*; B. mosaicivectura, B. nigriensis and B. manihotis* from cassava (Mound and Halsey, 1978). This followed proof that certain key taxonomic traits such as the appearance of dorsal setae were under environmental (especially host related) influence (Mound, 1963; Mound and Halsey, 1978).

Perring *et al.* (1993) and Bellows *et al.* (1994) demonstrated distinct molecular, biological and behavioural differences, as well as mating incompatibilities between the A-biotype and the highly pestilent B-biotype (also silverleaf or poinsettia strain). They elevated the latter to a new species *Bemisia argentifolii* (Bellows *et al.,* 1994). However, the validity of this binomen is still in doubt (Brown *et al.,* 1995; Rosell *et al.,* 1997). Distinct differences have been shown between several other geographical and host associated populations (Burban *et al.*, 1992; Costa *et al.*, 1993; De Barro and Hart, 2000; De Barro *et al.*, 2000). There are no differences between the karyotype of biotypes (Blackman and Cahill, 1998). Presently, such populations are referred to as variants within *B. tabaci* sl. (Naranjo and Ellsworth, 2001).

2.1.2 Taxonomic characters

Martin (1987) has provided a detailed key for the identification of forty-six common whitefly species of pest importance based on the morphology of the pupal case. The key characters were described based on slide-mounted specimens, and a description of the leaf morphology of the specimen sources given. However, the potential extent of host plant influence on the pupal morphology needs to be considered in using this key. Host-correlated variation in the morphology of pupal integument had been extensively demonstrated by Mound (1963) on tobacco and cassava and by Azab *et al.* (1969) on cabbage, cotton, *Euphorbia pulcherrima* and *Lantana camara.* Mohanty and Basu (1986) and Basu (1995) demonstrated the combined effect of host leaf topology and seasonal factors on pupal morphology. Guershon and Gerling (2001) observed that artificial tomentosity influenced larval pupal case morphology. The almost exclusive use of mounted larvae in the taxonomy of the family Aleyrodidae means that the genera are difficult to define and the biological species concept based on reproductive isolation difficult to test directly (Martin, 1987).

A few adult morphological features have been used to differentiate members of the family Aleyrodidae. Size is principally used to differentiate the families Aleurodicinae from Aleyrodinae, and a basis of proposal of the subfamily Udamoselinae, perhaps the family of the largest whiteflies (Byrne and Bellows, 1991). Also, members of the genus *Aleurodicus* are the largest while those of the genus *Bemisia* are the smallest (Byrne and Bellows, 1991). Within Aleurodicinae, *Trialeurodes* is slightly larger than *Bemisia,* but (like *B. afer*) orients the wings horizontally to cover the abdomen at rest. The morphology of the cement gland and setal pattern of the gonapophyses allow the identification of the genera *Aleurothricus, Aleyrodes, Bemisia, Trialeurodes* and *Dialeurodes* (Guimaraes, 1996).

Biochemical characteristics have been essential in differentiating morphologically indistinguishable species of aleyrodids. The two greenhouse whitefly pests, *B. tabaci* and *T. vaporariorum* were differentiated on the basis of their surface lipid composition. The carbon-chain length of the dominant post eclosion long chain alcohols, alkanals and major wax esters were all distinctly longer in *B. tabaci* (Nelson *et al.*, 1994).

2.1.3 Biotypes and host races of *B. tabaci*

Great variability has been demonstrated in *Bemisia tabaci* populations. Host range for instance varies from nearly monophagous e.g. in *Jatropha* and Ivorian cassava biotypes to polyphagous as in the *Sida* and B biotypes (Burban *et al.*, 1992; Brown *et al.*, 1995). Sympatric populations may occupy different ecological niches defined by their host preference as reported in Ivory Coast and Puerto Rico (Burban *et al.*, 1992; Brown *et al.*, 1995). Such populations are named based on their host plant affiliations and are called host races or phytophagic forms (Perring, 2001).

Host races are populations that show no clear diagnostic differences in morphology, but do show some differences in survival and development on a particular host (Claridge *et al.*, 1997). Host tagging of *B. tabaci* populations facilitates communication associated with pest status, specific crop damage and virus vector capabilities. Identifying populations by host races though convenient, may not be useful for highly polyphagous species such as the B-biotype, and may be confusing since the reported host range is ever widening.

At a similar level of distinction as a host race is the biotype, designating populations that lack morphological distinction but that possess other characteristics which serve to separate them from other populations (Claridge *et al.*, 1997). So far, host association and

utilisation, virus transmission, development rates, fecundity, mating behaviour; morphometrics and molecular details have been used to delineate populations (Brown *et al.*, 1995; De Barro and Driver, 1997). Presently, partly due to the complexity of host associations, recognised biotypes are named using alphabetical letters from A to S based on non-specific esterase electromorphs (Perring, 2001).

Geographical origin, distribution and affiliations of various biotypes have been used to characterise *B. tabaci* biotypes. Such affiliations have been supported by molecular and biological evidence revealing phylogeographic relationships between various populations and associated behavioural characteristics (De Barro *et al.*, 2000). For instance, while *B. tabaci* does not attack cassava in South America where the crop originated, the African populations readily colonised the varieties introduced from there (Oliviera, 2001). Also, the Mediterranean S biotype, African okra biotype and the B biotype are considered phylogenetically close and all are polyphagous (Cervera *et al.*, 2000).

Out of 41 distinct populations of *B. tabaci* studied so far, 24 have been named as specific biotypes, 20 of them based on the non-specific esterase patterns on polyacrylamide gel electrophoresis (PAGE) (Banks and Markham, 2000). Biotypes have also been named on the basis of phytophagic and geographical affiliations (Table 1). Thus host races may be a biotypes described on the basis of specific host associated parameters, perhaps confusing biologically, but convenient in the description of crop pests.

Table 1: Biotypes of *Bemisia tabaci*

Biotype	**Location**	**Host Range**	**Plant Virus** [a]
A	Arizona, US	Polyphagous	Old and New World geminiviruses and LIYV.
B	Arizona, US	Polyphagous	Old and New World geminiviruses and LIYV (poorly).
E	Benin	*Asystasia* spp.	AGMV
J	Nigeria	Polyphagous	TYLCV-Ye
N	Puerto Rico	*Jatropha gossypifolia*	JMV
Non-cassava	Brazil	Polyphagous not cassava.	New World geminiviruses and LIYV.
Cassava	Ivory Coast	Cassava, egg plant	ACMV
Okra	Ivory Coast	Polyphagous, not cassava.	Old World geminiviruses not ACMV.
Sida	Puerto Rico	Polyphagous	New World geminiviruses not AGMV

[a] **LIYV**, Lettuce Infectious Yellow Virus; **AGMV**, Asystasia Golden Mosaic Virus; **TYLCV-Ye**, Tomato Yellow Leaf Curl Virus - Yemen; **JMV**, Jatropha Mosaic Virus; **ACMV**, African Cassava Mosaic Virus (Brown *et al.*, 1995).

Molecular analyses have now enabled comparison of genetic relationships among biotypes. Phylogenetic trees have been produced on genetic similarities from randomly amplified polymorphic DNA (RAPD) PCR and amplified fragment length polymorphism (AFLP) analyses (Guirao *et al.,* 1997; Banks and Markham, 2000; Cervera *et al.*, 2000). Molecular phylogenies have been prepared from sequences of mitochondrial and nuclear genes (Frohlich *et al.,* 1999; Banks and Markham, 2000; De Barro *et al.,* 2000), to show evolutionary relationships between biotypes worldwide. Unfortunately, some genetic studies have sought to identify various geographic and phytophagic races (e.g. Guirao *et al.,* 1997; De Barro *et al.,* 2000) without reference to the conventional naming of biotypes. This has made it difficult to relate these phylogenies to established biotypes.

There has been great dispersal of *B. tabaci* populations worldwide, and present geographical location alone is insufficient in describing the origin of populations. Possibly, some biotypes could be no more than single host-location restricted demes within a wider polymorphic population which, being reasonably reproductively isolated by geographical barriers, may have differences distinct enough to detect on molecular markers. The best information should, therefore, use multiple techniques that consistently place a particular biotype in a certain location (Perring, 2001) and which describe most biotypes using the same conventional parameters (Perring, 2001).

2.1.4 Clusters of world *B. tabaci* biotypes

Current genetic and behavioural evidence supports the existence of seven phylogenetic clades within the *B. tabaci* species complex as summarised in Table 2. These may perhaps indicate the geographical origins of the various biotypes.

Table 2: Phylogenetic clades of *B. tabaci* populations

Group	Biotypes	Geographic Location	Other attributes
Group 1. New world biotypes	A, C, N, R	Southwestern Mexico, US, Costa Rica, Puerto Rico	
Group 2. Cosmopolitan biotypes	B (*B. argentifolii*) B2 (Yemen)	Global distribution	Highly pestilent and polyphagous
Group 3.	E, S	Spain and Benin	
Group 4	H (cotton and water melon)	India (non SSL)	Possible Old World Origin
Group 5	L, Unknown Egyptian, Q, J.	Sudan, Egypt, Spain, Nigeria	Vegetable pests
Group 6	M (non SSL), Hainan unknown, Korean unknown	Turkey, Hainan, Korea	Non SSL, not crossing with B. K or D.
Group 7	AN (Australian native)	Australia	On cotton, little pest importance

* adapted from Perring (2001)

This cladogenesis does not resolve some biotypes. Biotype D is morphologically close to B biotype, yet neither crosses with it nor causes squash silverleaf disorder (SSL). Biotype K is non-SSL inducing, but does not cross with biotypes B, D or M. It has morphological similarities with B, E and K, but limited genetic similarity (De Barro *et al.*, 2000; Perring, 2001).

A number of studies have questioned the integrity of *B. tabaci* as a single taxon. Mating incompatibilities, the basis of the biological species concept, has been demonstrated between a number of biotypes presenting a case of cryptic species within a species complex undergoing evolutionary change (Oliviera *et al.*, 2001; Perring, 2001). Cryptic species are reproductively isolated populations hence have separate gene flow without much associated morphological differences (Lane, 1997). These populations are not conceptually different from other species, except on the characters that are used to distinguish them (Lane, 1997). Brown *et al.* (1995) has reviewed literature on the species and concluded that it is a suite of highly cryptic sibling species.

2.2 Ecology

2.2.1 Origin and distribution

The evolutionary affiliations of *Bemisia* taxa within the family Aleyrodidae suggest that *B. tabaci* may have originated in tropical Africa and was introduced quite recently into Neotropics and Southern North America (Campbell *et al.*, 1996). Other evidence points to Indo-Pakistan as the native home of *B. tabaci*. There are two closely related species *B. capitata* and *B. graminosus* that are endemic in India (Brown *et al.*, 1995). The area to the north and west of Pakistan shows the greatest diversity in parasitoids of *Bemisia* reputedly an indication of a genus epicentre (Brown *et al.,* 1995). Present distribution of *B. tabaci*

biotypes shows geographical affiliations that may indicate the origin of the parental stock of the biotype clusters (Perring, 2001).

The present day worldwide distribution of *B. tabaci* is presumably related to its close association with agricultural monocrops (Brown *et al.,* 1995) and climatic conditions (Byrne and Bellows, 1991). It occurs throughout the tropical and sub-tropical regions of the world, in a band between 30 and 35 degrees latitude around the world (MacKinlay *et al.*, 1992). Low winter temperatures limit North and Southward extension of this band. Indeed, they are important pests in protected agricultural systems especially greenhouse horticultural crops in most temperate regions presently including Europe, Japan and North America. Thus, whiteflies are regarded as the tropical equivalents of aphids (Byrne and Bellows, 1991).

B. tabaci feeds and develops on a wide range of, mostly herbaceous, cultivated plants and weeds. In contrast most other Aleyrodidae infest woody plants and are usually monophagous or oligophagous. Up to 600 plant species belonging to over 74 families are infested by the tobacco whitefly (Mound and Halsey, 1978). Cotton and okra (Malvaceae), tobacco, peppers, eggplant and garden egg (Solanaceae), cassava (Euphorbiaceae), brasiccas (Cruficeraceae) and some legumes are commonly attacked (Mound and Halsey, 1978). Weeds from the same families including *Amaranthus* spp., *Solanum nigrum, Datura stramonium* (Solanaceae); *Malva parlviflora* (Malvaceae) and *Lantana camara* (Verbenaceae) are alternative hosts, especially in the crops' off-season (Mound and Halsey, 1978; Muniz, 2000). Several *B. tabaci* biotypes are oligophagous or nearly monophagous and are associated with specific hosts, while others are polyphagous.

The choice of host plants for feeding and oviposition within the same species is influenced by host-associated parameters such as nutritive suitability, age, growth pattern and morphology (Legg, 1994). Sweet cassava varieties are preferred to bitter varieties which have higher levels of cyanogenic glucosides (Legg, 1994). Higher tissue nitrogen often an index of higher protein content and nitrogen fertilisation, lower water potential and high sugar level also encourage *B. tabaci* preference and fecundity on cotton, cassava, *Chrysanthemums* and poinsettia (Mor, 1987; Bentz and Larew, 1992; Bentz *et al.*, 1995). Whitefly body weights tend to decrease as the relative concentration of essential amino acids decreases in the plant (Blackmer and Byrne, 1999). Such changes occur during plant growth and may influence variations in pest densities during the growing season.

The age of the host influences the leaf chemistry variously during growth. In cassava, *B. tabaci* populations peak at the sixth week after planting after which the populations decline (Fishpool *et al.*, 1995). This gradual fall coincides with tuberisation and hence diversion of photosynthates from the leaves towards the storage organs, which may render the leaves less suitable for feeding (Legg, 1994). Apparently high populations associated with periods of rapid plant growth could be a response to better nutritive balance at the same time.

Physical characteristics such as host morphology also determine host acceptance. Leaf hairiness has been variously related to oviposition and feeding preference (Legg, 1994; Simmons, 1994). Both tomentose and glabrous leaves have been associated with high infestation in different host-plant species. Cassava and cotton varieties with wide leaflets support greater numbers of *B. tabaci* than those with narrow leaflets and shorter plants are also preferred as are plants with leaflets slightly flexed downwards about the midrib

(Legg, 1994). Perhaps the degree of sheltering and sheer feeding space are important here. On upland cotton, okra-leaf cultivars are preferred by the B biotype, as are leaves with denser vascular bundles (Chu *et al.,* 1999). Even though morphological cues are important in determining landing preference, initial probing and oviposition, final host acceptance occurs only after some probing (Isaacs *et al.,* 1999).

In the presence of a mixture of possible host plants, whiteflies spend less time in one position, and spend longer periods of time moving, suggesting some greater level of restlessness and difficulty in making a choice (Bernays, 1999). Female whiteflies also showed reduced performance when presented with hosts in mixtures than when the host plants were presented alone (Bernays, 1999).

2.2.2 Distribution on the hosts

Distribution of all stages of *B. tabaci* within the host plant is aggregated and related to adult feeding and oviposition preference. All stages are found on lower surfaces of leaves and rarely on petioles. Adults, eggs and first instar nymphs occur predominantly on younger leaves nearest the bud since young leaves are preferred for feeding and oviposition. Middle instar nymphs are found in the middle-aged leaves while most pupae occur on older leaves on cassava, cotton and poinsettia (Abisgold and Fishpool, 1990; Liu *et al.*, 1992). Most of the nymphs are found between the 7th and the 20th leaves on cassava and on the first four leaves of the fourth and fifth branches in cotton (Krishna and Lingappa, 1990).

2.2.3 Climatic determinants of *B. tabaci* population trends

Extreme relative humidity, both low and high are unfavourable for the survival of immature stages (Gerling *et al.,* 1986; Horowitz *et al.*, 1984). Temperature influences oviposition, egg hatching, nymphal development and adult longevity. Eggs fail to hatch at temperatures outside 16°C and 36°C range while development time is shortest between 25°C - 27°C (Butler *et al.*, 1983).

Heavy rainfall may dislodge and kill adults and nymphs. In the Ivory Coast, Fishpool *et al.* (1995) observed that rainfall is negatively correlated with population size, possibly because of its contribution to reduction in oviposition. In coastal Kenya, whitefly populations were found to increase during the high rainfall months; and in areas with higher annual rainfall possibly as a result of the increased vegetative growth (Robertson, 1985). In Uganda, no direct relationship has been found between the two parameters, although the dependence of cassava growth on soil moisture affects whitefly populations (Legg, 1994).

Wind speed and direction do not affect whitefly populations directly, but facilitate dispersal (Legg, 1994). Whiteflies cannot fly against an air stream of 0.4 m/s or faster above the canopy, being able to make only 0.2 m/s themselves (Yao *et al.,* 1987). Other meteorological parameters such as light intensity and duration possibly influence whitefly populations through their effect on the host plants.

2.2.4 Population Dynamics

Like most whitefly species, *B. tabaci* is multivoltine with 11 - 15 generations per year (Brown *et al.*, 1995), and breeds continuously so long as temperature conditions permit

(Coudriet *et al.*, 1985; Gerling *et al.,* 1986). Reproduction continues throughout the year in warmer tropics under intensive monocropping systems. Fecundity of whiteflies varies greatly depending on the biotypes, host plant, host suitability and environmental conditions as shown in controlled environments (Brown *et al.,* 1995). The Sudanese and B- biotypes have the highest fecundity of over 200 eggs per female compared to 80 eggs per female for the A biotype. Gerling *et al.* (1986) speculated that the Sudanese strains were probably induced to increase oviposition rate under insecticide stress, a phenomenon Dittrich *et al.* (1990) called hormogilosis. But Castle (1999) attributed it to agronomic practices like increased fertiliser use, late planting dates and other production technologies.

The key mortalities affecting the population occur between adult emergence and oviposition and during the fourth nymphal instars. Host switching may also initially hinder population growth. Mid season exponential increase on cotton is related to adaptation of the population to cotton as a host (Brown *et al.*, 1995).

2.2.5 Dispersal

B. tabaci disperses principally by adult movement to new feeding sites and oviposition on new host plants by females. *B. tabaci* has low wing loading of 0.00174 to 0.00532 g/cm^2 and wing-beat rate of 105 to 224 Hz, quite higher than insects of the same wing loading (Byrne *et al.,* 1988). Thus, they should be able to be more efficient fliers, but are still classified as weak fliers. Two morphs - the migratory and the trivial flying morph exist (Byrne and Houck, 1990).

Sustained flight of up to 7 km away from the site of release has been demonstrated in Israel (Cohen and Ben-Joseph, 1986). Whiteflies have been caught up to 1800 m above the irrigated cotton-growing areas of the Sudan Gezira scheme (Legg, 1994). Sustained flight duration of up to two and a half hours against an air-stream has been observed in a flight chamber (Blackmer and Byrne, 1993), which involved response to skylight, and ignoring vegetation cues for a time (Byrne, 1999).

Dispersal is therefore not always passive or in a direction solely dictated by prevailing winds, but mostly by drifting about in the manner of aerial plankton (Byrne and Bellows, 1991). Though it does not meet the conditions of migratory flight, the periods involved, distance and ability to select cues and to orient towards them during flight means that the flight is persistent (Byrne, 1999).

2.2.6 Biotic interactions

Other leaf feeding pests like *Mononychellus tanajoa* (Bondar) and cassava mealy bug *Phenacoccus manihoti* Mat-Ferr compete directly with *B. tabaci* for space and plant nutrients (Legg, 1999). The ecological implications of such interactions are not documented. Indirect competition by the red and green spider mites occurs, where, damaged or infested leaves are less attractive to *B. tabaci* (Robertson, 1985). In the Ivory Coast the presence of the green mites on the undersides of the leaves discouraged oviposition by *B. tabaci* (Fishpool and Burban, 1994), while in Uganda, mite damage in the upper five leaves was negatively correlated with whitefly numbers (Legg, 1994).

Predators of *B. tabaci* have been recorded in four insect families: Chrysopidae, Miridae, Anthocoridae and Coccinellidae, and two mite families: Phytoseiidae and Stigmatidae

(Mound and Halsey, 1978; Lopez-Avila, 1986). Twenty parasitoid species were recorded from the genera: *Encarsia*, *Aphelosoma* and *Eretmocerus* (Aphenilidae) and *Amitus* (Platygasteridae) (Lopez-Avila, 1986). These natural enemies are ineffective in suppressing *B. tabaci* populations in the crop fields though some success in greenhouses has been achieved with *Encarsia formosa* (Kajita *et al.*, 1992; Matsui, 1992).

Some natural enemies have been observed in Africa (Table 3) but little is known about their role in population regulation. In Uganda, a significant reduction in the proportion of parasitised whitefly nymphs has been observed during extended dry periods in Savannah ecosystem, perhaps contributing to the high incidence of the pest in these areas (Legg, 1994).

Four species of entomopathogenic fungi *Paecilomyces furmosoroseus*, *P. farinosus*, *Erynia radicans* and *Aschersonia aleyrodes* infect whiteflies. In the laboratory, *P. farinosus* caused 90% mortality of adults, but field observations have not been reported in Africa (Legg, 1994).

Table 3: Recorded natural enemies of *B. tabaci* on cassava in Africa*.

Country	Natural enemy	Family
Nigeria	*Serangium cinctum* (Wse.)	Coccinelidae
	Encarsia sp	Aphenilidae
	Typhlodromid mite	Phytoseiidae
Kenya	*Encarsia transvena* (Timberlake)	Aphenilidae
	Eretmocerus mundus (Mercet)	Aphenilidae
	Predatory mites	Phytoseiidae
	Thrips (*Scolothrips* sp?)	
	Coniopterygid larvae	
	Cecidomyiid larvae (*Theradiplosis* ?)	
Ivory Coast	*Euseius* sp.	mite
	Stethorus jejunus Casey	Coccinellidae
	Holoborus pallidicornis (Cameron)	Staaphylinidae
	Scolothrips latipennis Priesner	
Malawi	*E. transvena*	
	Eretmocerus sp.	Coccinellidae
	Scymnus sp	Coccinellidae
	Semidalis sp.	Coniopterygidae

* adapted from Legg (1994).

2.3 Biology

2.3.1 Life cycle

Whiteflies undergo unusual hemimetabolous metamorphosis in which the nymphal stages appear different from the adults, and with a quiescent pupa-like last nymphal stage (Figure 1). The nymphal stages have thus been referred to as larvae while the quiescent last nymphal stage is called the pupa (Martin, 1987).

Females lay eggs on the under surface of leaves. The first eggs are laid on older leaves from which the adults themselves emerge before migrating up the plant to younger leaves, which are also the preferred feeding sites for the adults. The eggs are ovoid and about 0.2 mm on the longer axis with a peg like extension of the chorion called the pedicel (Byrne and Bellows, 1991). Eggs are inserted vertically into leaf stomata or slits cut into the leaf epidermis.

The crawler, the active first instar nymph of Aleyrodidae before it settles, is the only mobile immature form of whiteflies. They move about the leaf to select appropriate feeding site on vascular bundles of the right size, after which they do not move again until they eclose as adults, except for brief moments and very short distance during moulting (Byrne and Bellow, 1991; Thompson, 2000). Crawler movement is affected by positive response to light and is negatively correlated with availability of suitable feeding sites (Simmons, 2002). The first instar is of translucent white colour anteriorly and green posteriorly with black dot like eyes, which are not easily visible except at high magnification (Thompson, 2000). The second and third instar nymphs are oval, scale like and covered a wax coating. Sizes vary from about 0.4 mm (second instar) to 0.6 mm (early

fourth instar). As they develop, the nymphs become bigger, more opaque and whiter or brownish towards the last nymphal stage.

In the last instar, red eyes are clearly visible through the integument. It is commonly referred to as a pupa, implying holometabolism. However, the stage is divided into three sub-stages, the first during which feeding occurs and hence is not a true pupa (Gill, 1990; Thompson, 2000). At this stage the body looks swollen and opaque white with some green patches developing (Thompson, 2000). A transitional stage during which apolysis occurs follows and in the last sub-stage, there is no feeding (Byrne and Bellows, 1991).

Conventionally, therefore, the term pupa could be used to refer to the last sub-stage while the earlier feeding stage is called the fourth nymphal stadium (Byrne and Bellows, 1991). Adults emerge through an inverted T-shaped slit on the dorsum of the nymphal integument (Thompson, 2000).

The life cycle parameters of *B. tabaci* vary with environmental conditions, and may be different in dissimilar biotypes and on different hosts (Thompson, 2000, 2002) but averages about 21 days (Bethke *et al.*, 1991; Drost *et al.,* 1998; Thompson, 2000). The teneral period varies with temperature, but *B. tabaci* females do not mate within 12 hours of their emergence though they may be attracted to the males, and courting may be initiated (Li *et al.*, 1989; Byrne and Bellows, 1991). The pre-oviposition period is decreased with temperature and varies between biotypes, and on different hosts, generally between one to six days (Drost *et al.*, 1998).

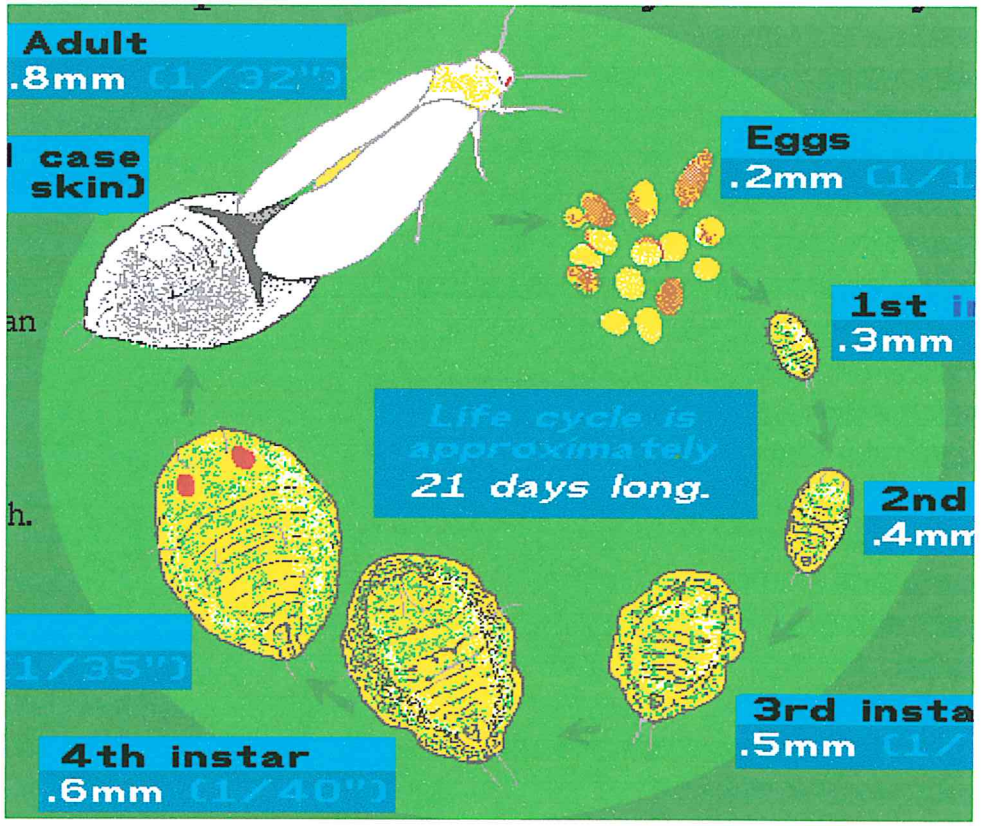


Figure 1: Life cycle of *B. tabaci*

(Adapted from USDA [undated] Whitefly Knowledgebase)

2.3.2 Reproduction

Whiteflies reproduce by arrhenotokous parthenogenesis. Fertilised eggs develop into females while unfertilised eggs develop into males (Byrne and Bellows, 1991; De Barro and Hart, 2000). Sex ratios are variable on different hosts and environmental conditions and also depend on the age of the females and the frequency of mating (Byrne and Bellows, 1991).

Mating involves a complex courtship pattern that does not involve long-range sex pheromones (Byrne and Bellows, 1991). Males may initiate courtship to females of a different biotype but sometimes mating does not occur. Perring *et al.* (1993) observed pre-mating isolation between A and B biotypes, while Costa *et al.* (1993), Ronda *et al.* (1999) and De Barro and Hart (2000) report limited fertilisation between biotypes with the production of female offspring, but did not determine the fertility of these females. Occurrence of mixed-biotype mating in nature is in doubt.

2.4 Agricultural Importance

2.4.1 Crop Losses

B. tabaci was first described as a pest of tobacco in Greece. It is now one of the most important pests of herbaceous crops worldwide (Oliviera *et al.*, 2001). Outbreaks of the pest occurred on cotton fields from late 1920s and early 1930s in Indo-Pakistan region, Sudan and Iran (1950s), El Salvador, Mexico and Brazil (1960s), Turkey, Israel and Thailand (1970s) and in Ethiopia and the USA in 1980s (Oliviera *et al.,* 2001). These were associated with continuous cropping, heavy fertiliser use and effect of insecticide resistance (Eveleens, 1983; Dittrich *et al.,* 1990; Castle, 1999)

B. tabaci is now a major pest of field, horticultural and greenhouse crops especially vegetables and ornamentals. The grave pest status of *B. tabaci* is attributed to its highly polyphagous nature, great intraspecific variability, short life cycle and high reproductive rate, as well as its ability to spread fast and adapt to new environments. Its dispersal has been facilitated in part by human trade on vegetative parts of ornamental plants (De Barro and Driver, 1997). In fact, whiteflies are increasingly becoming pests of prior non-hosts.

Reliable estimates of the economic importance of *B. tabaci* on a world scale may be difficult to calculate or harmonise because of the extensive areas affected, number of crops and ornamentals involved and different monetary and loss assessment systems used (Oliviera *et al.*, 2001). Direct crop losses are usually accompanied by other socio-economic losses. Gonzalez *et al.* (1992) concluded that for every one million dollars of primary induced crop loss, a 1.2 million dollars loss of personal income and elimination of 42 jobs occurred in California. In Africa, cassava mosaic disease leads to loss of income and staple food with inestimable economic and social distress. The use of pesticides to prevent crop losses leads to undeclared losses in the production chain such as increased cost of production or commodity prices. Estimates of the agricultural damage caused by *B. tabaci* are therefore based on specific crop losses or extra expenditure (Table 4).

2.4.2 Crop damage

2.4.2.1 Feeding damage

Direct feeding damage is caused by adults piercing and sucking sap from foliage of plants weakening them and reducing vigour (Berlinger, 1986). Water is absorbed from plants by all stages including eggs resulting in water stress but rarely serious wilting.

Table 4: Selected landmark crop losses attributed to *B. tabaci* in history

Location	Year	Damage	Reference
Arizona, Texas, Florida	1991	US$ 200m on poinsettias	Oliviera *et al.*, 2001
	1992	US$ 500m	Bellows *et al.*, 1994
	1994 - 1998	$ 154m control costs on cotton	Ellsworth, 1999
Mexico (Mexicali)	1991 - 1992	3862 ha abandoned (cotton)	Oliviera *et al.*, 2001
Mexico (Sonora)	1995 - 1996	65% reduction of cotton acreage	Hilje *et al.*, 2001
Guatemala	1996	40% losses on melon. 50% rise in production cost.	Hilje *et al.*, 2001.
Brazil	1995 - 2001	$ 5b on beans, tomatoes, cotton, melons, watermelons okra and cabbage.	Lima *et al.*, 2000
Sudan	1980/81	Near-total crop failure, Higher expenditure on chemicals	Abdeldaffie *et al.*, 1987
Africa	1990s	Up to 90% losses due ACMD	Fauquet and Fargette, 1990
Ghana	1990s - date	Trebling costs of insect control on exported vegetables	Gerken *et al.*, 2001

Phytotoxic disorders are probably induced by plants' reaction to whitefly salivary secretions, though most are of unknown aetiology (Oliviera *et al.*, 2001). The squash silverleaf symptom (SSL), uneven ripening of tomatoes, pumpkin white stem blanching in lettuce and eggplants are associated with the B-biotype and related. Similarly, the Red Cotton Syndrome in Central and West Africa is an emerging effect of *B. tabaci* infestation that is perhaps a reaction to whitefly secretions.

Phytotoxic disorders may be confounded by virus symptoms like those of *Criniviruses* (Yokomi *et al.*, 1990; Costa *et al.*, 1993). However, phytotoxic disorders are usually associated with increases in field populations of the pest as opposed to virus disease symptoms that may not have a direct relationship. Physiological disorders reduce yield and quality of produce directly or through reduction of plant vigour.

2.4.2.2 Crop contamination

Up to 10% losses in cotton quality, reduction in quantity and marketability of melons, vegetables and ornamentals may be caused by honeydew secretion (Strolz, 1992; Riley and Palumbo, 1995). Honeydew accumulated on leaf surfaces serves as a substrate for sooty moulds that taint produce, darken leaf photosynthetic surfaces and cause contamination and stickiness of cotton (Oliviera *et al.*, 2001; Abdeldaffie *et al.*, 1987). It also reduces colour grade, harvesting handling and ginning quality (Ayars *et al.*, 1986).

2.4.2.3 Virus transmission

Whiteflies transmit over 100 viruses from seven distinct virus groups (Jones, 2003). Geminiviruses e.g. genus *Begomovirus* and Clestoviruses of genus *Crinivirus* are the most

important in agriculture (Duffus, 1996). Numerous cultivated crops are attacked by the viruses that are especially destructive to tomatoes, beans and cassava (Table 5).

Clestoviruses of the genus *Crinivirus,* transmitted by *B. tabaci* and three other whitefly species, cause extensive damage in North America, Mediterranean Basin and the Middle East. The Lettuce Infectious Yellow Virus (LIYV) is transmitted very efficiently by the A biotype hence its damage level is associated with the presence of the biotype (Duffus *et al.*, 1996).

Cucurbit Yellow Stunting Disorder Virus (CYSDV), Lettuce Chlorosis Virus (LCV), Tomato Chlorosis Virus (ToCV), Sweetpotato Chlorotic Stunt Virus (SPCSV) are also transmitted by *B. tabaci* (Oliviera *et al.*, 2001). Clestoviruses cause chlorosis and stunting of crops quite similar to phytotoxic disorders accompanying heavy infestation.

Virus vector biotype relationships are complex. In southern California, the competitive exclusion of the A biotype by the B biotype of *B. tabaci* has resulted in the natural decline of the LIYV problem, due to reduced transmission (Duffus *et al.*, 1996). Also, many viruses are still unknown hence their presence, symptoms and transmission mechanisms are difficult to establish accurately.

Table 5: Important whitefly transmitted viruses

Virus	Host	Damage level	Distribution
Tomato Yellow Leaf Curl Virus (TYLCV)	Tomatoes	Total crop losses	Tropics
Tomato Mottle Virus	Tomatoes	20% yield loss	Worldwide
Bean Golden Mosaic Virus (BGMV).	Beans	Near total crop loss. Constraint to acreage	Americas
Bean Golden Yellow Mosaic Virus	Beans	Near total loss. Constraint to acreage.	Americas
Cassava Mosaic Diseases (complex of viruses)	Cassava	Up to 95%	Africa
Cotton Leaf Curl Virus	Cotton	75% decline in production	Asia, Sudan
Okra Leaf Curl Virus	Okra	Up to 75% yield loss	West Africa
Mung bean Yellow Mosaic Virus	Grain Legumes	80% loss of yield. $ 300m yearly	Southern Asia.

* Source Oliviera *et al.* (2001).

2.5 Control

The primary importance of *B. tabaci* is its role as a vector of viral diseases, and hence control approaches are aimed at both insect and virus control. The direct feeding and phytotoxic effects of whiteflies are density dependent, fitting well into the economic thresholds described by Stern (1973). Vector effect involves vector-host and vector-pathogen interactions that are not directly density dependent. Whitefly management encompasses the main foci of integrated pest management (IPM): host plant resistance, biological control, chemical control and cultural practices (Hilje *et al.*, 2001). Plant resistance and cultural control are preventive and hence more effective in preventing whitefly-associated viral diseases as well. Chemical control is the commonest approach worldwide due to its apparent ease and efficiency, while biological control is still not well developed on field crops.

2.5.1 Cultural control practices

These are measures that deliberately manipulate some component of the agro ecosystem (e.g. soil, flora, the crop etc) to make the environment less favourable for pest infestation, reproduction and survival (Herzog and Funderbuk, 1986). Such measures include the manipulation of current components of or introducing new components into the agro ecosystem to reduce pest damage to non-economic levels (Hilje *et al.*, 2001). Cultural control approaches may be classified according to the ecological mechanism underlying them as well as the scale in which they operate (Table 6) (Hilje *et al.*, 2001).

Table 6: Cultural approaches to whitefly control and the scope of their operation

Mechanism	**Scale**	**Examples**
Temporal Avoidance	Regional	Crop free periods, crop rotation, and manipulation of planting dates.
Spatial Avoidance	Local	Screen houses, floating row covers, high plant densities
Behavioural manipulation	Local	Intercropping and mulching
Host suitability	Individual	Fertilisation Irrigation.
Removal	Individual	Overhead irrigation, Vacuum removal.

2.5.1.1 Temporal avoidance

These are aimed at separating in time the host crops from the sources of whiteflies such as alternative hosts, previous season's and volunteer crops. The measures also disrupt synchrony between the most susceptible period of crop development and high-density periods of the vector.

Crop free periods

The cropping patterns are synchronised over a wide area to avoid continuous availability of whitefly hosts and whitefly-transmitted virus host plants. This creates a period of dearth of suitable reproductive or virus hosts reducing the population densities and virus inoculum in the area. Complete elimination of all *B. tabaci* hosts in an area is difficult since the insect is polyphagous and can reproduce on a wide variety of crops and weeds (Mound and Halsey, 1978; Greathead, 1986; Muniz, 2000).

However, pestilent biotypes and viruses with known host range may be controlled by judicious removal of the specific hosts. Also, closed seasons reduce the population of whiteflies even on weed hosts delaying attainment of high pest densities very early in the cropping season when whitefly damage is greatest. Further, direct migration of whiteflies directly from one crop to another is minimised (Hilje *et al.*, 2001).

Area-wide closed seasons have been successful in the control of cotton leaf curl virus in Gezira, Sudan in the 1920s (Bailey, 1930); and tobacco leaf curl in the Dominican Republic (Avilla *et al.*, 1998). It has also been used in Israel on ornamentals and vegetables, and on cotton in the USA.

Disposal of crop residue

Removal of crop residues and off-season tillers from fields during the dead season prevents carry over infestation through unintended hosts. Such plants may not be capable of maintaining high pest population but could maintain an effective virus inoculum encouraging early infection of the new crop. High early infestation with whiteflies carrying tomato yellow mottle virus (ToMoV) results when old and new fields are located within the flying range of the vector (Hilje *et al.*, 2001) from each other.

Adjustment of planting dates

Uniform planting facilitates the implementation of crop-free periods for specific large-scale crops such as cotton. On a local scale, early or late planting may facilitate avoidance of high vector populations and inoculation peaks. This has been successful in okra, tomatoes and beans in Egypt (El-Gendi *et al.*, 1997), okra and cotton in Mexico (Hernandez-Jasso and Pacheco-Covarrubias, 1998) and tobacco in India (Patel and Patel, 1996).

Weed control

This generally aims at reducing the availability of alternate hosts of *B. tabaci* and associated viruses. Its role in the control of whitefly-associated problems varies with cropping system, host range and preference of the specific whitefly biotypes and viruses involved. In Israel, the weed *Cynanchus acutum* (Asclepiadaceae) serves as a source of TYLCV and facilitates perennation of the virus between seasons (Cohen *et al.*, 1988). Marginal feeding hosts may also act as an inoculum source for migrating whiteflies that stop to feed on them (Hilje *et al.*, 2001).

Weeding reduces possible virus infected or alternative hosts of whiteflies, and the proportion of viruliferous vectors at the onset of the season (Cohen *et al.*, 1988). A full understanding of the various weed host preferences of *B. tabaci* biotypes and viruses is important for the efficient application of this approach.

2.5.1.2 Spatial avoidance

These practices are aimed at reducing the opportunities for contact between whiteflies and crops on local or individual scale. Whiteflies are either physically excluded from the crop or through plant density manipulation, the overall damage on individual crops is minimised.

Exclusion

Whiteflies are physically excluded from the susceptible hosts (or host stages) using physical barriers like insect-proof screens, or solid plastic, which allow air and light penetration (Bell and Baker, 2000). Screen house mesh, greenhouse plastics and screening are used in protected agriculture especially for horticultural crop production to protect crops throughout the life cycle. Physical protection of nurseries is important for seedlings and plants in early development, which are usually more susceptible to whitefly and virus damage (Hilje *et al.*, 2001).

Floating row covers are lightweight materials laid directly on the growing plants without support, which allow light penetration and plant growth as they screen out whiteflies. They are most useful in the protection of young and growing field-sown seedlings usually in the early part of the growing season. Row covers have been used on tomatoes, cucurbits and

bell pepper to delay or reduce whitefly-associated problems (Costa *et al.*, 1994; Avilla *et al.*, 1998); and in the protection of tomatoes from geminiviruses (Iannou, 1997).

Insect screening containing ultraviolet- absorbing additives that block a greater portion of the ultraviolet light spectrum achieve a greater protection to whiteflies and whitefly-borne viruses in greenhouses (Antignus *et al.*, 1998; Costa and Robb, 1999; Antignus, 2000). It is speculated that the elimination of certain portions of the ultraviolet spectrum interferes with the ability of insects to orient properly and find plant hosts (Antignus *et al.*, 1996; Antignus, 2000).

Barriers

Barriers of insect mesh, screen and tall non-host species such as sorghum, maize and elephant grass (*Pennisetum purpureum*) around a crop field impede adult whiteflies migrating into a field. However, because whiteflies migrate at height above plant canopy e.g. 7 metres above the ground (Isaacs and Byrne, 1998), barriers only delay infestation (Hilje *et al.*, 2001). *B. tabaci* transmits viruses in a persistent and semi-persistent manner (Nault, 1997) but not trans-ovarially, so, effective delay needs to last the period of adult longevity to ensure natural death of viruliferous adults. Thus, whitefly barrier plants function more by reducing the overall numbers migrating into the crop rather than reducing the inoculation percentage of the vectors (Hilje, 2000).

Planting sorghum around tomato fields in Brazil resulted in reduced adult whitefly density and increased density of their predators, while in Mexico, a maize barrier was associated with reduced viral infection on bell pepper (Hilje *et al.*, 2001). However the success of this approach is inconsistent (Cohen *et al.*, 1988; Smith and McSorley, 2000). Perhaps, the

behaviour of the populations involved and the environmental conditions may influence the success of this approach.

High planting density

Lower damage per plant (or a total proportion of the world) for a given size of pest population is achieved owing to reduced pest pressure per plant (Broadbent, 1969), enabling a greater number of plants to escape the direct damage or infection. Percentage infection of cassava with ACMV in usually highest at the lowest plant density (Fargette and Fauquet, 1988; Fargette *et al.*, 1990). However, because most viral infection is persistent and not directly dependent on vector density, the effectiveness of this control method may achieve low pest pressure but not necessarily accompanying reduction in virus infection. Phytotoxic disorders and feeding damage are expected to reduce under high plant density. However, higher interplant competition leads to a reduction in yield.

2.5.1.3 Behavioural manipulation

Modification of the whitefly host searching behaviour, orientation, visual and olfactory host evaluation influences the ability of the pest to locate, assess and accept a feeding host.

Intercropping

This refers to planting two or more plant species in a given pattern, in close proximity to each other, within the same plot. Crop associations modify the environment and manipulate the host seeking behaviour of the pest. They may also create a refuge for the natural enemies of the pest contributing to greater natural mortality. Non-host intercrops obscure host finding, causing the insects that land in response to general physical cues to search in a different location, having had unsuccessful attempts by prospective probing.

Intercropping maize, cowpeas or peanuts with cassava reduces whitefly incidence on cassava and protects the crop from ACMV (Fargette and Fauquet, 1988; Ahohuendo and Sakar, 1995).

Trap cropping

A primary host is intercropped with a more preferred but less economically important (or less damaged) species to attract whiteflies away from the primary (Hilje *et al.*, 2001). The more attractive host is treated with insecticides before senescence to prevent the remigration to the host plant. The trap crop also increases the overall density of the crop stand and lowers the average damage to crops without serious effects of competition between plants. Cucumber, a preferred feeding host but not susceptible to TYLCV, has been used as a trap crop between rows to attract whiteflies away from tomatoes and reduce TYLCV infestation in Jordan (Al-Mousa, 1982). Others include melon or the, *Physalis weighti,* with cotton (Ellsworth *et al.*, 1992; Castle, 2001), green beans, and squash or eggplants with tomatoes (Hilje *et al.,* 2001).

The trap crop may attract whiteflies into the vicinity of the main crop increasing the risk of dispersal into the protected crop if it encourages landing and settling down of migrating populations. The success of trap cropping depends on the host preference of the local biotypes of *B. tabaci* and their interaction with the available plant communities. Also, it is not applicable in commercial scale because of the logistical implications of maintaining two or more crops.

Mulching

Mulches interfere with the ability of whiteflies to locate the crop usually by disrupting the visual cues or suicidal attraction to sun-heated mulch (Cohen and Berlinger, 1986). Reflective materials, plastic and sawdust are used as inert mulches while plant residue is used as organic mulches. Shorter cover crops are used as live mulches when planted between rows of the main crop. The incidence of Tomato Mottle Virus (ToMV) in Florida and TYLCV in Jordan was reduced using aluminium or silver reflective mulches (Csizinsky *et al.*, 1995). The total effect of mulches on the plant physiology may contribute to their apparent association with increased yield.

Living mulches cause whiteflies to fly away from tomato plots without feeding on tomato plants (Hilje *et al.*, 2001) perhaps by complicating the success in locating the appropriate host after initial landing in much the same way as non host intercrops (Finch and Collier, 2000).

2.5.1.4 Host suitability

Irrigation and fertilizer application induce physiological changes in plants that make them less suitable for reproduction and survival of whiteflies. The nutritional acceptance of a host, weight of whiteflies, population growth rate and survivorship increase with more fertilizer application and concentration of essential amino acids (Bentz and Larew, 1992; Bentz *et al.*, 1995; Blackmer and Byrne, 1999; Bi *et al.*, 2001) However, oviposition acceptance and stage specific survival are independent of fertilisation status (Blua and Toscano, 1994). Vigorous development associated with these practices may make plants more tolerant to whitefly damage and engender compensatory growth. Water stressed cotton had higher nymph density in Israel (Mor, 1987). Reduction of water stress by

manipulation of irrigation frequency or the method of water application decreases whitefly infestation (Flint *et al.*, 1994, 1995, 1996). Drip irrigation promotes growth of shorter plants with fewer leaves hence reduces whitefly adult population (Sharaf *et al.*, 1984).

Selection and breeding of less susceptible or acceptable hosts to whiteflies and viruses provides a sustainable means of control. Genetically influenced morphological characteristics such as okra leaf trait in cotton, hirsutism and leaf shape have been recommended. Virus resistance has been used in the control of the African cassava mosaic diseases (Legg, 1999). The diversity of viruses, whitefly diversity and the virus transmission dynamics have however limited the impact of this approach.

2.5.1.5 Physical removal

The disruptive effects of physical forces are used to reduce whitefly numbers on plants. Overhead irrigation at high frequency retards egg, nymph and adult numbers on cotton, as does rainfall (Castle *et al.*, 1996; Castle, 2001). This may result from the negative effect of physical impact or increased relative humidity on nymphs (Gerling *et al.*, 1986) or higher incidence of entomopathogenic fungi. This approach is more suitable for intensive crop production.

Rogueing out heavily infested and virus-infected plants reduces the virus inoculum source early in the season, and destroys whitefly nymphs. But significant reduction of whitefly infestation is not achieved, since populations build up fast, and as a result of the mobility of whiteflies on a local scale.

2.5.2 Biological control

Augmentative biological control, combined with reduced pesticide application or the use of pesticides that are less toxic to natural enemies, such as botanicals, insect growth regulators and microbials, is a viable tool for tackling the *B. tabaci* problem in intensive agriculture (Simmons and Minkenberg, 1994). Nymphal parasitoids of the genus *Eretmocerus* have good potential for such programmes because of high natural parasitisation observed in nature. Simmons and Minkenberg (1994) reported high parasitisation and higher yield in field cage tests with *Encarsia* nr. C*alifornicus.* Predators and entomopathogens also have a potential. However, whitefly problems are worst in dry conditions while most pathogens are most infectious in humid conditions, limiting the potential of their use to greenhouses. Also, the efficacy of biological control agents may vary among different biotypes.

2.5.3 Chemical control

The use of insecticides is the primary strategy employed in the control of *B. tabaci* and other whiteflies worldwide (Palumbo *et al.,* 2001). Rapid build up of populations encouraged by warm dry climates, and overlapping availability of multiple crop and weed hosts throughout the year has necessitated repeated insecticide use especially in hot irrigated and intensive growing areas (Palumbo *et al.*, 1999).

2.5.3.1 Traditional toxicants

The effective control of *B. tabaci* using insecticides has not been sustainable in the long run. Conventional toxicants such as organophosphates, pyrethroids and carbamates have been most employed. More recently, synergised pyrethroids (Horowitz and Ishaaya, 1996; Prahabker *et al.,* 1998) and mixtures of pyrethroids with organophosphates, carbamates,

formamidines and cyclodienes have provided greater protection where individual compounds failed (Palumbo *et al.*, 2001). Greater potency results from the inhibition of resistance mechanisms.

Although both adults and nymphs are susceptible to these compounds, immature stages are inherently difficult to control with contact insecticides because they are sessile and reside on the lower leaf surfaces, which are difficult to cover effectively with sprays (Prahabker *et al.*, 1992; Palumbo and Coates, 1996). Also, the nymphal hydrophobic wax integument provides added protection from water-based sprays. Continuous immigration of adults into fields necessitates frequent foliar spraying to prevent virus transmission and direct crop damage (Schuster *et al.*, 1996; Berlinger *et al.*, 1993).

2.5.3.2 Novel insecticides

Novel insecticide chemistries such as nicotinoids and growth regulators may give greater control as a result of more efficient application methods and different modes of activity. Nicotinoids such as imidalocoprid are systemic, have a long residual activity and can be applied in diverse ways hence very effective especially against whiteflies of all stages and other sucking insects (Kagabu, 1999; Yamada *et al.*, 1999).

Insect growth regulators (IGRs) such as Buprofezin (chitin inhibitor) and Pyriproxyfen (juvenile hormone analogue) provide an alternative toxicity mechanism, being non-neurotoxic. Both are selective against some homopterans including *B. tabaci* (Kanno *et al.*, 1981). They act specifically on immature stages causing mortality during ecdysis but may reduce fecundity and egg hatch of female whiteflies (Ishaaya *et al.*, 1988; Beevi and Balasubramaniam, 1995). Other novel insecticides include selective respiration inhibiting

Diafenthiuron (Horowitz *et al.*, 1999) phago-depressant Pymettrozine (Fluckiger *et al.*, 1992; Kayser *et al.*, 1994) and botanical extracts of *Melia* spp. (Hammad *et al.*, 2001) These provide new opportunities to the control of whiteflies resistant to conventional insecticides.

2.5.3.3 Resistance to insecticides

The development of resistance to insecticides in *B. tabaci* has been typically associated with intensive agricultural systems and certain biotypes worldwide. It was first reported in the early 1980s for the populations infesting Sudanese cotton (Abdeldaffie *et al.*, 1987; Cahill *et al.*, 1995). Organophosphate, carbamate and pyrethroid resistance has been demonstrated in other countries as well (Dittrich *et al.*, 1990; Byrne *et al.*, 1992).

The ability of *B. tabaci* to develop resistance to novel insecticides under high pesticide pressure has been demonstrated. In Almeria, Spain, resistance to imidacloprid has been reported, arguably developed due to heavy use of the insecticide (Cahill *et al.*, 1996).

2.5.4 Novel Approaches

Sterile insect technique has been developed and tested against *B. tabaci* (Calvitti *et al.*, 2001). It slows down pest population growth by decreasing mating efficiency among fertile whiteflies and producing higher embryonic mortality (Calvitti *et al.*, 1997, 1998). It shows promise in closed environments and where the threat of virus transmission by releases vectors is limited.

2.6 Techniques of Characterisation of *B. tabaci* Biotypes

2.6.1 Morphology

Morphological taxonomic characters have been used to classify Aleyrodidae to species level based on the characteristics of the 4th larval instar. Some are however labile depending on the environment and host morphology (Martin, 1987). At the intraspecies level, such morphological characters are less obvious. Some consistent morphological characters have been observed to differentiate the B biotype from the A biotype. The marginal wax projections from the thoracic and posterior tracheal folds of the A biotype are narrow with short wax filaments, while in the B biotype, they are wider and more robust (Bellows *et al.*, 1994). However, Rosell *et al.* (1997) analysed these and other characteristics of ten *B. tabaci* populations from different hosts but were unable to obtain a reliable phylogram.

2.6.2 Mating studies

Demonstration of reproductive incompatibility has been used in the delimitation of evolutionary relationships between *B. tabaci* biotypes. Restricted gene flow represents one of the fundamental parameters of the biological species concept (Diehl and Bush, 1984). *B. tabaci* is arrhenotokous hence mating incompatibility is observed in a shift in sex ratio of the offspring to favour males. Fertility of the F_1 female crosses is used to infer on genetic compatibility and stability of such crosses in nature (Perring *et al.*, 1993; Ronda *et al.*, 1999; De Barro and Hart, 2000).

Varying levels of reproductive isolation and genetic incompatibility have been demonstrated between biotypes of this species. Incomplete and prolonged courtship have been observed (Perring *et al.*, 1993; De Barro and Hart, 2000). Incompatible mating has

been observed between A, B, AN, D, K, L and M biotypes (Costa *et al.*, 1993; Bedford *et al.*, 1994; Byrne *et al.*, 1995; De Barro and Hart, 2000). Successful interbreeding between biotypes has been demonstrated between the Australian and Nauru biotypes (De Barro and Hart 2000). Greater natural mating with backcrossing possibilities may suggest closer co-ancestry of biotypes. Moya *et al* , 2001).

2.6.3 Phytotoxic disorder assay

Whitefly-host interactions are variable between sets of biotypes and plants. Phytotoxic disorders induced by feeding nymphs are also variable between cultivars of a crop. These disorders have been used to characterise and screen whitefly populations. The squash silverleaf (SSL) disorder, tomato uneven ripening and white stem streaking in cole crops are consistent in differentiating between B and non B biotypes (Brown *et al.*, 1991; Moya *et al.*, 2001; Perring, 2001).

2.6.4 Host range

Host transfer experiments are the oldest method of characterising *B. tabaci* biotypes (Bird, 1957). In fact, variation of host range is usually the most striking and readily measurable difference between putative whitefly biotypes. It is practical with inference to agricultural and description of biotypes. Several biotypes have thus been named according to their host range (Brown *et al.*, 1995).

Feeding, oviposition and development preferences have been used in describing host-range of *B. tabaci*. No-choice feeding bioassay is the most basic means with the assumption that survival after a given duration confirms acceptability. However, the acceptability of a new host may be influenced by the previous host or the rearing host of

the insect tested. A more practical criterion is the total development of a bisexual population (Burban *et al.*, 1992).

2.6.5 Molecular and biochemical characteristics

In addition to evidence accumulated in Aleyrodid taxonomy, a number of studies have yielded biochemical and molecular data. Multilocus enzymes (also called isozymes) are multiple molecular forms of an enzyme as defined by the substrate specificity. These may result from the presence of more than one locus coding for the enzyme e.g. carboxylesterases (Loxdale and Lushai, 1998). Allozymes of a given enzyme are products of different alleles at a specific locus (Richardson *et al.*, 1986).

Most isozymes are fixed characters within all or most individuals in a taxon hence are valuable genetic markers (Andrews and Chilton, 1999). Banding patterns of non-specific esterases are variable enough among populations to provide an invaluable method of determining biotypes of *B. tabaci* (Burban *et al.*, 1992; Perring *et al.,* 1993; Brown *et al.*, 1995; Moya *et al.*, 2001; Ryckewaert and Alauzet, 2001). Allozymes show heterozygous banding pattern, and segregate according to Mendelian genetics, hence can be used to electrophoretically detect the proportion of allelic differences (Loxdale and Lushai, 1998).

2.7 Molecular Methods in *Bemisia* Taxonomy

Presently, the only reliable methods to distinguish between the different biotypes and dead adult material are those based on the variability in protein or DNA. Variability such as allozyme markers have been used to distinguish between biologically well characterised biotypes (Brown *et al.*, 1995) However, the techniques require fresh or frozen material hence provide a limitation since alcohol preservation is usually more convenient in field

conditions (De Barro and Driver, 1997). DNA based techniques require only minute amounts of insect material, hence may be used for juveniles which often do not produce sufficient esterases to allow visualization using allozyme electrophoresis (De Barro and Driver, 1997).

2.7. 1. Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is an *in vitro* technique for the enzymatic synthesis of selectively discrete segments of DNA sequences using oligonucleotide primers that hybridise to opposite strands flanking the target region (Erlich, 1989). Repetitive cycles of denaturation, annealing and extension enable exponential accumulation of copies of the target fragments to quantities detectable by electrophoresis. Because amplification products of one cycle serve as templates for the next cycle, copies of the target fragments nearly doubles every cycle hence very small quantities of template DNA are needed to start with.

Initially, PCR used the Klenow fragment of *E. coli* DNA polymerase I, but this enzyme was irreversibly denatured at high temperatures needed to denature the DNA template necessitating addition of enzyme after each cycle (Erlich *et al.*, 1991). Thus, PCR was laborious and more liable to error. Presently, a thermostable DNA polymerase (*Taq* polymerase) isolated from *Thermus aquaticus* is used. This enzyme can stand repeated exposure to high temperatures needed for template denaturation without significant loss of activity, and is thus added to the reaction only once (Gelfand, 1989, Saiki *et al.,* 1989). Further, the automation of thermal cycling conditions by programmable devices has now made PCR less tedious and more accurate (Oste, 1989).

The PCR reaction mixture contains the polymerase enzyme, template DNA, dNTPs oligonucleotide primers and suitable buffers and ions (Saiki, 1989). These are passed through cycles of three-step temperature conditions optimum for denaturation (>90°C) annealing and extension (72°C). The extension temperature lies within the optimum activity range for the Taq polymerase, while the annealing temperature is set about 2-5°C below the melting temperature of the primers. Higher annealing temperature increases the specificity of hybridisation hence product fidelity. The melting temperature depends on the length and composition of the primer estimated as follows: Tm = [4 (G + C) + 2 (A + T)] (Thein and Wallace, 1986).

During amplification, the primer that anneals to a complementary portion of the template DNA strand is extended by addition of dNTPs producing a short DNA strand complementary to the template strand and defined by the ends of the primers. Often, PCR is used to amplify a known sequence of DNA, hence the primers are designed to anneal to sequences flanking the region of interest. For taxonomic diagnosis, the sequences amplified are usually within highly conserved regions that do not vary within a taxon or group.

2.7.2 Random Amplification of Polymorphic DNA – Polymerase Chain Reaction (RAPD –PCR)

RAPD –PCR amplifies segments of DNA, which are essentially unknown to the scientist. The primers used are of arbitrary sequence and may anneal to several segments of the template DNA strand (Welsh *et al.*, 1991; Williams *et al.*, 1990). When two random primers orient in opposite directions at a reasonable distance away from each other, the region flanked by them is amplified. Since random primers are usually of intermediate size

(about 10 bp) multiple amplifiable fragments from different loci are usually present for each set of primers in each genome (Lynch and Milligan, 1994). These products are then separated by electrophoresis.

RAPD PCR has several advantages in diversity studies. Since primers consist of random sequences and do not discriminate between coding and non-coding regions, the technique is likely to sample the genome more randomly (Lynch and Milligan, 1994). Also it requires no prior knowledge of the target DNA sequence, and is technically simple. Further a large number of potential markers can be generated using readily available markers.

The inherent problems of RAPD PCR are associated with its sensitivity to contamination by foreign DNA, low repeatability and reproducibility of products and co migration of amplicons from different regions of the DNA (Lynch and Milligan, 1994). Also, being a dominant marker, it is limited in the number of "allelles" revealed per locus (Aman, 1995). Systematic error can be minimised by a number of practical procedures to improve the reliability of these markers (Hadrys *et al.*, 1992; Gawel and Bartlett, 1993; Lima *et al.*, 2002). Still, comparison of results between different laboratories, amplification conditions or DNA purification procedures is still impractical.

RAPD PCR is useful in analysing genetic variation within species. Amplicons generated when treated as discrete loci markers has been used to estimate the quantitative genetic structure of populations (Excoffier *et al.*, 1992; Lynch and Milligan, 1994; Lima *et al* 2002), genetic mapping, DNA fingerprinting (Caetano-Anolles *et al.*, 1991) and taxonomic studies. In fact, the technique has been used to differentiate between insect

species, clones of aphids (Cenis *et al.*, 1993) and biotypes of *B. tabaci* and other species (Black *et al.*, 1992; Gawel and Bartlett, 1993; Gadelseed, 2000). Reproducible RAPD profiles have recently been generated and used for diagnosis and identification of *B. tabaci* biotypes (De Barro and Driver, 1997; Lima *et al.,* 2002) and in interbreeding studies (De Barro and Hart 2000; Ronda *et al* 2000).

2.7.3 Amplified Fragment Length Polymorphism (AFLP)

This is based on selective PCR amplification of a fraction of the fragments obtained after DNA restriction allowing a higher resolution of genetic differences (Cervera *et al.,* 2000). Primers are designed that anneal to the ends of the restriction fragments using adapters previously ligated to the fragments. It is among the most polymorphic marker techniques and has been used to study genetic differences in plants (e.g. Danquah *et al.,* (2002) and animals including *B. tabaci* (Cervera *et al.,* 2000).

2.7.4 Other PCR methods

Specific genes have been coded in comparative studies of *B. tabaci* biotypes. Campbell (1993) and Campbell *et al.* (1996) differentiated A and B biotypes based on the 18S rRNA genes. Frohlich *et al.* (1999) have differentiated *B. tabaci* populations based on variability of the 16S rDNA and cytochrome oxidase 1 (CO1 gene). Analysing the ITS1 gene sequence, De Barro *et al.* (2000), constructed a phylogeny based on 31 populations of *B. tabaci* throughout the world. The COI gene coding has been used to detect minute genotypic differences and determine the phylogeographic relationship of *B. tabaci* populations (Sseruwagi *et al.*, 2003).

Microsatellites (also called Simple Sequence Repeats, SSRs) are short tandem repeats of sequence units less than 6pb in length, thought to be produced by errors in DNA replication when the DNA polymerase slips when copying in the repeats region changing the number of repeats in the region (Robinson and Harris, 1999). They are unique in that are closely associated with conserved regions that contain coding regions (Loxdale and Lushai, 1998). Base changes in these repeats occur at a higher rate than in the coding regions and the polymorphic length variation is recordable. Microsatellite loci are highly variable and, hence an ideal and simple means of in studying variation (Robinson and Harris, 1999). Some microsatellite probes for *B. tabaci* have been isolated but are still of limited use especially in some biotypes (De Barro *et al.*, 2003, Tsagkarakou and Roditakis, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Experimental Material

3.0.1 Insects and sampling sites

The populations used for molecular characterisation were collected from four host plants (cassava, okra, tomatoes and garden eggs) in three locations - University of Ghana farm – Legon, Sinna's garden, and Pokuase (Table 7). The University of Ghana farm, Legon and Crop Science Department experimental garden (Sinna's garden) are about three kilometres from each other but separated by non-host vegetation. The Ministry of Food and Agriculture - Plant Protection and Regulatory Service Directorate (MOFA/PPRSD) Station, Pokuase is 21 km from the first two, much farther than the 7-kilometre annual migration distance of *B. tabaci* (Byrne, 1999). The geographical co-ordinates of the sampling sites were taken using a Garmin 12 XL 12 channel Global Positioning System (Garmin Olathe, Kansas, USA). At each site, the different host plants were no more than 50 metres away from each other (within the daily flight range of *B. tabaci*). The host populations were labelled as shown in Table 7.

Insects used for morphometric studies and cage oviposition bioassays were collected from natural infestation at Sinna's garden. Those used for cage host-preference assays and mating studies were collected from and reared on cassava and okra, respectively.

Table 7. Details of sampling sites and populations used for molecular characterisation

Location, co-ordinates	Host plant	Code
University Farm, Legon	Cassava	C1
N: 05.66008	Tomato	T1
W: 000.19325	Garden egg	G1
	Okra	K1
Sinna's Garden, Legon	Garden egg	G2
05.64963	Cassava	C2
000.18474	Tomato	T2
	Okra	K2
PPRSD, Pokuase	Cassava	C3
N: 05.69737	Garden egg	G3
W: 000.28519	Okra	K3

3.0.2 Test plants

Test plants were maintained in whitefly-proof screen cages. Four test plants: cassava, *Manihot esculenta* (Euphorbiaceae), tomato *Lycopersicon esculentum,* garden egg *Solanum integrifolium* (Solanaceae) and Okra, *Abelmoschus esculentus* (Malvaceae) were used for oviposition bioassay. These were the same species as the field hosts from which collection was done. For rearing studies, additional host plants: eggplant (*Solanum melongena*), Sweet pepper (*Capsicum annum*) (Solanaceae), Cowpea *Vigna unguiculata* (Leguminosae) and cabbage *Brassica oleraceae capitata* (Cruciferaceae) were tested. The following varieties were used: Garden egg: Legon 1, Okra: Clemson Spineless; Tomato: Wosowoso; Eggplant: Long purple. Cultivars of the other crops were not ascertained.

3.1 Molecular Characterization

Adult insects were aspirated into sampling bottles in the morning (5 to 6 a.m.) or in the evening (6 to 7 p.m.). At such times, it was cool and the insects were assumed to have settled for feeding or had not moved from the previous night's feeding sites. The aspirator head was removed and the bottle inverted to allow vigorous adults to move to the top while the mortally injured or dead adults were allowed to fall off before the bottle was closed (Plate 1). Insects were brought to the laboratory alive, freeze-killed at –20 °C and stored at that temperature until ready for use.

3.1.1 DNA extraction

The DNA extraction protocol described by Cenis *et al.* (1993) was adapted and used to isolate DNA from individual adult whiteflies. Unsexed individuals were pulverised singly in 1.5 ml microfuge tubes in 10 µl of the extraction buffer (0.2 M Tris-HCl pH 8.5, 0.025 M NaCl, EDTA pH 8, 0.5% SDS). Using disposable micropestle tips (Plate 2)

Micropestle tips were improvised by cutting back the ends of 200 µl micropipette tips about 8 mm, melting them slightly and pressing into the bottom of a 1.5 ml microfuge tube to fit. On cooling, the moulded tip was twisted out and smoothened over a naked flame. These pestle tips were washed and autoclaved before use.

After homogenisation, 90 µl of the extraction buffer was added to the lysate and incubated at 65 °C for 30 minutes in a water bath. The lysate was then allowed to cool on the bench after which 50 µl of 3 M Sodium acetate (pH 5.2) was added and mixed by tapping the tubes. The tubes were kept at –20 °C for 10 minutes, and then centrifuged (Biofuge 13, Heraeus Instruments) at 13,000 g for five minutes. The supernatant was transferred into a fresh tube. An equal volume of isopropanol was added and mixed by gentle inversion several times, then left for 30 minutes at room temperature to precipitate the nucleic acids. DNA was pelletted by centrifugation at 13,000 g for twenty minutes. The supernatant was discarded and the pellet washed twice with 250 µl of 70% ethanol, each time centrifuging at 13,000 g for five minutes and tipping the ethanol onto tissue paper. The pellet was dried on the bench at room temperature.

DNA was initially re-suspended on ice in 18 µl of double distilled water, with gentle aspirations after 30 minutes, for checking. Three microlitres were loaded onto 1.5% agarose in TAE gels and subjected to electrophoresis at 50 V for three hours. Bands were examined under ultra violet light for clarity and consistency. Very low yield or degraded DNA was discarded. Selected samples were further diluted to 50µl, with double distilled water and stored at -20 °C till ready for use. Each insect, therefore, produced enough DNA for over 40 reactions, hence for all primers, the same insect DNA was used as a given accession.

3.1.2 PCR reactions

RAPD PCR reactions were performed in a total reaction volume of 25 µl. A composite master mix containing, in every 24 µl: 21.5 µl autoclaved double distilled water, 1.5 µl of 25 mM $MgCl_2$ and 1 µl random primer solution. 24 µl of this mixture was added to each tube of Ready-To-Go™ PCR beads (Amersham Pharmacia Biotech, New Jersey) containing when reconstituted to a final volume of 25 µl: ≈ 2.5 units of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs and stabilisers. Magnesium chloride concentration was adjusted to 3.5 mM using 25 mM $MgCl_2$ solution. To this preparation, 1 µl of template DNA solution was added. These reagents were assembled on ice, centrifuged lightly to seat at the bottom of the tubes, and layered with light mineral oil on top prevent evaporation.

Amplification was done in a Progene thermocycler (Prog 05 D, Techne Cambridge Ltd) using the following programme, modified from Cenis *et al.* (1993): Initial block heating at 40 °C for two minutes, initial denaturation at 94 °C for three minutes then 45 cycles of a minute each of denaturation at 94 °C, annealing at 35 °C and extension at 72 °C, followed by a final extension cycle at 72°C for five minutes. The products were stored for 4 °C until ready for use.

12 µl of the amplification products were separated by electrophoresis at 2.5 V/cm potential gradient for 4 hours using 2% agarose in 1 x TAE gel stained with ethidium bromide. The running buffer was 1x TAE (0.04M Tris-acetate, 0.001M EDTA). A lane of 100 bp DNA ladder (Life Technologies, 15628-50) was included in each gel as a molecular size marker. Bands were photographed under ultra violet light on a 365 nm UV transilluminator (Transill UVP, TM 20E) using Polaroid (DS 34, Direct Screen) camera on Polaroid paper.

The electrophoregrams were scanned into Microsoft Photo Editor Version 3.0™ and converted into negative image to make bands more distinct for presentation.

3.1.3 Primers

Decamer oligonucleotide primers of arbitrary sequence supplied by Operon Technologies (Alameda, California) were used for the study. Twenty primers selected from the A, B, C, D and E kits previously used in identifying biotypes of *B. tabaci* (Gawel and Bartlett, 1993; De Barro and Driver, 1997; Guirao *et al.*, 1997; Gadelseed, 2000; Moya *et al* 2001) were screened for number, reproducibility and polymorphism of RAPD bands. Five primers (Table 8) were chosen and used in this study.

3.1.4 Scoring bands

Individual bands were scored manually from the electrophoregrams as present (1) or absent (0) and entered into binary data matrix. Band sizes were estimated graphically ± 25 base pairs, using the size migration curve of the 100 bp marker of the same gel as a reference. Gel scores were pooled into a composite matrix combining scores from all primers.

Table 8. Base pair sequence of RAPD primers used

Primer	Sequence 5' – 3'	Tm °C
OPA 02	T G C C G A G C T G	34
OPB 08	G T C C A C A C G G	34
OPC 05	G A T G A C C G C C	34
OPD 16	A G G G C G T A A G	32
OPI 16	T C T C C G C C C T	34

The melting temperature (Tm) of the primers was calculated by: Tm = 4(G+C) + 2(A+T) °C (Thein and Wallace, 1986)

3.2 Morphometrics

Insects used for morphometric studies were collected at the quiescent red-eyed larval stage to ensure their antiquity (Burban *et al.*, 1992). Each leaf with larvae was placed in a petri dish lined with moist filter paper to retain its turgidity until the adults emerged. Adults emerging were placed in sample bottles, inactivated in a freezer at –20°C for a minute then placed in 80% alcohol till ready for use. They were later examined on temporary slides.

3.2.1 Slide preparation

Permanent slides were prepared following a method previously described by Kyerematen (1996) with some adaptations. Fourth instar nymphs were detached from host plants and heated just below boiling point in 8% Potassium hydroxide for about 20 minutes to macerate the musculature and fat or till they looked rather translucent. The macerated body was neutralised in glacial acetic acid at room temperature for 15 minutes. They were then passed through a series of ethyl alcohol from 50% through to, and ending with, absolute alcohol twice. The specimens were then transferred into cedar wood oil layered with and cleared for 30 minutes until the cloudiness reduced. They were then mounted dorsum up in DPX mountant in groups of five, on the same slide under a stereo-microscope with the positions marked out on the label. The dorsum was identified by the presence of seven pairs of setae described by Martin (1987). Slides were dried in an incubator at 40 °C for 24 hours and then at room temperature for 72 hours.

3.2.2 Measurements

Specimens were measured using a Nikon Optiphot phase contrast microscope equipped with a drawing tube. The microscope's eyepiece graticule was calibrated using a 10-mm stage micrometer divided into 100 units, as shown on Table 9.

Table 9: Calibration of the microscope used in measurements

Objective lens	Graticule units	Stage-micrometer units	Unit length
X 04	50	100	20.0
X 10	74	59	7.98
X 20	82	32	3.90
X 40	70.5	14	1.98
X 100	64	5	0.78

The length corresponding to a single eyepiece graticule unit was calculated by simple proportions.

3.2.3 Drawing

Large pencil drawings of the various structures were made under a Nikon microscope equipped with a drawing tube. The drawings were reduced to appropriate sizes by photocopying and pasted on A2 cards. They were then inked on tracing paper and further reduced using to A4 paper size by photocopying. Drawings were scanned into Microsoft Photo Editor™ and the size reduced as appropriate for printing.

3.2.4 Morphometric features

Morphological and morphometric measurements and ratios followed features earlier used to delineate biotypes A and B (Bellows *et al.*, 1994), and those used to analyse morphological differences between biotypes of *B. tabaci* (Rosell *et al.*, 1997). Features mentioned in Martin (1987) and found to vary within species of Aleyrodids in response to

host morphology were avoided. Ratios followed some suggestions from Bellows *et al.* (1994) and Rosell *et al.* (1997).

3.2.5 Morphology and terminology

Terminology used is originally described by Martin (1987) and used in later work (Bellows *et al.,* 1994, Rosell *et al.,* 1997). Morphometric measurements and ratios were based on the features used to delineate A and B biotypes (Perring *et al.*, 1993) and those used to analyse morphological differences between biotypes of *B. tabaci* (Rosell *et al.*, 1997). Additional features described by Martin (1987) in the taxonomy of Aleyrodid pest species are also used. Ratios followed some examples from Kyerematen (2000) and Billah (1997) as modified for whiteflies.

3.2.5.1 Fourth instar nymphs

- Total length (TL) – Length along the longest axis from the apex to the base of the caudal setae through the middle of the dorsum (Figure 2).
- Body width (BW) the distance across the widest point on the dorsum.
- Space between the caudal setae – distance between the bases of the caudal setae.
- Width of vasiform orifice – the distance across the widest point of the vasiform orifice.
- Length of ligula: distance between the point the operculum joins the ligula to the top of the ligula
- Length of the caudal tracheal fold – distance between the tip of the ligula to the posterior tip of the larva.
- Width of the caudal furrow – distance across the caudal furrow mid-way between the ligula and the base of the caudal setae.

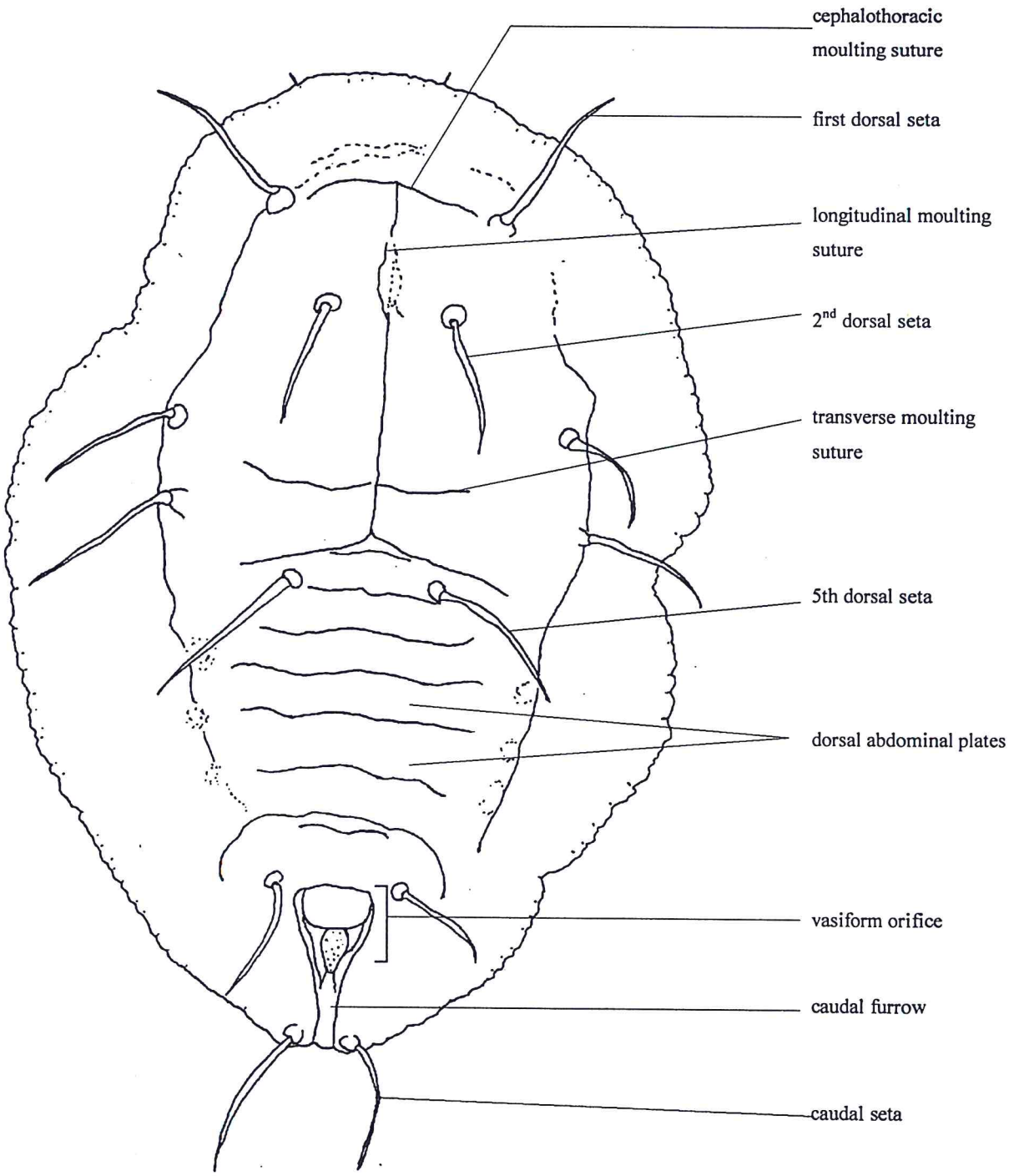


Figure 2: Features of the fourth-instar nymph of *B. tabaci* used in comparative morphometric analysis

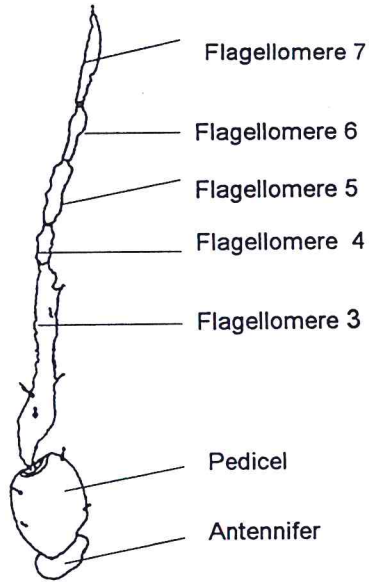
- Head capsule – the distance between the transverse moulting suture and the cephalothoracic suture along the longitudinal moulting suture.
- Head depth – distance between the last thoracic suture to the cephalothoracic moulting suture along the longitudinal moulting suture.
- Width of thoracic plates 1 and 2 – width measured along the dorsal central line.
- Distance between setae: distance between the bases of the respective dorsal setae pairs: DS1, DS2, DS5.
    - DS1 – located in front of the head near the eyespots and the cephalothoracic moulting suture.
    - DS2 – pair of setae just behind the eyes on the dorsum
    - DS5 – located on the fourth thoracic segment area of the dorsum.

3.2.5.2 Adult features

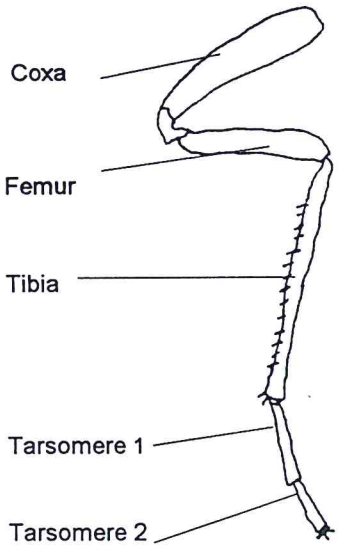
Features that were variable enough between the populations of *B. tabaci* are boldfaced and assigned character states 1 to 3. Features that were not variable are assigned states 1 and 2 and used to delineate *B. tabaci* from the outgroup (*Aleurodicus dispersus*) and the sister group (*Bemisia* nr. *afer*) (Figure 3).

Antennae: All lengths of the antennal segment were measured in ventral view.

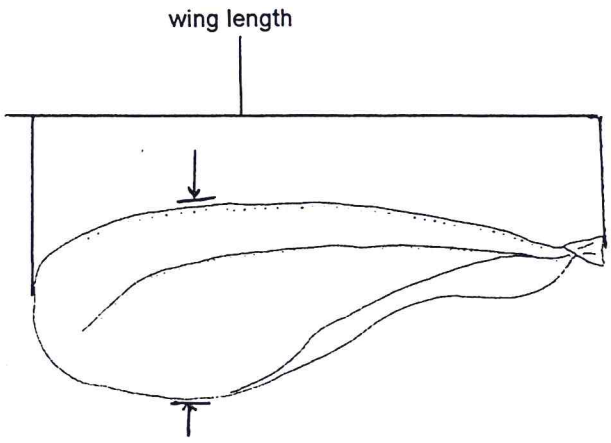
- Length of pedicel
- Length of flagellomeres III to VII.
- Wing length: distance between arculus and wing tip
- Width of the wing along the widest axis
- Length of femur tibia, tarsus are illustrated
- Length of claspers



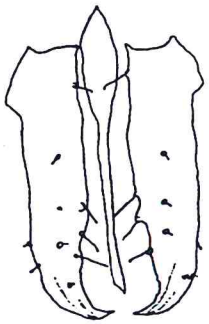
a - antenna



c - hind leg



b- forewing



d - male claspers

Figure 3: Some features of *B. tabaci* imago used in comparative morphometric analysis

3.2.6 Trends used for morphometric comparison (Table 10)

Adults: males T1- 24, females 25 – 48; 4th instar nymph: 49 - 52.

T1. Body length <700 μm (0) 701 – 800 (1) 801 – 900 (2) > 901 (3)

T2. Femur length < 179 (0) 171 – 183 (1) 801 – 1000 (2) > 1001 (3)

T3. Wing length < 700 (0), 700 – 800 (1) 801 – 1000 (2) > 1001 (3)

T4. Clasper length < 65 (0) 65 – 75 (1) 75 – 90 (2), > 90 (3)

T5. Length of thorax < 235 (0), 235 – 254 (1) 255 – 300 (2) > 300 (3)

T6. Pedicel length < 40 (0) 40 – 43 (1) 43 – 50 (2) > 50 (3)

T7. Pedicel width <262 (0) 26 – 27.9 (1) 28 – 35 (2) > 35 (3)

T8. Antennal Ratio < 0.4 (0) 0.4 – 0.45 (1) 0.45 – 0.49 (2), > 0.5 (3)

T9. Length of tibia of rear leg < 330 (0) > 331 (1)

T10. Length of first tarsomere < 105 (0), > 106 (1)

T11. Length of second tarsomere < 80 (0) > 81 (1)

T12. Length of third tarsomere < 25 (0), > 25 (1)

T13. Wing width < 300 (0), > 331 (1)

T14. Length of Abdomen < 360 (0), > 360 (1)

T15. Thorax length < 270 (0), > 270 (1)

T16. Length of flagellomere 3 < 102 (0) > 102 (1)

T18. Length of flagellomere 4. < 20 (0), > 20 (1)

T19. Length of flagellomere 5 < 35 (0), > 35 (1)

T20. Length of flagellomere 6 < 30 (0) > 30 (1)

T21. Length of flagellomere 7.< 40 (0) > 40 (1)

T22. Total antennal length > 321 (0), > 321 (1)

T23. Antennal ratio2. < 0.8 (0)< 0.8 (1)

T24. Antennal ratio 3. 0.35 (0), > 0.35 (1)

T25. Femur length < 185 (0), 185 – 194.9 (1) 195 – 250 (2) > 250 (3).

T26. Length of tarsomere 1 < 102 (0), 102 – 110 (1), 110 – 130 (2), >130 (3)

T27. Length of tarsomere 2 < 69 (0), 70 – 75 (1) 75 – 80 (2) > 80 (3)

T28. Wing length <800 (0), 800 – 920 (1) 920 – 1000 (2). 1000(3)

T29. Abdomen length < 400 (0) 400 – 480 (1) 480 – 500 (2) > 500 (3)

T30. Thorax length <270 (0) 270 0 300 (1) 300 – 400 (2) > 400 (3)

T31. Pedicel length, 40 (0) 40 – 46 (1) 47 – 55 (2) > 55 (3)

T32. Length of 7^{th} antennal flagellomere < 37 (0), 37 – 40 (1) 40 – 45 (2) > 45 (3)

T33. Leg ratio <3.19 (0), 3 – 3.2 (1), > 3.2 (2).

T34. Antennal ratio < 0.4 (0) 0.4 – 0.459 (1), >0.46 (2)

T35. Length of tibia of rear leg <340 (0) > 340 (1)

T36. Length of first tarsomere < 110 (0), > 110 (1)

T37. Length of second tarsomere < 80 (0), > 80(1)

T38. Length of third tarsomere < 28 (0), >28 (1)

T39. Wing width < 360 (0) > 360 (1)

T40. Length of Abdomen < 480 (0) > 480 (1)

T41. Thorax length < 290 (0), > 290 (1)

T42. Length of flagellomere 3 < 105 (0), > 105 (1)

T43. Length of flagellomere 4. < 25 (0), > 25 (1)

T44. Length of flagellomere 5 < 35 (0), > 35 (1)

T45. Length of flagellomere 6 < 35 (0), > 35 (1)

T46. Length of flagellomere 7. <40 (0) > 40 (1)

T47. Total antennal length < 280 (0), > 280 (1)

T48. Caudal wax fringe: absent (0) narrow (1) wide (2)

T49. Caudal wax fringe: narrow (0) wide (1)

T50. Thoracic wax fringe invisible (0), short (1) medium (2) long (3)

T51. Thoracic wax fringe invisible (0), narrow (1) medium (2) wide (3)

T52. Dorsal setae present (0) basal marks, no setae (1)

Ratios

Leg ratio: length of tibia/ total length of leg

Antennal Ratio 1: Length of flagellomere 3/ total antennal length

Antennal Ratio 2: Length of 3rd flagellomere / total length of flagellomeres 4 – 7

Antennal ratio 3: Length of pedicel/ length of 3rd flagellomere

Body ratio: Length of nymph / width of nymph

Table 10. The character states for character 1-52 in *B. tabaci* populations, *B.* nr. *afer* and *A. dispersus*

Polymorphies: A=0&1, B = 1&2, C=2&3, D = 0&2, E = 0&1&2; Missing = ?. Features 1 to 24 (male adults) 25 – 48 female adults.

Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
A. dispersus	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3
B. nr. *afer*	B	2	2	2	B	2	B	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	A	A
Cassava	A	A	A	B	B	E	E	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	A	E	E
Garden egg	A	A	1	A	A	E	E	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	A	0	0
Okra	A	A	A	A	0	E	B	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	A	A	A
Tomato	A	A	A	B	A	E	E	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	E	A	A

Population	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**
A. dispersus	3	3	3	3	3	F	A	2	2	2	2	2	2	2	2	2	2	2	2	2	2	?	?	?	?
B. nr. *afer*	2	A	1	2	A	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	B	0	0	0
Cassava	B	A	0	1	A	1	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1,	A	A	A
Garden egg	B	1	A	1	E	B	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	B	B	B
Okra	B	A	0	1	1	B	B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	B	B	B

3.3 Oviposition Host Preference Assay

Test plants were established in nursery boxes in top soil mixed with 0.1 part farmyard manure and enriched with NPK 17:17:17 fertiliser. Seedlings were watered daily and left in direct sunlight (Plate 3). Plants were used between two and four true-leaf stages.

3.3.1 Cage set up

A multiple choice oviposition bioassay cage was designed after Simmons (1994) with slight modifications. The cage consisted of a modified petri dish 10 cm in diameter by 2 cm depth and two fitting lids (Figure 4). Four evenly spaced 2 cm wide circular windows were cut in the bottom of each petri dish, 2 cm from the centre. A Whatman No. 3 filter paper was placed on the first lid underneath the petri dish and saturated with distilled water. In each choice set up, two leaves from each test plant were placed opposite each other on the filter paper and oriented to be centred beneath the windows with the lower surface exposed inside the chamber. In the second lid, a 1cm wide hole was cut at the centre for the introduction of whiteflies into the cage. This was then sealed with a piece of the mesh. The cage was secured together using two rubber bands placed perpendicular to each other round it.

3.3.2 Choice oviposition site preference bioassay

Adult whiteflies were aspirated from the rearing cages, chilled for one minute at –20 °C to inactivate and then transferred into the test cage (Simmons, 1994). Each batch contained 20 - 25 adult female whiteflies. Upon recovery, the cages were turned upside down so that all exposed leaves faced downward.

After 24 hours at ambient conditions, *B. tabaci* eggs on each exposed arena was counted under a dissecting microscope (Zeiss, Stemi 1000) at X 25 magnification. Up to six replicates were made, and scored only when the total number of eggs laid was more than twenty, else the set up was repeated.

3.3.3 Screen-cage host choice and oviposition tests

Feeding and oviposition choices of cassava and okra populations were tested on six host plants namely: okra, cassava, tomato, eggplant, garden egg and cowpea. Seedlings of the test plants were established in 15 cm diameter pots in top soil mixed with 0.1 part farm yard manure and fertilised once with 0.5 teaspoon NPK 17:17:17 fertiliser. They were maintained in whitefly-free screen cages until they produced 4 – 6 leaves (about three weeks after germination) till when they were ready for use.

Twenty-four plants (each crop comprising a treatment with four replicates) were arranged in a Completely Randomised Design (CRD) in a 120 cm by 150 cm screen cage (Plate 4). The screen cage conditions fluctuated as the ambient conditions (Temperature 20 – 34 °C, and 50 – 80% R. H.) monitored using a thermohygrometer.

About 700 adult whiteflies were aspirated from each of the discriminant hosts (okra and cassava) between 7 a.m. and 6 - 7 p.m. and released at the centre of the screen cage above the plant canopy. The aspirator's sampling bottle component containing whiteflies was held inside a clear plastic tumbler hung at the centre of the cage about 30 cm from the plant canopy. Whiteflies moved to the open top and flew away from the sampling bottle to approach the plants from above.

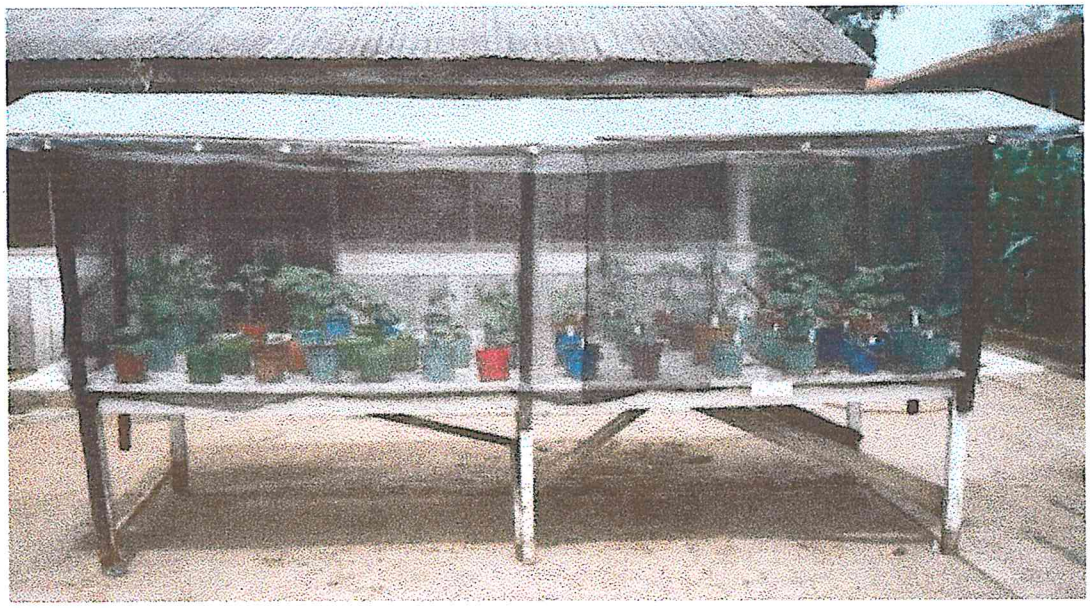


Plate 4: Field screen-cage used for multiple choice host selection experiments and as a rearing cage in host suitability experiments

The number of adult insects per plant was determined after 1 hour (landing response), 12, 36, 48 and 60 hours (settling and feeding response). To avoid whitefly movement between leaves and plants, counting was done under dim torchlight just before 6 a.m. and just after 6 p.m. At the end of the landing assay, whitefly eggs were counted on each leaf under a dissecting microscope (Zeiss, Stemi 1000) at x 20 magnification.

3.4 Rearing Suitability

This was a no choice bioassay of the two biotypes on eight hosts namely, okra, cassava, garden egg, eggplant, tomato, cowpea, and cabbage. Test plants were established singly in 15 cm diameter pots and reared to four true-leaf stage in a whitefly-free screen cage. They were transferred to the test screen cage (Plate 4). Insects were reared on the discriminant hosts (okra and cassava) exposed to plants in transparent plastic truncated clip cages designed after Muniz and Nombela (2001) (Plates 5 and 6).

Briefly, a crystal clear communion cup 3.6 x 2.6 cm diameter by 4 cm high (No. 6 – 000 – 3463 – 6, Southern Containers, Moorsville) was trimmed to remove the bottom using a hot knife. A mesh cloth (0.6 mm x 0.6 mm mesh) was cut to cover the wider open end of the cone and fixed to the rim with a transparent adhesive (UHU All Purpose Adhesive – A. W. Faber – Castell, Malaysia). An aluminium hair clip was prepared into a hinge by flattening the flanges and bending the bottom flange through 90° at the mid-point. A clear round plastic sheet 2.8 cm was cut and glued to the straight top flange to form the lid, while the bottom flange was attached to the side of the cone. The lid covered the narrower end of the clip cage and could be opened clear of the entrance to introduce the whiteflies (Plate 5).

Adult whiteflies were collected from the host plants and introduced into the cages without narcosis using a mouth aspirator. The aspirator's sample bottle component was held under an open clip cage below a fluorescent tube light so that whiteflies moved to the top and escaped into the clip cage. Thus only healthy and vigorous individuals were used in the experiment. Each batch comprised of about 30 adults in approximately 1:1 sex ratio.

After 24 hour access to the leaf surface, the cage arena was marked using a waterproof fine tipped felt pen and the adults gently dislodged. Eggs laid within the cage arena were counted under a stereo-zoom microscope (Zeiss Stemi 1000). As potential mortalities were unknown, all eggs within the laying arena were used as the starting population for the test. The mid-point of the oviposition exposure period was used as the starting point of this experiment after Muniz (2000). Three replicates were made per plant.

Targeted egg-laden plants were held in a whitefly-free screen cage (Plate 4) at ambient temperature and humidity conditions. Three replicates per host were set up. Temperature and humidity in the cage were monitored throughout the rearing period using a thermohygrometer.

Eggs and nymphs were observed and counted daily until adult emergence. Each stage was coded on the leaf next to the anterior side using a fine water-proof felt pen on first observation to avoid repetitive counting. A simple code: dot, horizontal bar, plus sign and two dots, was used for each successive instar respectively. Stage transition was assessed by the presence of the exuvium of the last larval moult and morphological characteristics described by Thompson (2000) as shown on Plates 7 and 8.

Emerging adults were harvested daily between 7 a.m. and noon representing the peak emergence period with of up to 80% of the population (Butler *et al.*, 1983) and used to determine sex ratio and morphometric characteristics. The body length (head to abdomen), wing length and length of the hind tibia of 10 randomly collected whiteflies (where this was possible, or all adults emerging) were measured under a compound microscope (Nikon Optiphot 2) equipped with an eyepiece graticule.

3.5.1 Determination of survival parameters

Survival between each successive stage was determined as a percentage of the number of individuals entering that stage thus:

$$\text{Percentage survival} = \frac{\text{Number entering the next stage}}{\text{Number leaving the previous stage.}} \times 100$$

Mortality at each stage was determined as a reciprocal of the survival at that stage as follows:

$$\% \text{ mortality} = (100 - \text{Percentage survival}).$$

The additive mortality factor between the various stages was determined as follows:

Mortality factor = Log_{10} Number entering stadium– Log_{10} Number leaving stadium.

(Log_{10} Y+1 was used to transform data where there was total mortality at an instar).

The development time was determined from daily counts of adult emergence.

3.6 Insecticide Tolerance

A glass vial technique initially developed for testing resistance to pyrethroid insecticides in the tobacco budworm (Plapp *et al.*, 1990) and modified for testing resistance in whiteflies (Costa *et al.*, 1993) was used in this study. Fifty-millilitre glass test tubes (r= 2.5 cm; h = 10 cm) were used to provide contact exposure to insects (Plate 10).

Desired stock concentrations were prepared by dissolving commercial grade insecticides in various quantities of HPLC grade acetone (99.9% pure, Sigma – Aldrich). Two insecticides were used: Karate 25 EC (25 g/l λ-cyhalothrin) and Dursban (480 g/l Chlorpyrifos). A range of concentrations that produced between about 4% and 95% mortality in six hours was determined by preliminary survey for each population. At least eight serial concentrations within this range were then constituted by dissolving a given amount of insecticide in a known amount of acetone. Chemical residues were achieved by transferring 200 µl of the stock solution into the test tubes and rotating horizontally to provide uniform coverage until all the acetone had evaporated. The vials were allowed to dry for a further 2 hours with the caps open. The control vial was coated in 200 µl of acetone.

Insects were collected using a mouth aspirator to minimise injury to test insects. Collection targeted the first three fully open leaves in each crop previously determined to be the most preferred feeding and oviposition sites of whiteflies (Gadelseed, 2000), and courting pairs. The gentle leaf turn method was used to minimise disturbance to whiteflies during collection (Plate 9). This strategy ensured the testing of relatively young and robust individuals, while avoiding both the newly emerged and very old whiteflies.

Plate 9: Collection of samples from okra by gentle leaf turn method using a mouth aspirator.

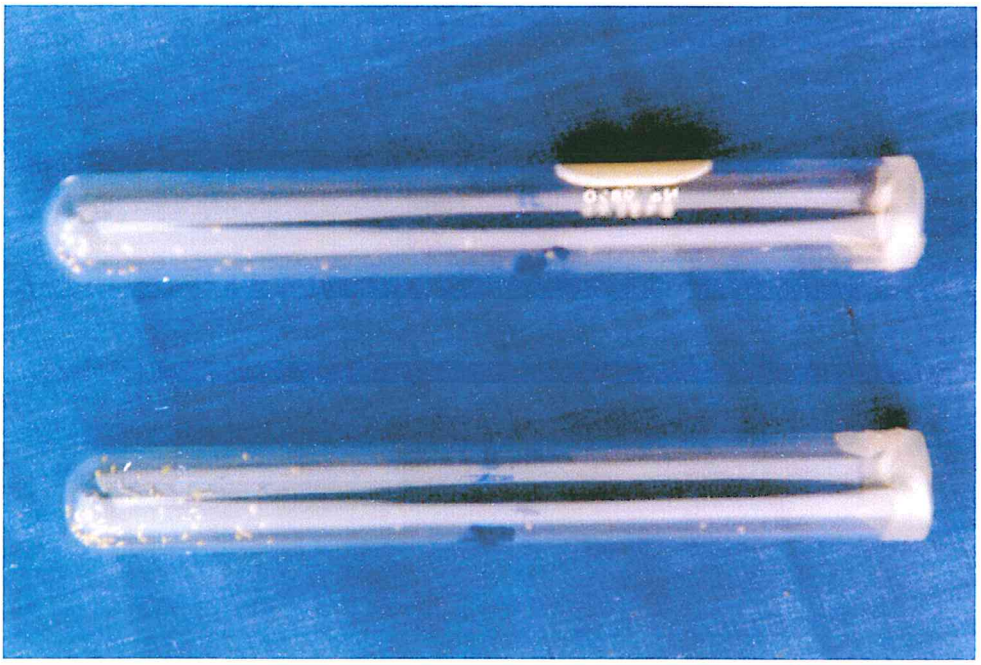


Plate 10: Glass test tubes for contact exposure of whiteflies to insecticides in toxicological tests

Between 30 – 40 unsexed adults were then transferred to the test vial by gently blowing them in an inverted vial. Insects were exposed to the toxicant-treated vials for six hours in a shade at about 25± 2 °C for six hours after which mortality was determined. Dead and moribund insects were counted as 'dead' in tabulating whitefly responses. Insects were considered moribund if they could not make oriented movements when the bottle was gently turned and tapped. Each treatment was replicated four times.

3.6 Mating Interactions

3.6.1 Inter-biotype breeding

Strict production of pure populations of each biotype was ensured by using insects reared on the two hosts. Adult whiteflies were allowed to oviposit on rearing plants continuously until red-eyed nymphs were observed. All adult whiteflies were then removed and the plants kept in respective screen cages per biotype. As whiteflies younger than 12 hours do not mate (Li *et al.*, 1989; Byrne and Bellows 1991; De Barro and Hart, 2000) adults used in this experiment were less than six hours old to ensure their virginity and allow time for clipping.

As whiteflies do not eclose between 6pm to 6 a.m.(Butler *et al.,* 1983), around sunset prior to the day of clipping, all adult whiteflies were blown off a rearing plant or leaf selected at random, and the plant transferred to a whitefly free cage. Between 7 a.m. and 10 a.m. the following day, covering the peak emergence period (Butler *et al.,* 1983), newly emerged adults were collected using a small mouth aspirator (Plate 9), sexed without narcosis or freezing under a compound microscope and introduced into clip cages. Newly emerged males were aspirated similarly and introduced into the cages to achieve the mating combinations desired.

Five virgin females and five male whiteflies were enclosed together in a clip cage (Plate 6). The sex ratio of 1:1 followed the design of Ronda *et al.* (2000) and De Barro and Hart (2000). Cages were clipped on young leaves of eggplant, which had been found to be a satisfactory common host of the two biotypes (Burban *et al.,* 1992). Any dead males were replaced using males of the same biotype to maintain a sex ratio of 1:1; but dead females were not replaced. Insects were kept for four days at each clipping leaf then moved to a new leaf. Thus the period of 8 to 12 days covered the teneral and peak oviposition periods of *B. tabaci* (Drost *et al.*, 1998).

Reciprocal cross set-ups for this experiment were instituted with two controls as follows: Okra biotype males + Cassava biotype females; Cassava biotype males + Okra biotype females; Okra biotype males + females, Cassava biotype males + females. Two female performance control set-ups of unmated females of each biotype were made. Egg hatchability and adult emergence were determined as already described in the rearing suitability experiments. The progeny was monitored daily till emergence of the first red eyed nymphs. Emerging adults were collected daily from 7 a.m. to sunset: at 9 a.m., midday and 6 p.m., and sexed under a stereomicroscope.

3.6.2 Fertility of female progeny

The abdomens of female progeny were examined under a dissecting microscope for any deformation. The females were assumed potentially fertile if their abdomens had the normal plump structure (De Barro and Hart, 2000). Tibial length, wing length and abdomen length of ten randomly selected females from each crossing plan were measured. Abdominal ratio (length/width) was used to statistically compare the abdomen shape.

Three randomly selected female progeny from each cross were enclosed in clip cages on eggplant leaves and allowed to oviposit for five days. The eggs were counted and compared with those from intra-biotype crosses. Females were assumed fertile if they laid eggs at all. As most imagines were saved for sex ratio determination and morphometric comparison, only two replicates were made. Thus, females were assumed to be fertile if they oviposited without statistical comparisons.

3.6.3 Data transformations

Oviposition data was transformed into per female per day basis. Other data were transformed into percentages to normalise for the starting numbers and to compare proportions between each stage. Egg hatchability, and cohort survival were determined as already explained in section 3.5 (rearing suitability studies). To eliminate the cumulative effect of hatchability on the total survival, the larval survival was determined as a proportion of the first instar nymphs undergoing complete eclosion.

3.7 Statistical Analysis

3.7.1 RAPD PCR data

Similarities were calculated from the binary data set using the Dice coefficient:

$$S_{ij} = \frac{2a}{2a + b + c}$$

Where: S_{ij} = similarity coefficient between A and B.

a = positive matches (number of bands in both a and B)

b = number of bands in A but not B

c = number of bands in B but not A

Cluster analysis using unweighted pair-group method with arithmetic averages (UPGMA) Sokal and Michener, (1958) using NTSYS-PC software (Rolf, 1993) and phenograms constructed. Mean genetic similarity within and between populations, genetic distance and gene flow, RAPD allelic frequencies, and Chi-square test of association between the bands and crops, hosts and reaction batches were analysed using Popgene Version 1.31 software (Yeh *et al.*, 1999).

Partitioning of the observed genetic variation and the corresponding F- statistics was carried out by means of analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992). AMOVA incorporates information on haplotype divergence into an analysis of variance (ANOVA) format. A hierarchical model was used according to the differences between putative biotypes (F_{CT}), among populations within biotypes (F_{SC}) and between individuals within populations (F_{ST}). These populations were regrouped into two biotypes according to RAPD patterns and six populations within the biotypes. As genetic distances, Euclidean distances:

$$D_{ij} = \sqrt{2\,(1 - s_{ij})}$$

where D_{ij} = Euclidean genetic distance between the ith and jth entity.

S_{ij} = coefficient of similarity between ith and jth individual.

The contribution of the three partitions to the total variance as well as the three F-statistics were tested statistically by randomisation tests based on 1000 permutations. These analyses were repeated partitioning variance between and within host population and

geographical locations independently. Finally, population pair-wise genetic distance Φ_{ST} was done. These analyses were computed using WINAMOVA version 1.55 (Excoffier, 1993) with data input files prepared using AMOVA Prep (Miller, 1998)

3.7.2 Morphology and morphometrics

3.7.2.1 Discriminant function analysis

Discriminant function analysis explores the potential between several independent variables and their interactions in predicting group membership. The predictor function model is built in two possible ways: by simultaneous entry of variable or by stepwise entry. The former considers all variables together and computes a model based on the most significant, while the latter enters parameters in their order of significance and tests the significance of the model at each step. In this case, the significance of the model keeps reducing with addition of more parameters. The last parameter to be added is one that just violates the criterion. Thus, a final step of removal of the least suitable variable and testing follows to restore the significance of the model. Group membership is predicted based on canonical functions, the first of which is size related. The rest are ratios computed from the relationships between the variables and are more shape-based. These are therefore useful in taxonomic analysis of groups and species based on the nominalistic species concept.

In this study, dicsriminant analysis was used to classify the host populations based on the measurable characters and to isolate the characters that were strongly associated with each group for character weighting in parsimony analysis. Stepwise classification using Wilk's method was done on SPSS Version 10.0 for Windows^{TM} (SPSS Inc., 1999). Entry and removal criterion for character assessment were set at F probability 0.05 and 0.1 respectively. Prior probabilities were computed from group sizes by the programme.

Combined group plots were based on the first four canonical functions. This analysis was done separately for nymphs, adult males and females. To eliminate exaggeration of the classification functions and obtain characters most useful in biotype separation, this analysis was repeated without the sister group since it was morphometrically different in most characters.

3.7.2.2 Parsimony analysis

Cladogenesis is an approach to systematics that seeks to infer evolutionary relationships based on functional similarity between individuals depicted by shared characters (Forey *et al.*, 1993). The evolutionary origin rather than the present functional state of the characters is essential (see Forey *et al*, 1993 for review). Because cladogenesis invariably leads to several tree diagrams attempting to define the relationship, the parsimony analysis principle is used to resolve the apparent conflict, selecting the solution that requires the least number of steps, while violating as few of the options as possible.

In this study, data ranges were examined and character states defined for the measurable characters using arbitrarily judged limits to define the various groups from the data set. Three subgroups were included: adult males, adult females and fourth instar nymphs were entered separately into the same data matrix on MacClade 3.06 (Maddison and Maddison, 1996). Parsimony analysis was done using PAUP 3.11 (Swofford, 1993) on Power Macintosh 5600/160™. Character weights were assigned generally following the weight of the characters in cluster analysis of the groups revealed by SPSS (SPSS Inc, 1999). Thus polymorphies were weighed lower than monomorphic characters and while monomorphic ratios.

Cluster analysis followed stepwise procedures, with different character statuses. Initially analysis was done with all the characters unordered and un-weighted. The characters were then ordered but un-weighted and the analysis repeated. Third analysis was done with weights assigned to the ordered characters. Finally, parsimony analysis was attempted using a priori assumed restraint tree. All searches were heuristic with 1000 iterations. At each tree-building step, both strict consensus and 50% majority rule consensus were used.

3.7.3 Host selection assay

3.7.3.1 Two-choice oviposition surface preference

Egg counts were compounded per host leaf and converted to percentage preference in each choice set up. Paired mean preferences per treatment were compared using two-tailed t-test at 95% level of significance. This analysis was done using SPSS Version 10 (SPSS Inc., 1999).

3.7.3.2 Multiple choice landing and oviposition host preference

The landing preference at each of the 12 hour time intervals and the $\log_{10}$ transformed number of eggs per leaf were compared between hosts using one way ANOVA, with host species as the factor, at 95% level of significance, using Genstat Software Version 5 Release 3.2 for Windows Rothamsted Agricultural Station, (1995). This was done separately for the each biotype. Significant means were separated using Tukey's LSD at 0.05 error limit level. The correlation between the proportion of eggs laid per leaf with the relative preference of the leaves was carried out and its significance tested $p < 0.05$.

3.7.4 Host suitability

Host plants were compared on their host suitability based on the survivorship at each stadium, total survival, larval, development time and sizes of the imagoes. ANOVA was used at $p < 0.05$ using Genstat 5 Release 3.2 for Windows (Rothamsted Experimental Station, 1995). Two-way ANOVA with interaction was used to assess host-biotype effects on (arcsine transformed) egg and first instar survivorship. The rest were separated and analysed by one way ANOVA per biotype since total mortality of some biotypes on specific hosts was observed. The two indicators of host suitability (development time and survivorship) were used to calculate an index of host suitability based on the rearing suitability index (Howe, 1971).

3.7.5 Probit analysis

Probit analysis was done to linearize dose –response data and obtain a relationship used to characterise the populations tested. Data was corrected for natural mortally using Abbott's formula (Abbott, 1925). Dose-response data was analysed by probit analysis after transforming mortality to probit units and dose to Log_{10} (Finney, 1964). SPSS version 10 (SPSS Inc., 1999) was used to carry out the probit regression. Median lethal concentrations (LC_{50}) were read directly from data reconverted from Probits to concentrations by SPSS software. Fiducial limits of effective concentrations of the toxicants were estimated at 95% confidence interval by SPSS, and their significance inferred after a maximum of 20 iterations. The resistance factor at the median lethal concentration (RR_{50}) was calculated by dividing the LC_{50} of each population by that of the most susceptible population.

3.7.6 Mating interactions

The fecundity of females was compared under different mating systems. The performance of the progeny was compared across mating patterns based on egg viability, total survival and nymphal survival. F1 females from each cross were compared based on the three measurements and the abdominal shape and body size. One way analysis of variance was used to compare the crossing plans based on the variables above at $p< 0.05$ using Genstat 5 release 3.2 for PC/Windows NT™ (Rothamsted Agricultural Station, 1995). Data presented as percentages were arcsine transformed to homogenise their variance before analysis on Genstat. Significantly different means were separated using Tukey's LSD.

CHAPTER FOUR

RESULTS

4.1 Amplification Products

4.1.1 Primers

Five primers (OPA 02, OPB 08, OPC, 05, OPD 16 and OPI 16) yielded reproducible fragments some of which were polymorphic between various host populations. A total of 89 bands were scorable, with fragments predominantly ranging in size between 400 and 1800 bp (Table 11). Polymorphic fragments were distributed across the entire size range.

4.1.2 RAPD Profiles

Two unique sets of amplification product profiles were generated by each of the five primers used, associated with cassava and okra/garden egg, but there were no profiles associated with the geographically separated populations within the biotypes (Figures 5 – 7). The cassava whitefly RAPD pattern was shown by all whiteflies collected from cassava and some individuals from tomato. The okra whitefly RAPD pattern was shown by individuals from all the hosts except cassava.

Table 11: Characteristics of amplified random fragments

Primer	Size of bands (bp)	Maximum No. of bands	Average No. of bands
OPA 02	400 – 2072	18	10.36
OPC 05	400 – 1800	17	10.55
OPB 08	400 – 1800	20	11.00
OPD 16	300 – 1800	19	9.33
OPI 16	400 – 1800	15	8.11
Mean		**17.2**	**9.87**

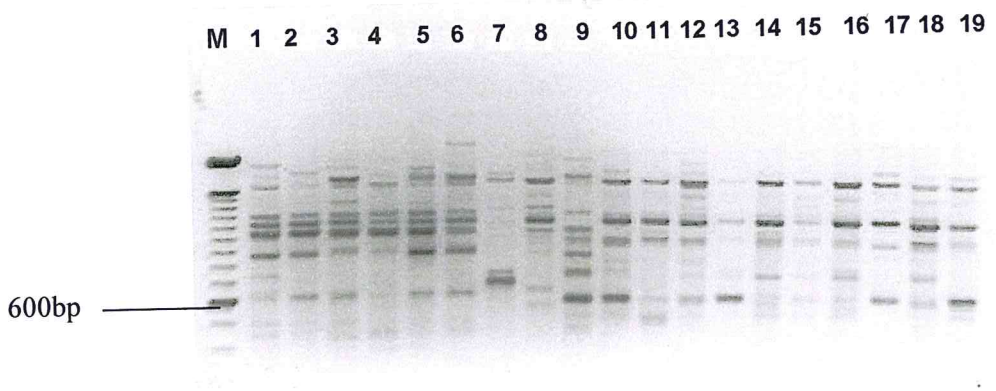


Figure 5. RAPD profiles from 19 whiteflies from four hosts generated by primer OPB 08 Accessions: Lane M is 100bp marker, Lanes 1 – 5 (Cassava); 6 – 9 (Tomato); 10 – 14 (Garden egg); 15 - 19 (Okra). Lanes are stratified by location within hosts thus: first two (farm); next two (Sinna's garden); last one (Pokuase).

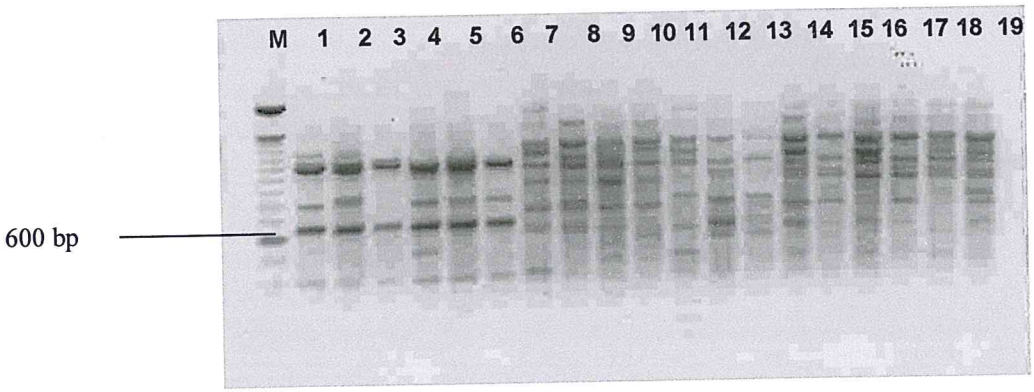


Figure 6. RAPD profiles from 19 whiteflies from four hosts generated by primer OPD 16 Accessions: Lane M is 100bp marker, Lanes 1 – 5 (Cassava); 6 – 9 (Tomato); 10 – 14 (Garden egg); 15 - 19 (Okra). Lanes are stratified by location within hosts thus: first two (farm); next two (Sinna's garden); last one (Pokuase).

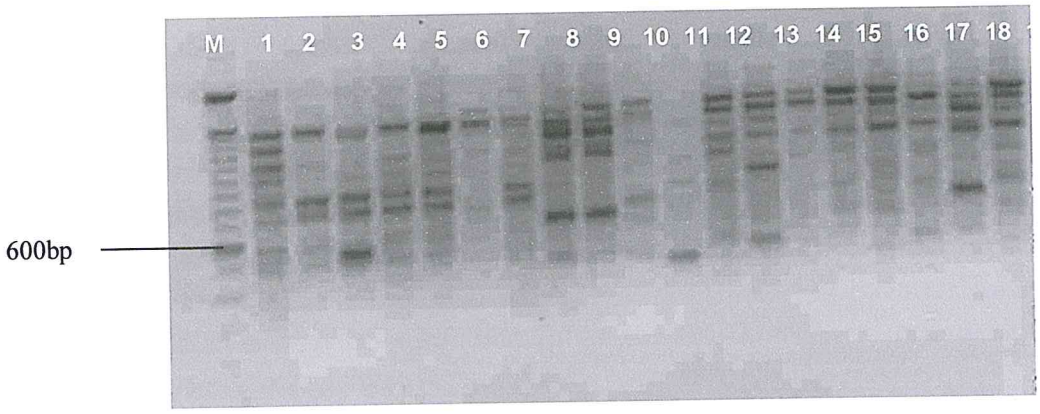


Figure 7 RAPD profiles from 19 whiteflies from four hosts generated by primer OPI 16 Accessions: Lane M is 100bp marker, Lanes 1 – 5 (Cassava); 6 – 9 (Tomato); 10 – 14 (Garden egg); 15 - 19 (Okra). Lanes are stratified by location within hosts thus: first two (farm); next two (Sinna's garden); last one (Pokuase).

4.2 Genetic Diversity

4.2.1 Polymorphism

Polymorphism varied between 53.3%, in cassava population from Pokuase, to 92% in the tomato population from Sinna's Garden. The proportion of polymorphic bands varied among geographical populations and host races (Tables 12 and 13). Polymorphism was highest between host populations at the same location. Populations collected from the same host had higher proportion of polymorphism between different sampling sites than within the same site. Similarly, whitefly populations from different hosts had as high gene diversity as were populations from different hosts in different sites.

Table 12. Percentage RAPD polymorphism and average Nei's gene diversity index ***(h)*** within sub-populations

Host/Location	% Polymorphism	Mean *h*	Standard deviation of *h*
Cassava			
University Farm	73.03	0.2787	0.2031
Sinna's Garden	73.03	.02713	0.1910
Pokuase	53.93	0.2041	0.2062
Garden egg			
University Farm	79.78	0.2994	0.1869
Sinna's Garden	74.16	0.2951	0.1994
Pokuase	61.00	0.2873	0.2171
Tomato			
University Farm	85.00	0.3760	0.1381
Sinna's Garden	92.00	0.3450	0.1545
Okra			
University Farm	79.78	0.3172	0.1885
Sinna's Garden	87.64	0.3404	0.1700
Pokuase	75.28	0.3151	0.2011

Table 13: Percentage RAPD polymorphism and average Nei's ***(h)*** gene diversity index within host populations of *B. tabaci*

Host/Location	%Polymorphism	Mean h^* ± SD
Cassava	85.39	0.3123 ± 0.1735
Garden egg	87.64	0.3359 ± 0.1721
Okra	91.01	0.3566 ± 0.1603
Tomato	98.88	0.3854 ± 0.1195

h = Nei's (1973) gene diversity, * Mean ± SD

4.2.2 Biotype affiliations

Cluster analysis was performed first with each primer based on the UPGMA of the Dice similarity matrix. Each of the primers OPA 02, OPC 05, OPD 16 and OPB 08 as well as a combination of all the five primers used revealed two distinct clusters. The first cluster contained all cassava whiteflies with some tomato and a few garden egg whiteflies; while the second grouped together all okra whiteflies with some tomato and most garden egg whiteflies (Figure 8). No haplotypes clustered outside their distinct group. Two individuals, one each from tomato and garden egg were loosely associated with cassava and okra-garden egg cluster respectively. Similarity between the two RAPD types revealed by each primers was 45% (OPA 02), 49% (OPB 08), 42% (OPD 16) and 46% (OPC 05), and 45% with all the primers combined. Primer OPI 16 did not reveal clear separation of the populations, but clustered them stepwise at different levels, apparently not associated with any hosts or geographical populations.

4.2.3 Genetic distance, differentiation and gene flow between populations of *B. tabaci*

The gene differentiation was generally greater between local populations than between populations from the same host but different geographical locations. Gene differentiation was greatest for populations with widest geographical separation (Pokuase and both Legon populations) ($G_{ST} > 0.05$) than within the local host populations. The index of gene flow was in all cases greater between similar host-races at the same location than between those separated by space or between different host races (Table 14).

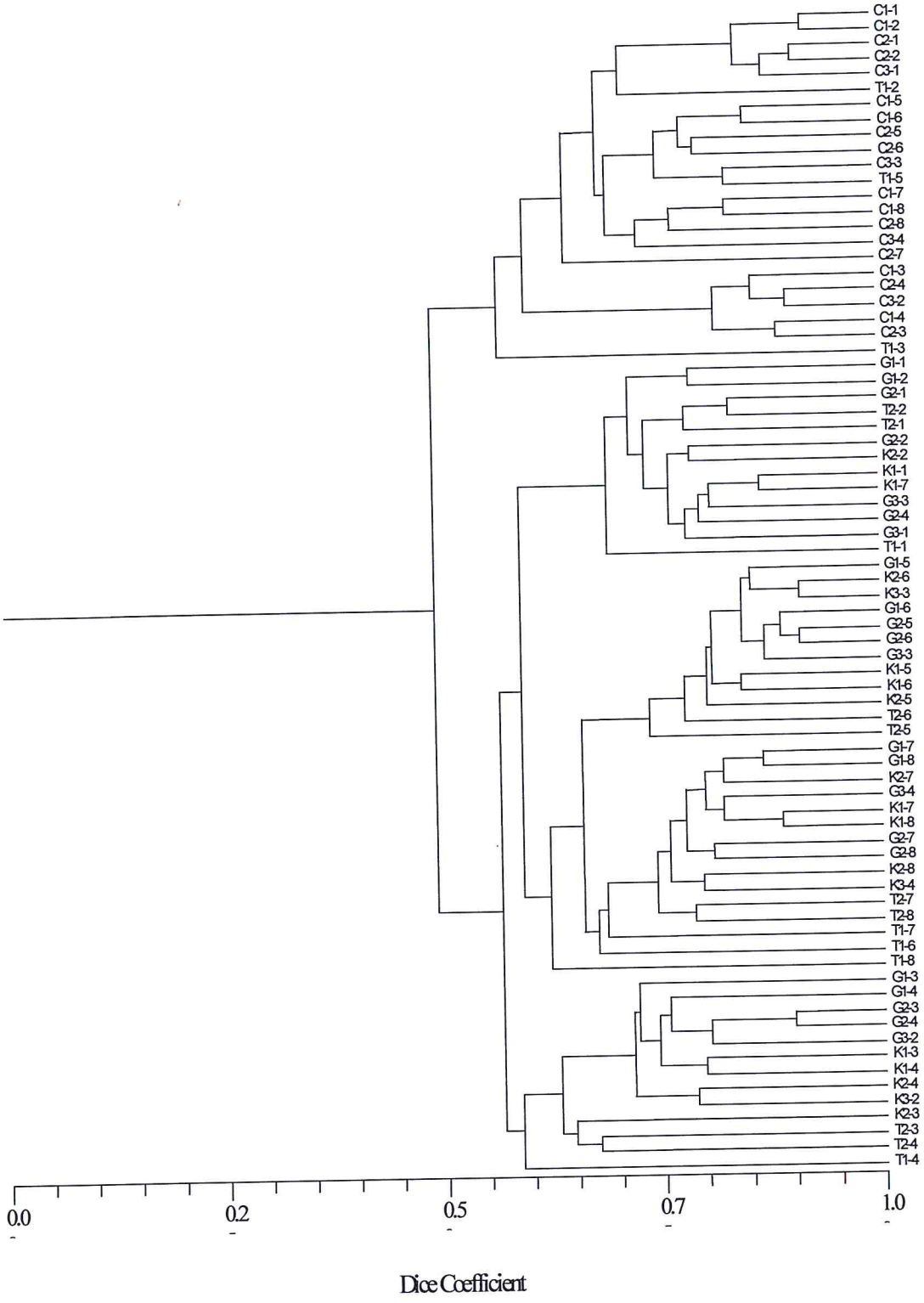


Figure 8: Phenogram of genetic similarity between *B. tabaci* from four hosts in three locations based on UPGMA method of cluster analysis of the Dice coefficient.

Accessions are labelled according to codes described in Table 7.

4.2.4 Analysis of molecular variance (AMOVA)

Analysis of the molecular variance observed revealed significant variation between and within host races and populations (Tables 15 and 16). However, nested analysis of molecular variance revealed significant variation between biotypes, within biotypes and within populations, but no significant differences between populations within biotypes. Thus, geographical isolation did not contribute significantly to the observed variation.

The differences within populations and within biotypes contributed more to the observed variation than geographical variation and differences between the biotypes. Also, the genetic distance between geographically isolated populations of the same biotype were not significant in all cases, while the distance between different biotypes were highly significant (Table 17).

4.2.5 Homogeneity of RAPD profiles

To identify RAPD bands associated with various biotypes, individual insects were assigned to the two biotypes represented by the major clusters on the dendrogram. Chi-square and G square tests of association were carried out using Popgene Version 1.31, to assess the homogeneity of distribution of the on diploid dominant markers revealed bands that were highly associated with each biotype mostly between 600 and 1200 bp in size, the region within which repeatability was highest. No band was present in one biotype and absent in the other, but frequency levels of over 80% in one biotype and below 10% in another were observed (Table 18).

Table 14: Gene differentiation (G_{ST}) and gene flow index (N_m) between different populations

Category	G_{ST}	N_m
Locations	0.1643	2.5426
Hosts	0.2713	1.3432
Within local host populations	0.0210	23.3118

Table 15: Hierarchical analysis of molecular variance of the accessions

Source of variation	d.f.	SSD	MSD	Variance component	Φ
Host races	3	264.5954	88.198	19.95^{***}	0.173^{***}
Populations	7	90.9375	12.991	-2.69^{ns}	-0.034^{ns}
Within populations	65	1076.1250	16.556	82.74^{***}	0.199^{***}
Total	75	1431.6579			

*** $p < 0.001$.

Number of permutations = 1000

Table 16: Analysis of molecular variance based on populations and biotypes

Source of variation	d.f.	SSD	MSD	Variance component
Among biotypes	1	232.8983	88.198	29.43^{***}
Within biotypes	74	1198.7596	16.199	70.57^{***}
Among populations	5	287.1676	57.434	17.52^{***}
Within populations	70	1144.4903	16.356	82.48^{***}
Total	75	1431.6579		

*** $p < 0.001$; Number of permutations = 1000

Table 17: Genetic distance (Φ_{ST}) between pairs of populations

(Biotype)		Cassava			Okra		
Population		Farm	Sinna's	Pokuase	Farm	Sinna's	Pokuase
Cassava	Farm	0.0000					
	Sinna's	-0.0083 ^{ns}	0.0000				
	Pokuase	-0.0058 ^{ns}	-0.0324 ^{ns}	0.0000			
Okra	Farm	0.2622 **	0.2879**	0.2719**	0.0000		
	Sinna's	0.2827 **	0.3106**	0.3033**	-0.0016 ^{ns}	0.0000	
	Pokuase	0.2705 **	0.3249**	0.2943**	-0.0337 ^{ns}	-0.0251 ^{ns}	0.0000

Probability Random distance (Φ_{ST}) > Observed distance: ** - highly significant $p < 0.001$

Distances =Φ_{ST} between pairs of populations; Number of iterations: 1000

Table 18: Frequency of RAPD bands highly associated with cassava and okra whiteflies

Primer	**Fragment size (bp)**	**Frequency of band**		X^2	**p value**
		Cassava	Okra		
OPA 02	600	0.6388	0.0287	35.5751	0.00000
	700	0.6388	0.0993	24.1180	0.00001
	900	0.0115	0.8626	38.7133	0.00000
OPC 05	650	0.7915	0.2996	15.6901	0.00008
	800	0.0445	0.6929	26.9734	0.00000
	1100	1.0000	0.4172	22.5807	0.00000
	1500	1.0000	0.1645	46.0484	0.00000
OPD 16	400	0.7915	0.2352	20.6205	0.00001
	650	0.7051	0.0190	43.0682	0.00000
	800	1.0000	0.0684	61.1661	0.00000
	1050	0.0220	0.7621	35.3480	0.00000
	1300	0.1659	1.0000	59.1303	0.00000
OPI 16	700	0.7915	0.2352	20.6205	0.00001
	800	1.0000	0.2109	40.3554	0.00000
	1000	1.0000	0.4506	20.4871	0.00001

4.3 Morphology and Morphometrics

4.3.1 Statistical inferences

In each case, a majority of the individuals were statistically predicted to belong to their correct groups based on the crop hosts. Correct classification of nymphs, females and males were 79.5%, 66.0% and 63.0% respectively. Out of the 24 features measured in both males and females, only five were informative in this classification when *B.* nr. *afer* was included. Without *B.* nr. *afer*, three features, abdominal claspers, antennal flagellomere 4 and femur were informative among males, while the lengths of body, second tarsomere and femur of hind leg were useful. In the nymphs, five features (body length, body width, distance between first and second pairs of the dorsal setae, width of the vasiform orifice and length of the caudal furrow) were informative. Combined group plots based on canonical functions for nymphs, female and male imagoes are shown on Figures 9 – 11.

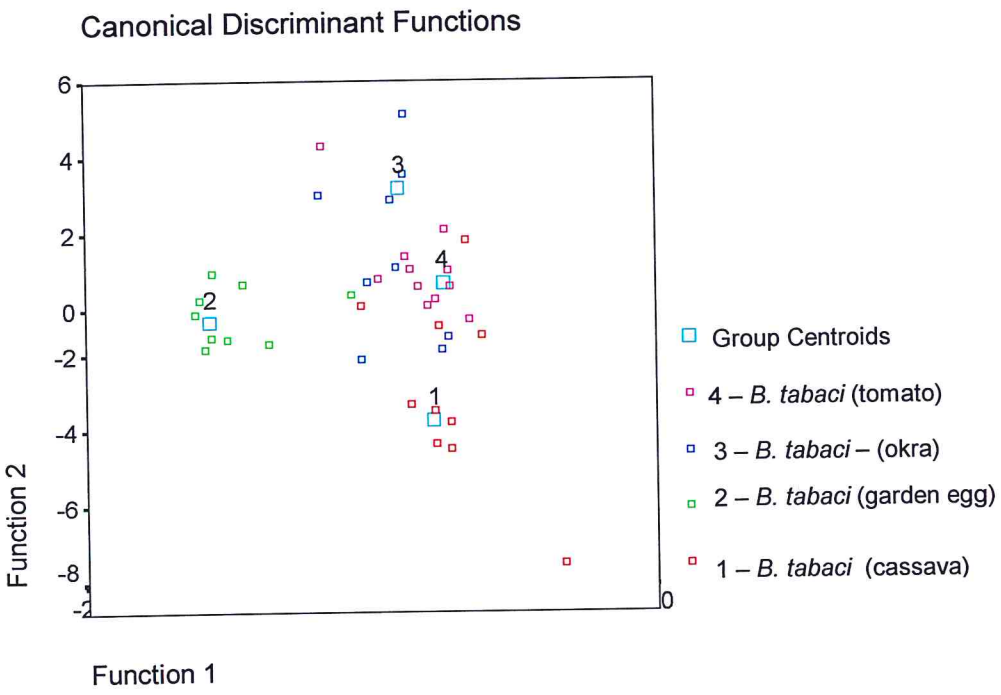


Figure 9: Combined plot for classification of nymphs from four populations of *B. tabaci* based on canonical discriminant functions

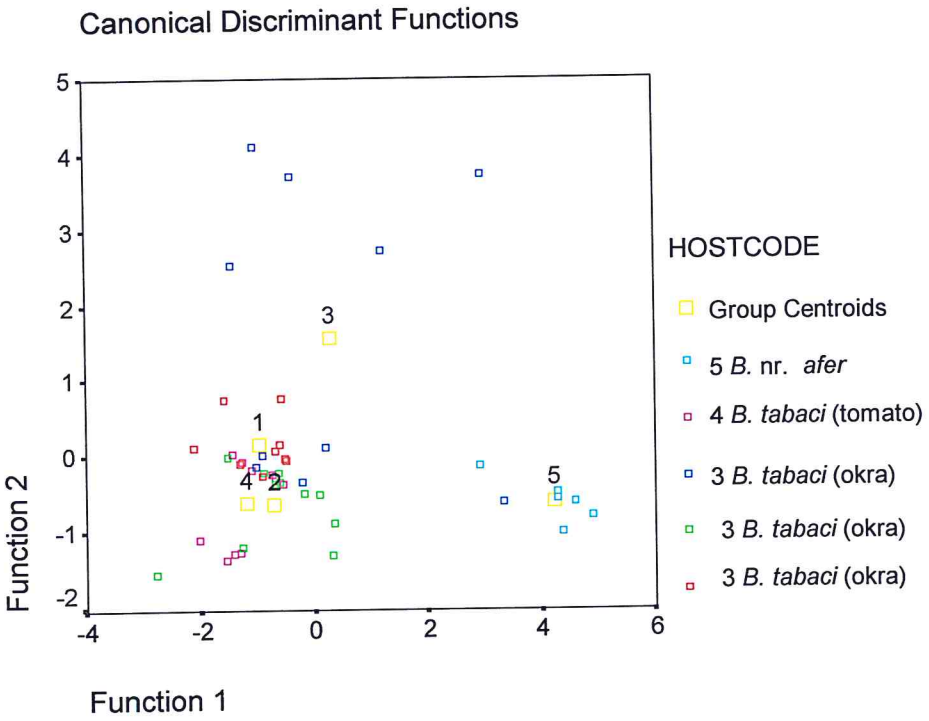


Figure 10: Combined plot for classification of male imagoes from four populations of *B. tabaci* and *B.* nr. *afer* based on canonical discriminant functions

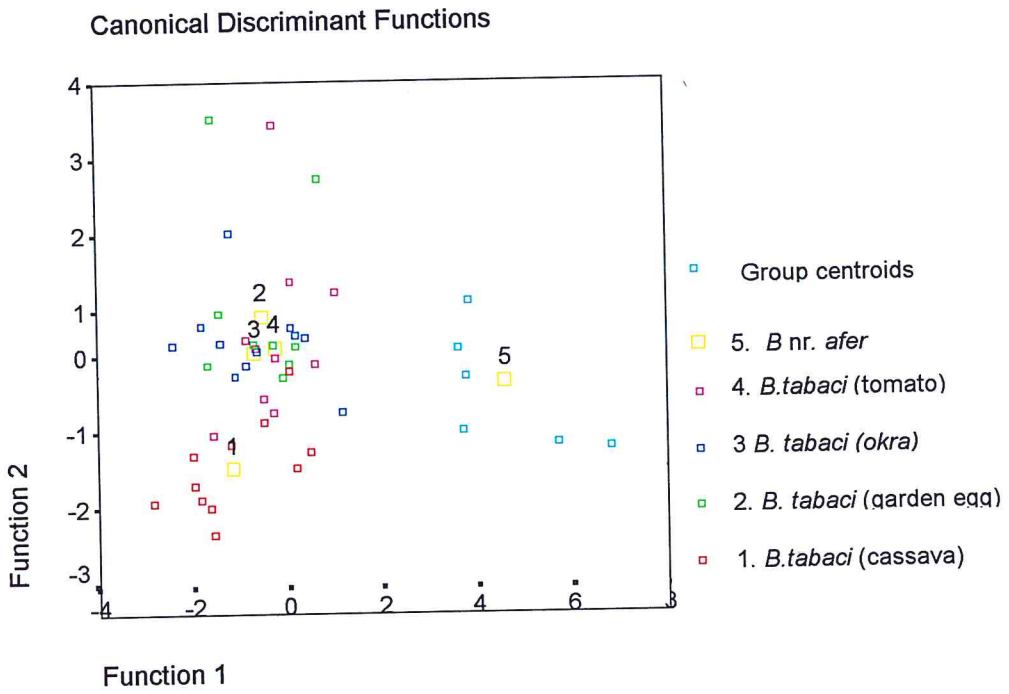


Figure 11: Combined plot for classification of female imagoes from four populations of *B. tabaci* and *B.* nr. *afer* based on canonical discriminant functions

4.3.2 Cladogenesis and parsimony analysis

Initial tree search with unweighted and unordered character states yielded a basal polytomy, which clustered *B.* nr. *afer* together with the four *B. tabaci* populations. Repeated search using ordered and unweighted character states retained seven trees, with shortest length of 32, after 234 rearrangements, classifying the populations variously (Appendix C) The trees had consistency index of 1 and 0.556; homoplasy index of 0.125 and 0.800 before and after excluding uninformative characters respectively. The strict and 50% majority rule consensus of the seven trees yielded similar trees (Figure 12).

Further heuristic search with weighted and ordered characters yielded fifteen trees rooted with the outgroup (Appendix D). The rearrangements tried were 498, and the length of the shortest tree was 1125 steps. Strict and 50% majority rule consensus of the trees yielded the same tree structure (Figure 13). The tree was bootstrapped with 100 replicates treating character weights as repeated counts, with simple addition sequence.

After weighting, the sister group (*B* nr. *afer*) and all *B. tabaci* were grouped together in a basal polytomy rooted close to the sister group. The outgroup was clearly distinguished. Other non-consensus trees revealed various relationships between the taxa and populations. Tree number 3 in the unordered character type search and numbers 11 to 15 distinguished the cassava biotype from the other biotype. Also, trees number 15 and 11 grouped garden egg and tomato populations together, while cassava and okra populations were distinct.

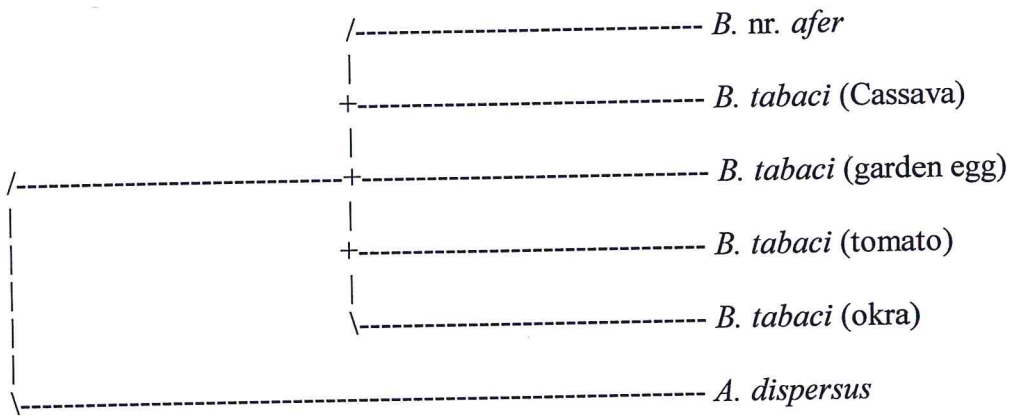


Figure12: Strict and 50% majority rule consensus of 7 trees

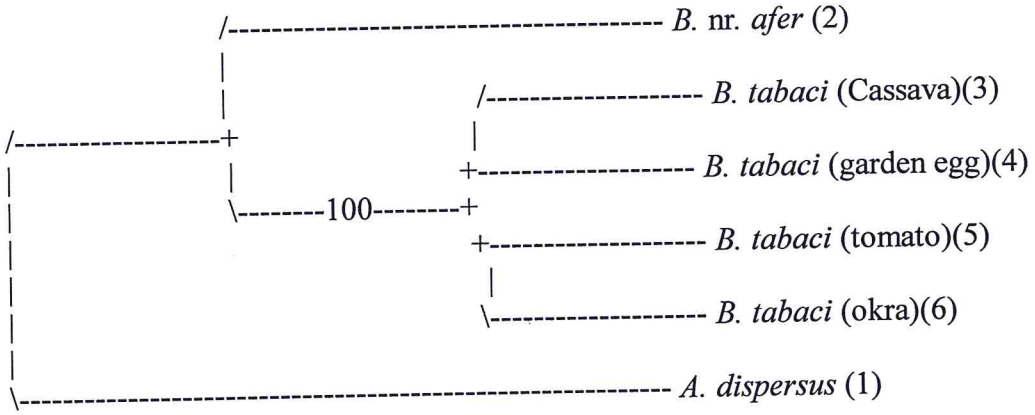


Figure13: Bootstrap 50% majority-rule consensus tree of 15 trees (characters weighted and ordered)

4.4 Oviposition Surface Preference

4.4.1 Leaf surface preference

The preference of the lower leaf surfaces (relative to the upper) of the four hosts to each whitefly populations summarised on Table 19. Generally, whiteflies preferred lower leaf surfaces to the upper leaf surfaces of the host plants. However, neither leaf surface of garden egg was significantly more attractive to cassava and tomato populations, as were the tomato leaf surfaces to garden egg whiteflies. In single-host leaf-surface preference tests, whiteflies collected from cassava oviposited less on okra, as did okra whiteflies on cassava, hence more tests were necessary. Nonetheless, there was some preference for lower leaves in cases when the total number of eggs was more than ten.

4.4.2 Two-choice host preference

Whiteflies preferred to oviposit on the hosts from which they were collected to any other hosts paired with them with a few exceptions (Table 20). Cassava whiteflies highly significantly preferred cassava ($p<0.001$) when paired with any other host. There was no significant difference in attraction to either tomato or okra when paired with garden egg, while tomato leaves were significantly more attractive to cassava whiteflies than okra leaves ($p<0.05$). Garden egg whiteflies preferred garden egg to other hosts except okra ($p<0.05$). Similarly, okra whiteflies preferred okra to any other plant except garden egg, but preferred any host paired with cassava. Each of tomato and garden egg was not significantly more attractive to okra whiteflies than the other, while any hosts plant paired with cassava was highly significantly preferred ($p<0.001$). Tomato whiteflies significantly preferred tomato to cassava and garden egg to okra ($p<0.05$) only, but did not show significant preference to any other of the paired hosts.

Table 19: Relative oviposition preference of four *B. tabaci* host populations for the lower leaf surfaces of four hosts

	Whitefly host- population			
Host	Garden egg	Okra	Tomato	Cassava
Okra	$64.61\pm5.77^{*}$	$81.42\pm5.01^{**}$	$72.22\pm10.04^{*}$	$71.36\pm8.54^{*}$
Cassava	$92.50\pm4.39^{**}$	$82.95\pm5.90^{**\S}$	$86.16\pm4.83^{*}$	$93.01\pm4.92^{**}$
Tomato	71.02 ± 11.05^{ns}	$87.69\pm4.14^{***}$	$74.35\pm6.38^{*}$	$83.09\pm6.81^{*}$
Garden egg	69.31 ± 9.77^{ns}	$76.57\pm4.11^{***}$	73.92 ± 9.32^{ns}	56.83 ± 9.20^{ns}

§ Between 10 and 20 eggs oviposited in each attempt.
Significantly different pairs (indicating significant preference) * $p<0.5$, ** $p<0.01$, ***, $p<0.001$.

Table 20: Oviposition preference of the *B. tabaci* populations on paired hosts

	Whitefly host-populations			
Choices	Cassava	Garden egg	Okra	Tomato
Cassava	$80.28\pm5.18^{***}$	$30.80 \pm 7.58^{*}$	$03.78\pm1.26^{***}$	$15.08\pm7.01^{*}$
Tomato	19.72	69.20	96.22	84.92
Cassava	$85.42\pm 6.00^{***}$	$0.457 \pm0.46^{***}$	$3.94\pm1.36^{***}$	59.26 ± 16.40^{ns}
Garden egg	14.58	99.54	96.06	40.72
Tomato	61.18 ± 9.96^{ns}	$18.19\pm40.72^{*}$	45.01 ± 9.57^{ns}	57.15 ± 24.74^{ns}
Garden egg	38.82	81.81	54.99	42.86
Cassava	$95.97\pm1.82^{***}$	$5.27\pm2.62^{***}$	$09.15\pm3.90^{***}$	37.89 ± 12.34^{ns}
Okra	4.03	94.73	90.85	62.11
Tomato	$73.57\pm 8.35^{*}$	$77.85\pm7.32^{*}$	$32.97 \pm4.33^{**}$	36.56 ± 3.56^{ns}
Okra	26.43	22.15	67.02	63.44
Garden egg	44.55 ± 9.10^{ns}	41.01 ± 12.12^{ns}	53.84 ± 4.07^{ns}	$8.52\pm6.09^{**}$
Okra	55.45	58.99	46.16	93.61

Significantly different pairs (indicating significant preference) * $p<0.5$, ** $p<0.01$, ***, $p<0.001$.

4.5 Multiple Choice Host Selection Assay

4.5.1 Landing and feeding preference

The trends of whitefly distribution between various hosts is shown on Figures 14 and 15. There was no significant difference between landing choice of the cassava biotype on the various hosts within the first 12 hours. However, landing was greater on cassava, eggplant and garden egg as compared to tomato, cowpea and okra. Between 12 and 60 hours, whiteflies shifted significantly to cassava, which eventually attracted 83% of all insects released (Figure 16). Okra attracted the lowest numbers of cassava whitefly while tomato, cowpeas and eggplant were marginally preferred.

Okra whiteflies were comparatively more evenly distributed. There was greater landing on cowpea than on any other host in the first 12 hours. Within 24 hours, insects shifted towards okra and away from cowpea. Cassava attracted the lowest number of landing whiteflies. After 60 hours, okra and garden egg had significantly higher numbers of whiteflies than other hosts and okra was significantly preferred (Figure 17). Cassava attracted the lowest number of okra biotype whiteflies.

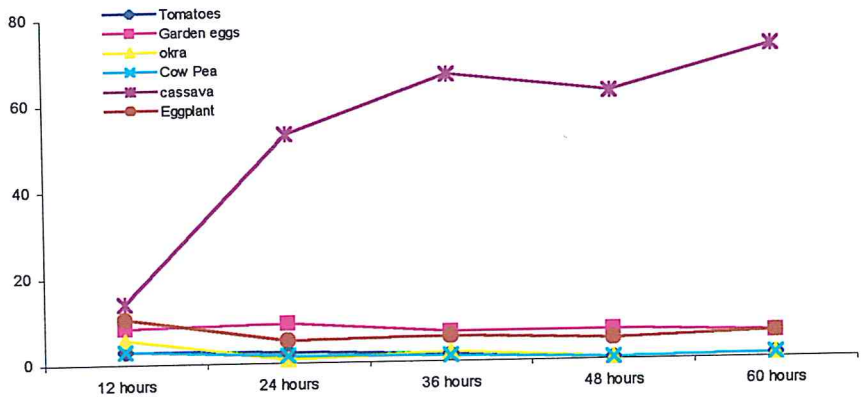


Figure 14: Landing and distribution of *B. tabaci* collected from cassava on six hosts within the first 60 hours after release

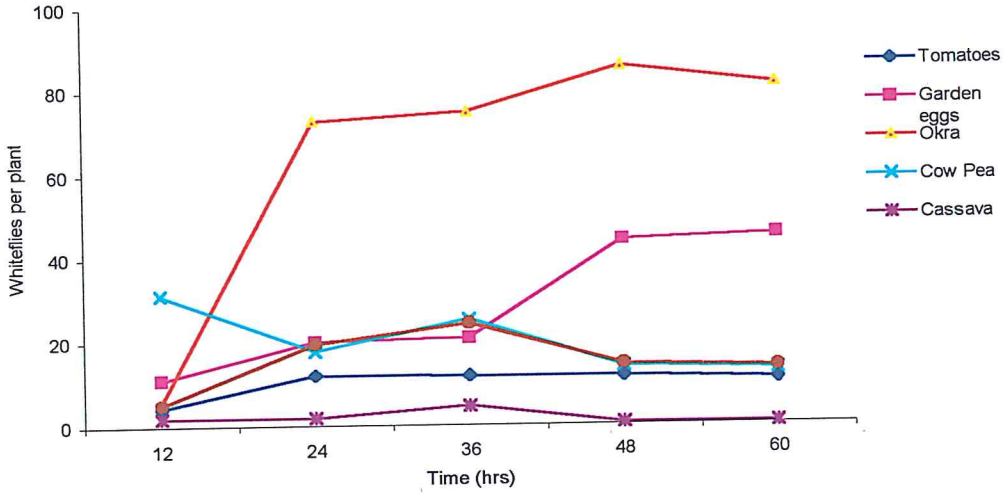


Figure 15: Landing and distribution of *B. tabaci* collected from okra on six hosts within the first 60 hours after release

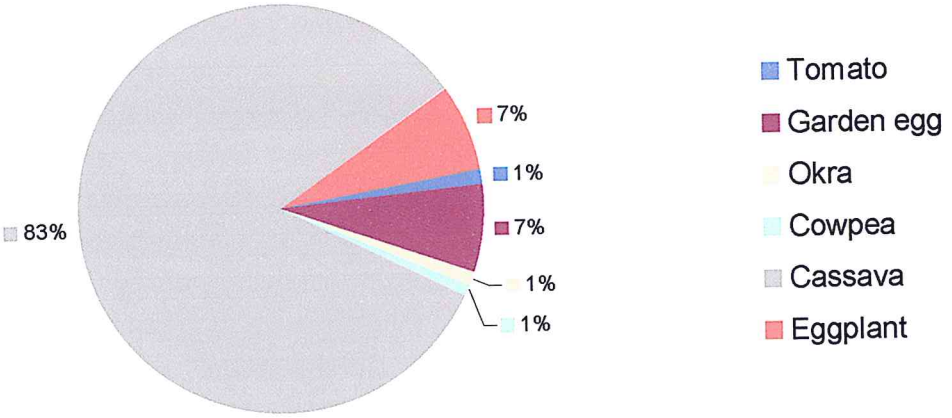


Figure 16: Distribution of *B. tabaci* collected from cassava among six hosts after 60 hours' exposure

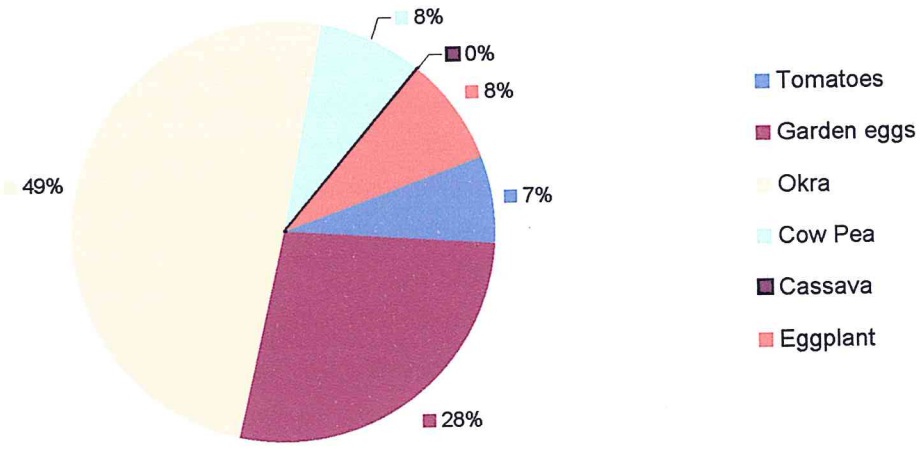


Figure 17: Distribution of *B. tabaci* collected from okra among six hosts after 60 hours' exposure

4.5.2 Multiple choice oviposition-host preference

Both biotypes oviposited significantly more on the preferred discriminant hosts, cassava and okra respectively (Figures 18 and 19). Okra biotype oviposited significantly less on cassava than other hosts even though some settling on cassava had been observed ($F = 3.36$, d.f. $= 5$, $p < 0.026$). Cassava biotype preferred cassava as the oviposition host ($F = 15.66$, d.f. $= 5$, $p < 0.001$) and did not oviposit on okra on which they did not settle either.

4.5.3 Egg distribution within plants

The youngest fully opened leaves of the main hosts of both biotypes were preferred by adults for feeding (settling after 60 hours) and oviposition. Minor and non-hosts had varied distribution, where, a few leaves were preferred. Egg distribution was correlated positively with 60 hour landing and feeding preference. The number of eggs per leaf was highly positively correlated with the 60 hour landing and feeding preference. The relationship is described by the models: cassava: $y = 1.1552x + 0.7578$, $r = 0.70$, and okra: $y = 1.1944x + 0.5720$, $r = 0.67$; $p<0.01$) (Figures 20 and 21).

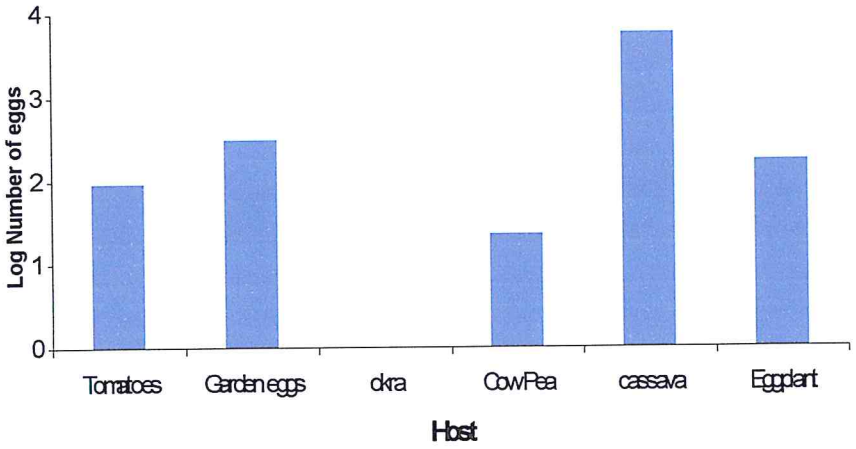


Figure 18: Oviposition preference of the cassava population on six hosts

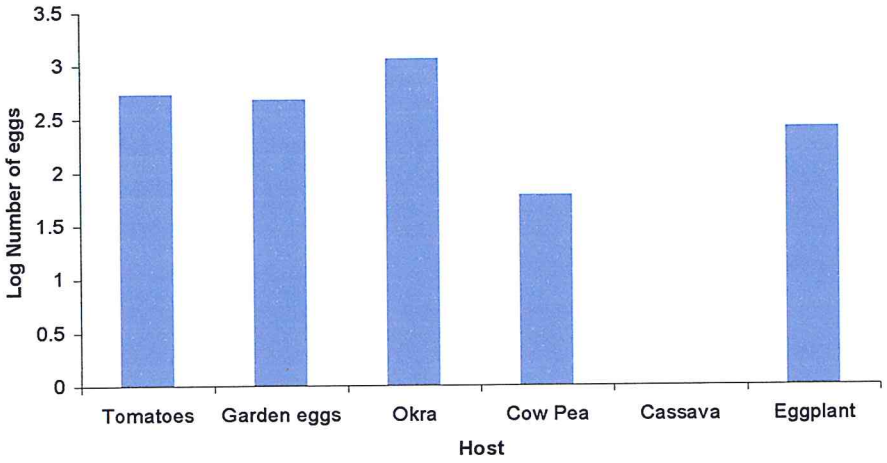


Figure19: Oviposition preference of the okra population on six hosts

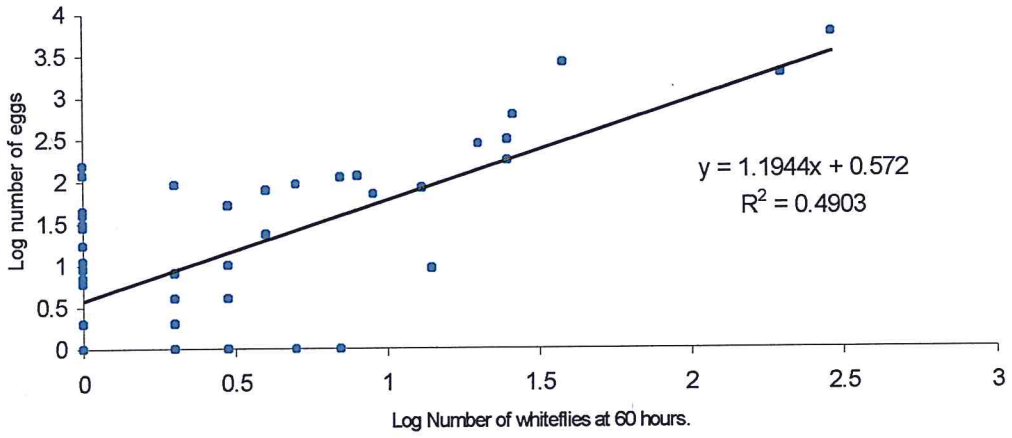


Figure 20: Association between oviposition and 60 hour distribution of the cassava population of *B. tabaci* (r = 0.700, p < 0.01, 2 –tailed)

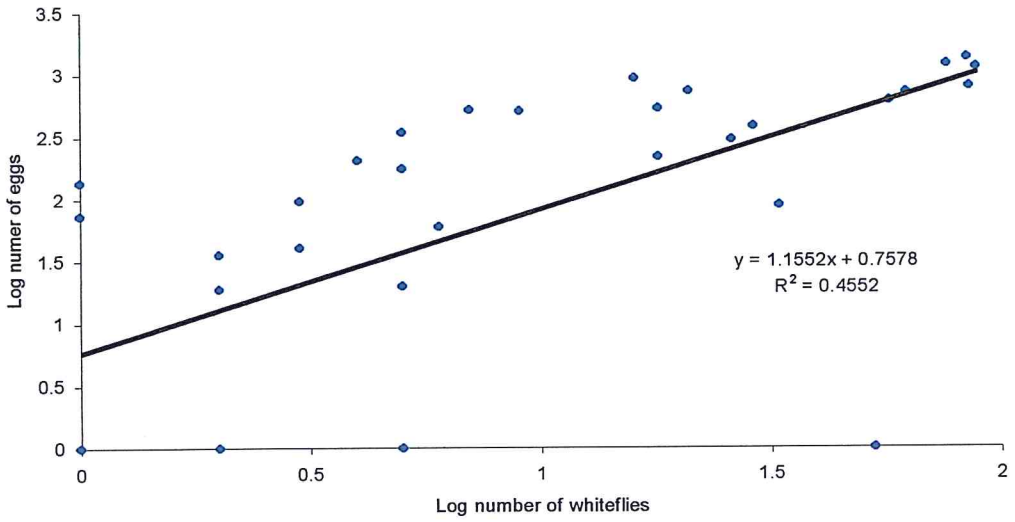


Figure 21: Association between oviposition and 60 hour distribution of the okra population of *B. tabaci.* (r = 0.700, p < 0.01, 2 –tailed)

4.6 Rearing Suitability

4.6.1 Okra whiteflies

The okra biotype developed to maturity on all hosts except cassava producing a bisexual population in each. But cabbage and pepper were minor hosts supporting less than 20% total survival, while the other hosts supported over 50% total survival (Table 21). There was no significant variation in egg hatchability and third instar nymph survival of this biotype on the different hosts. But, they did not survive on cassava beyond the first nymphal instar. Eggplant, okra and cowpea were the most suitable hosts for the survival of this stage, while pepper and cabbage were the least suitable (64.32% survival). Second instar nymphs survived best on eggplant (99.29%), but not significantly better than okra, cowpea and tomato, while pepper was the least suitable.

Pepper and cabbage were significantly less suitable than the other hosts for the development through the quiescent fourth instar nymphs ($F = 8.58$, d.f. $= 6$, $p < 0.001$), but the proportion of eclosed quiescent nymphs was not significantly different between the other hosts ($p > 0.05$). However, total survival through the development stages was highest on eggplant and okra, but okra was not significantly more suitable than cowpea. Pepper and cabbage supported a very low proportion of this biotype through to the adult stage. Similarly, okra whiteflies developed significantly faster on okra than on tomato, sweet pepper and cabbage ($F = 5.82$, d.f. $= 6$, $p < 0.001$).

Table 21. Stage specific survival and host suitability of various hosts to the okra population of *B. tabaci*

Host	% Hatch	1st instar	2nd instar	3rd instar	% Eclosion	% Survival	Development time (T)	Rearing suitability
Okra	93.40 ± 2.10^{a}	96.10 ± 2.09^{d}	96.10 ± 1.68^{cd}	96.21 ± 1.93^{a}	90.32 ± 5.35^{c}	74.91 ± 5.29^{dc}	16.13 ± 0.81	0.116
Garden egg	83.13 ± 1.07^{a}	82.16 ± 8.13^{c}	89.45 ± 3.95^{bc}	96.97 ± 2.48^{a}	90.52 ± 2.80^{bc}	53.48 ± 5.33^{c}	17.68 ± 2.41	0.098
Eggplant	92.46 ± 0.79^{a}	97.55 ± 0.85^{d}	99.29 ± 0.58^{d}	98.07 ± 0.39^{a}	94.83 ± 1.83^{c}	83.31± 2.88^{e}	17.19 ± 1.44	0.112
Cowpea	86.47 ± 6.39^{a}	92.07 ± 3.32^{d}	93.94 ± 3.55^{cd}	92.56 ± 1.57^{a}	92.38 ± 1.54^{c}	64.58 ± 8.87^{cd}	17.63 ± 1.41	0.099
Tomato	86.73 ± 3.17^{a}	82.65 ± 5.32^{c}	93.84 ± 2.70b^{cd}	90.26 ± 3.82^{a}	88.63 ± 1.33^{c}	54.42 ± 7.31^{c}	18.05 ± 1.03	0.096
Cabbage	81.11 ± 3.31^{a}	64.32 ± 8.37^{b}	84.72 ± 2.38^{ab}	85.56 ± 5.75^{a}	50.62 ± 0.62^{a}	19.39 ± 3.45^{b}	18.85 ± 0.99	
Cassava	90.79 ± 4.62^{a}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}	-	-
Pepper	90.00 ± 2.72^{a}	54.73 ± 1.98^{b}	77.57 ± 8.11^{a}	86.96 ± 10.66^{a}	60.92 ± 3.09^{ab}	20.67 ± 4.90^{b}	18.50 ± 1.91	0.071

% Survival: the percentage of eggs developing into adults.

Rearing suitability index: Log S / T (Howe, 1971)

Means within a column followed by the same letter are significantly different at $p<0.05$

Table 22: Stage specific survival and host suitability of various hosts to cassava population of *B. tabaci*

Host	% Hatch	1st instar	2nd instar	3rd instar	% Eclosion	% Survival	Development time (T)	Rearing suitability*
Garden egg	90.99 ± 4.21^{bc}	83.73 ± 1.71^{c}	91.69 ± 1.62^{bc}	91.23 ± 4.95^{b}	88.19 ± 4.79^{a}	55.94 ± 3.62^{cd}	17.79 ± 1.57	0.098
Tomato	78.26 ± 8.76^{a}	57.55 ± 2.51^{b}	84.76 ± 4.27^{b}	64.91 ± 11.03^{a}	82.14 ± 8.99^{a}	20.78 ± 5.45^{b}	17.50 ± 2.38	0.075
Eggplant	83.60 ± 2.95^{b}	94.37 ± 1.22^{d}	96.70 ± 0.31^{cd}	96.54 ± 0.53^{b}	89.36 ± 4.95^{a}	65.67 ± 3.16d^{e}	17.72 ± 1.04	0.103
Okra	60.61 ± 4.29^{a}	4.51 ± 2.51^{a}	0.00 ± 0.00^{a}	-	-	0.00 ± 0.00^{a}	-	-
Cowpea	82.39 ± 4.18^{b}	81.51 ± 2.94^{c}	87.22 ± 5.59^{b}	93.18 ± 1.74^{b}	87.05 ±c 4.13^{a}	47.31 ± 3.70^{c}	17.81 ± 2.02	0.094
Cassava	95.95 ± 2.10^{c}	90.26 ± 1.68^{c}	97.84 ± 0.27^{d}	98.34 ± 0.83^{b}	92.30 ± 2.73^{a}	76.86 ± 2.61^{e}	17.11 ± 1.06	0.110
Cabbage	91.29 ± 1.98^{c}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}	-	-
Pepper	83.75± 6.17^{a}	0.00 ± 0.00^{a}	-	-	-	0.00 ± 0.00^{a}	-	-

% Survival: the percentage of eggs developing into adults

* Rearing suitability index: Log S / T (Howe, 1971)

Means within a column followed by the same letter are significantly different at $p < 0.05$

4.6.2 Cassava whiteflies

The host preference of the cassava biotype for the same eight hosts was significantly different from that of the okra biotype (significant interaction at $p < 0.001$) (Table 22). Pepper and cabbage did not support this biotype beyond the first instar, while the few nymphs surviving on okra (4.51 ± 2.51) all died within the second instar. On these hosts, the motile first instar nymphs were found near the margins of the leaf from which they had emerged away from the centre of the lamina. Egg hatchability varied with the host they were oviposited on ($F = 3.28$, d.f. = 7, $p < 0.01$). Curiously, it was comparatively greater on cabbage, garden egg, sweet pepper and cassava, but was lowest on okra (60.61± 2.95%). Very high mortality was observed on okra at this stage, while cassava and eggplant supported the highest proportion to the next stage. Apart from tomato, cassava biotype third instar nymphs did not have significant rearing preference for the hosts on which they emerged. Similarly, adult eclosion was not significantly variable on different hosts for this biotype at $p<0.05$.

Cassava was the best host for the development of the cassava biotype supporting 76.86% of the oviposited eggs till maturity. This was not significantly higher than on eggplant. No adult emerged from okra while only 20.78% of the eggs completed development on okra, significantly lower than all the hosts. All the hosts supported a bisexual population of whiteflies to eclosion (Table 23).

There was no significant difference in development time of the cassava whiteflies among these hosts. The lowest stage-specific survival was at the first instar nymph. Mortality was also high at the quiescent larval stage. Here, some of the dead nymphs appeared

mummified with a dull brown colour as opposed to the pale yellow colour of normal nymphs.

4.6.3 Rearing suitability index

Development was fastest on cassava and longest on garden egg. Cassava was the most suitable host for the cassava biotype, while tomato had a low index of suitability. The total development time from oviposition to adult eclosion was shortest (but not significantly shorter) on okra, and longest on pepper. The sex ratio on the two preferred hosts (okra and garden egg) favoured males, while females were favoured on the rest of the hosts.

Table 23: Pooled sex ratios of first generation of cassava and okra populations of *B. tabaci* reared on various hosts

	Sex ratio (mean ± s.e.)	
Host	**Okra race**	**Cassava race**
Okra	0.41	-
Garden egg	0.59	0.67
Eggplant	0.47	0.60
Cowpea	0.59	0.61
Tomato	0.67	0.58
Pepper	0.4	-
Cassava	-	0.55
cabbage	0.5	-

4.7 Toxicological Comparison of the Two Host Races of *B. tabaci*

Response patterns varied between populations in different locations, between biotypes and also between toxicants (Tables 24 and 25). The cassava biotype was significantly more susceptible than the okra biotype in all sites except for chlorpyrifos at Sinna's garden. At this site although the 95% fiducial limits overlapped, the okra biotype was still over ten-fold more tolerant than the cassava biotype to chlorpyrifos. The cassava population from Pokuase was the most susceptible to chlorpyrifos while the cassava population from the University farm was most susceptible to λ-cyhalothrin. The okra biotypes were at least 4.7 times more tolerant to λ-cyhalothrin and six fold more tolerant to chlorpyrifos than cassava biotypes.

All populations were relatively heterogeneous as indicated by the low slope of their dose response curves (less than 1.00). Okra biotypes generally displayed greater heterogeneity than the cassava biotypes even though the latter were generally less tolerant to the two toxicants. However differences within the same biotype were not highly significant even between locations as indicated in the overlapping of their 95% fiducial limits.

Table 24: Response of three populations of two *B. tabaci* biotypes to λ-cyhalothrin

Population	Host	LC_{50}	LFL	UFL	Slope ± se	RF_{50}
Sinna's Garden	Cassava	31.962	20.894	47.843	0.67±0.05	1.214
	Okra	222.868	144.011	341.825	1.06±0.05	8.465
Farm	Cassava	26.328	17.770	38.240	0.70±0.07	1.000
	Okra	125.719	83.785	188.152	0.85±0.08	4.775
Pokuase	Cassava	34.977	14.081	87.166	0.73±0.05	1.329
	Okra	231.131	147.128	358.153	0.64±0.06	8.779

LC_{50} between populations significantly different if their 95% fiducial limits do not overlap.

Table 25: Response of three populations of two *B. tabaci* biotypes to chlorpyrifos

Population	Host	LC_{50}	LFL	UFL	Slope ± se	RF_{50}.
Sinna	Cassava	1.78	0.39	4.71	0.53±0.04	1.23
	Okra	21.68	1.58	88.30	0.63±0.06	15.02
Farm	Cassava	1.52	0.67	3.31	0.55±0.04	1.05
	Okra	17.14	11.50	25.19	0.77±0.11	11.87
Pokuase	Cassava	1.44	0.62	3.22	0.55±0.04	1.00
	Okra	8.68	5.30	13.81	0.79±0.06	6.02

LC_{50} between populations significantly different if their 95% fiducial limits do not overlap.

4.8 Mating Interactions Between Cassava and Okra Biotypes of *B. tabaci*

4.8.1 Female reproductive performance

Table 26 summarises the population parameters as a result of the reciprocal crossing of the two biotypes of *B. tabaci.* Fecundity varied from 6.67 eggs per female per day in cassava biotype females mated to okra biotype males to 11.75 eggs per female per day in unmated okra biotype females.

There was no significant difference between the fecundity of the female whiteflies of the cassava biotype whether mated by okra or cassava males. Similarly, fecundity of mated and unmated cassava biotype females was not significantly different. Unmated okra biotype females were significantly more fecund than the mated okra biotype females. However the oviposition rate did not vary significantly between okra biotype females mated with males of either biotype. Oviposition rates of females of the two biotypes unmated or mated with males of the same or different biotype were not significantly different.

4.8.2 Survival

A significantly higher portion of eggs laid by mated females of the okra biotype hatched compared to those of the unmated females of the same biotype ($F = 0.022$, d.f. $= 5$, $p < 0.05$). However, the difference between the hatchability of eggs oviposited by females mated by males of the other biotype was not significant. The hatching rates of eggs oviposited by cassava biotype females were not significantly different whether the females were unmated or mated with males of either biotype. Eggs laid by the unmated cassava biotype females were more viable than those of unmated okra biotype females were.

The progeny of unmated females of the okra biotype females survived significantly less than those of cross-mated okra biotype females, but not differently from those of line-bred okra females (Table 26). This was also less than the survival rates of progeny of unmated cassava biotype females. Conversely, cohort survival did not vary significantly among the offspring of the cassava biotype females irrespective of their mating system.

Larval survival was measured as the proportion of the first instar nymphs undergoing successful eclosion (Table 26). In the okra biotype, it was significantly higher when females were line-mated that when cross-mated or unmated. But the survival of the progeny of unmated okra biotype females was not significantly different from that of the progeny of cross-mated females. Similarly, the larval survival of the progeny of the cassava biotype females did not vary significantly with the mating status or mate biotype paired with the female parent. Larval survival was significantly higher among the progeny of unmated cassava biotype females than those of the okra biotype.

4.8.3 Sex ratios

The sex ratios of the progeny from the various crossing plans, expressed as the proportion of females is summarised on Table 27. Line-bred females gave rise to significantly higher proportions of females than crossbred females of either biotype ($F = 104.29$, d.f. $= 5$, $p < 0.001$). Over half of these progeny were females. But there was no significant difference between the sex ratio of progeny from the two cross mating plans both of which were male dominated. Both negative controls of unmated females gave rise to males only.

Table 26: Effect of biotype interbreeding on population growth parameters

Cross	Fecundity	Hatch rate	Total survival	Larval survival
Okra♂ x Cassava♀	6.67 ± 0.81^{a}	83.47 ± 2.92^{b}	70.78 ± 2.89^{b}	85.08 ± 2.28^{ab}
Cassava♂ x Okra♀	8.72 ± 0.75^{ab}	73.11 ± 5.28^{b}	63.18 ± 5.96^{b}	84.97 ± 3.48^{ab}
Okra♂ x Okra♀	9.46 ± 1.35^{ab}	74.39 ± 10.19^{b}	58.36 ± 14.31^{ab}	98.68 ± 8.42^{c}
Cassava♂ x Cassava♀	9.56 ± 1.55^{ab}	76.51 ± 4.78^{b}	65.40 ± 4.50^{b}	86.24 ± 4.38^{abc}
Cassava♀ unmated	8.64 ± 1.50^{ab}	76.32 ± 5.93^{b}	66.96 ± 4.87^{b}	88.09 ± 1.91^{abc}
Okra♀ unmated	11.75 ± 1.49^{b}	56.55 ± 6.64^{a}	41.04 ± 2.80^{a}	76.17 ± 6.21^{a}

Means within a column followed by the same letter are not significantly different $p < 0.05$

Table 27: Sex ratios of offspring from cassava and okra biotype reciprocal crosses

Cross	Total counted	Mean sex ratio* ± se
Cassava♂ x Cassava♀	84	0.681 ± 0.019^{c}
Okra♂ x Okra♀	179	0.679 ± 0.047^{c}
Cassava♂ x Okra♀	574	0.275 ± 0.029^{b}
Okra♂ x Cassava♀	207	0.324 ± 0.026^{b}
Okra♀ Unmated	239	0.000^{a}
Cassava♀ Unmated	155	0.000^{a}

*Sex ratio: proportion of females. Means followed by the same letter are statistically significant at $p < 0.05$

4.8.4 Fertility of F1 crosses

The results of morphometric measurements of three body parts of female progeny from two cross-mated and two line-mated plans studied are shown on Table 28. Female offspring from all the four possible crosses were not significantly different in size with respect to the abdominal length, width and length of the hind tibia. The shape of the abdomen as inferred from an abdominal ratio was also not significantly different among the various female progeny.

The number of eggs oviposited by two batches of females each containing three whiteflies is shown on Table 29. Both the line bred and crossbred females oviposited at nearly the same rate, and the females were therefore assumed to be fertile. Statistical comparisons were not made owing to the number of F1 females available for this test.

Table 28: Morphometric inference of the fertility of F_1 females

Cross	Tibia	Abdomen Length	Abdomen width	Abdominal ratio
Okra♂ x Okra♀	333.06 ± 1.04[a]	406.18 ± 6.23[a]	256.96 ± 2.61[a]	1.581 ± 0.020[a]
Cassava♂ x Cassava♀	333.45 ± 2.00[a]	414.56 ± 7.09[a]	262.94 ± 3.66[a]	1.579 ± 0.035[a]
Okra♂ x Cassava♀	333.63 ± 1.53[a]	412.06 ± 8.77[a]	265.52 ± 9.64[a]	1.559 ± 0.039[a]
Cassava♂ x Okra♀	333.06 ± 1.16[a]	405.38 ± 2.59[a]	256.56 ± 2.66[a]	1.582 ± 0.023[a]

All measurements are in micrometres

Means in the same column followed by the same letter are not significantly different ($p<0.05$)

Abdominal ratio: length of abdomen / width of abdomen

Table 29: Six-day oviposition by F_1 females

Cross	Rep	Day 1 to 4	Days 5 – 6	Total	Eggs/female/day
Okra♂ x Okra♀	1	101	46	157	8.72
	2	104	51	255	14.17
Cassava♂ x Cassava♀	1	135	31	167	9.28
	2	98	28	126	7.00
Okra♂ x Cassava♀	1	160	11	171	9.50
	2	122	11	133	7.39
Cassava♂ x Okra♀	1	106	23	129	7.17
	2	96	19	115	6.39

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION.

These results confirm the presence of two morphologically similar populations of *B. tabaci* delineated by their phytophagic preferences, associated with the four crops. The first is oligophagous preferring cassava while the second is polyphagous but does not infest cassava. The two races may attack garden egg, eggplant, tomato and cowpea. This largely confirms the findings of Burban *et al.* (1992) and Gadelseed (2000), who used host transfer experiments, population parameters, and enzyme electrophoresis to identify cassava and non-cassava (okra) biotypes in Ivory Coast and Ghana, respectively. In Uganda, Legg (1994) observed that the cassava biotype was restricted to cassava.

Morphological and morphometric studies

Morphological and morphometric studies did not reveal distinct differences between the host populations or the two biotypes. While parsimony analysis yielded polytomies under three different algorithms, cluster analysis tended to group a proportion of the whiteflies under the various hosts. A few phylograms concurred with the classification inferred from behavioural studies, clustering cassava biotype away from the other three populations, or cassava away from okra with tomato and garden egg populations in between (Appendix III). These could have been a result of features such as size, setal space and length, which are amenable to leaf morphology (Azab *et al.*, 1969; Mohanty and Basu, 1986; Martin, 1987; Rosell *et al.*, 1997; Guershon and Gerling, 2001).

Potential diagnostic features for the two biotypes either at adult and last nymphal stadium were not revealed. But classification of larvae had less overlap than that of female or male imagoes. The size of the caudal and thoracic wax margins was noted to be variable. On cassava, and on nymphs developing on okra cotyledons, the two structures were barely visible on most individuals. Morphological differences have been discovered between A and B biotypes (Perring *et al.,* 1993). Therefore, a detailed study of the morphology of the pupal exuvium, which has been used to distinguish biotypes of *B. tabaci* (Rosell *et al.,* 1997), might be desirable in this case.

Molecular characterisation

RAPD PCR analysis is associated with inherent problems of low reproducibility of results and extreme sensitivity to contamination and changes in reaction conditions (Gawel and Bartlett, 1993; Aman, 1995; Guirao *et al.,* 1997). Measures were taken to minimise systematic error such as using predispensed beads from a single supplier, careful primer screening, and optimisation of the amplification conditions, replication and scoring of only authentic bands. Still, an audit analysis by POPGENE revealed five bands that were highly associated with reaction batches in the very low (< 500 bp) and very high (> 1800 bp) fragment size regions. These could have arisen by chance, but could indicate the essence of optimisation in such reactions. Regardless, the results were consistent between primers and with behavioural studies.

RAPD PCR analysis revealed two distinct genetic clusters, one associated with cassava and the other with okra. The two had genetic similarity of 45%, comparable to that between the Spanish S and Q biotypes (Guirao *et al.,* 1997) but much lower than the 10% reported between A and B biotypes (Gawel and Bartlett, 1993). The genetic similarity

observed by this method depends partly on the phylogenetic relationship of the two populations and limited by the number of the diagnostic loci scored. Assuming indiscriminate sampling of the genome by the arbitrary primers and considering the consistent level of genetic diversity revealed by each of the primers, in this work, the influence of the number of loci seems to be a chance effect that refines rather than shifts the observable diversity. Lima *et al.* (2000), for example, used five random primers amplifying 79 loci to survey the genetic diversity of *B. tabaci* in Brazil, on a much wider scope than that of this study.

Cassava populations were less polymorphic than the okra populations. The latter has a wider host spectrum, and could represent a group of highly subdivided distinct populations. Wider host range may indicate greater ecological adaptation, while higher gene diversity implies greater ancestry (Moya *et al.,* 2001). This suggests that the introduction of the okra biotype could have preceded that of the cassava biotype in this region, or that it had been well adapted to these hosts elsewhere, before introduction of both locally. Phylogenetically, the okra biotype is close to the Spanish S and the polyphagous B biotype, while the cassava biotype clusters with the Ugandan cassava and the Spanish Q biotypes (Cervera *et al.,* 2000), which show similar host spectra.

Pokuase populations showed the lowest level of polymorphism, but not necessarily the lowest gene diversity possibly an effect of the smaller sample sizes (four individuals per host) in this location relative to the rest (eight individuals per host per location). This depicts the potential of RAPD markers to reveal population genetic parameters even with small sample sizes especially for heterogeneous populations. De Barro *et al.* (2000) screened four individuals per sampling site to analyse the diversity of Australian

populations of *B. tabaci*. Although large samples are likely to be more representative, the cost and statistically effective sample size needs to be determined objectively for each population owing to the potential for wide gene diversity.

AMOVA analysis showed that the variance component attributed to biotype differences (Φ_{ST} = 0.173, p< 0.001) and to differences among individuals within populations (Φ_{ST} = 0.199, p< 0.001) were significant while there was no significant contribution of differences between populations within biotypes (Φ_{ST} = -0.034, ns). The percentage variance component of each source of variation is 19.95% (biotypes); -2.69% (populations within biotypes) and 82.74% within populations. This structure is corroborated by the estimated gene flow index. The negative contribution (negative variance) is likely due to closer similarity between some individuals from different locations than they are to other individuals from their own population (Excoffier *et al.*, 1992). Negative F_{ST} values between pairs of populations within biotype; and F_{ST} values of above 0.05 between populations in the same biotype indicate heterozygote excess and deficiency respectively. Thus, geographical isolation did not have a significant contribution to the observed variability. This might be a consequence of migration and extensive dispersal of the two biotypes within the sampling range and the proximity of the sites. It would be interesting to study genetic diversity within each biotype from widely separated locations in Ghana.

Host selection behaviour

Cage oviposition tests revealed significant preference of most whitefly populations for the hosts on which they were collected, or to either of the two discriminant hosts - okra and cassava. However, reaction of both biotypes to tomato and garden egg depended on the other choice in the pair. For example, tomato was preferred by okra whiteflies when paired

with cassava but not when paired with garden egg. Such mixed response may indicate a mixture of the two biotypes on these crops in the field. Biotypes of *B. tabaci* have also been shown to coexist on the same hosts, but competition soon sets in (Burban *et al.*, 1992; Brown *et al.*, 1995; Guirao *et al.*, 1997). Burban *et al.* (1992) also observed that both biotypes reared together on eggplant separated when presented both hosts. The preference of each biotype for its main host is therefore genetically predetermined. The attraction of nearly equal proportions of the tomato whiteflies to okra when paired with cassava as to tomato when paired with okra seems to support this hypothesis.

The oviposition experiments described here lasted 24 hours, while polyphagous whiteflies need at least 72 hours to make final preference choices (Calvitti and Remotti, 1998). Therefore, quick recognition by morphological and olfactory cues is a more important determinant of host choice than exploratory feeding assessment of hosts in. Accordingly, greater oviposition would be expected on hosts that are more attractive for landing and initial feeding than those suitable for long-term survival. This possibly explains the observation of more oviposition by whiteflies on the non-preferred hosts in petri-dish cage tests as than in the three-day exposure in a field cage. Regardless, the cassava and okra populations showed clear response that seems to characterise them.

Insects are capable of learning their food types and experience is a factor in feeding preferences, which may modify immediate recognition of a substrate (Kwapong, 2003). Both prior experience and semiochemicals sequestered as juveniles may be involved in host preference as adults (Bernays, 1995; Kwapong, 1998; Goode, 2000). For example, Wickremasinghe and van Emden (1992) for instance discovered that *Aphidus rhopalosiphi* De Stefani Perez preferred to alight and remain on the same wheat cultivar as that from

which they emerged. Similarly, the parasitoid *Diadegma semiclausum* (Hellen) is attracted to host plants on which their oligophagous hosts *Plutella xylostella* L. were reared (Kwapong, 2003). Prior exposure might have played a role in *B. tabaci* oviposition host choices since adult insects were used in this study. The role of the rearing host on eventual adult behaviour could not be tested in this experiment. This would need the use of newly emerged adults from various hosts. Nevertheless, testing by exploratory probing may also be in play; hence, in the absence of known (previous) hosts, whiteflies would settle and oviposit on acceptable feeding hosts (Bernays, 1999; Muniz, 2000). The effect of rearing host of these biotypes on the subsequent host selection and other population parameters should be investigated to facilitate understanding of the effect of seasonal agro-ecological transition on these whitefly populations.

Ovipositional preference predominantly favoured the lower leaf surfaces, but the strength of this preference varied and was in a quarter of the instances insignificant. Preferences for the lower leaf surfaces is most likely a result of morphological features but may not fully explain the usually observed aggregation on and preference for the lower leaf surfaces for oviposition (Simmons, 1994; Gadelseed, 2000). The latter could partly be explained by the negative geotropic response of adult whiteflies influencing their feeding and oviposition site selection. Insignificant preference of the cassava, tomato and garden egg populations for the upper or lower leaf surface of garden egg is attributable to the little difference in the leaf surface structure compounded by the removal of the geotropic effect in this assay, since all leaves were turned downward. This is consistent with the observation of Simmons (1994).

Okra and cassava populations were significantly attracted to the lower surfaces of their rearing hosts. Physical characteristics of the leaf surface such as hairiness, shape, presence of sticky glandular trichomes and leaf microclimate influence the acceptability of these leaves for oviposition (Berlinger, 1986). Tomentosity has been positively correlated with oviposition and feeding (Bernays, 1995). The four hosts used in this study varied in their leaf morphology. Cassava is glabrous on either leaf surface, while garden egg is pubescent on both surfaces. Okra and tomato vary significantly in leaf hairiness on the two surfaces. Yet, most populations did not significantly prefer either surface of tomato leaves, and both cassava and okra populations preferred the lower leaf surfaces of their non-host plants, okra and cassava respectively. Therefore, reaction to host morphology does not seem to be variable between the different populations of whiteflies examined.

In the multiple-choice landing assay, adult *B. tabaci* seemed to make final host choices gradually and were randomly distributed among the hosts within the first 24 hours after landing. The cassava biotype settled faster on its preferred host compared to the okra population. The highest preference for cassava and okra by each biotype, respectively, may have been strengthened by acclimatisation to rearing hosts, but the sharp rejection of each other's preferred host is perhaps as a result of their poor host suitability. A higher proportion of the okra population accepted the other hosts compared to the cassava biotype. Both Burban *et al.* (1992) and Gadelseed (2000) observed similar host ranges, and therefore described them as monophagous cassava and polyphagous okra (or non-cassava) biotypes.

The oviposition pattern was concordant with the feeding preference of the two biotypes (cassava: $r = 0.70$, okra = 0.675; $p < 0.01$). It is likely that females oviposited more where

they preferred to feed. Similar preference has been observed in the A biotype (Costa *et al.*, 1991) while the B biotype prefers to oviposit on the hosts on which they were reared (Brown *et al.*, 1995). However, Bernays (1998) observed that females might prefer to feed on younger leaves with less nutritional reward than older leaves, as they are more suitable oviposition sites. Either way, it is unlikely that females would prefer one plant for oviposition and quite another for feeding. Oviposition acceptance is therefore a good indicator of host acceptance in choice assays.

Host suitability

In both biotypes, rearing suitability was not directly related to female oviposition or landing preference. Eggplant, for instance, supported 83.5% larval growth compared to cassava (74.9%), but was not a favoured landing host (7%). Gadelseed (2000) observed above 95% mortality of the cassava biotype clipped on tomato while Burban *et al.* (1992) did not find the cassava esterase pattern in tomato whiteflies after an extensive survey in the Ivory Coast. Tomato was, therefore, classified as non-host of the cassava biotype. In this study, it was a poor landing choice, but supported about 50% total survival producing a bisexual population, which is indicative of a host plant (Burban *et al.*, 1992). This apparent contradiction might have been a combined effect of host-acclimatisation, and the cost of host switching and distraction from other choices available. Tomato was obviously less acceptable in the presence of better feeding hosts, and either was not explored or was equally unacceptable for oviposition.

Perhaps, the effect of acclimatisation to the immediate former hosts influenced the acceptability of tomato while it remained marginally suitable for larval development. In this study, tomato harboured a mixture of the two biotypes as indicated by RAPD PCR in

this study. Exploratory landing on a non-host was suspected (J. L. Cenis, personal communication). Reaction to a host may be influenced by the options equally perceived and to physiological conditions such as egg (Miller and Stickler, 1984; Minkenberg *et al.*, 1992; Bernays, 1999). Since tomato is usually grown under irrigation throughout the year in many areas, it is likely that a no-option situation arise in the off-season triggering its role as a host. It would therefore, be more informative to investigate the effect of rearing the cassava whiteflies on tomato on their eventual adult performance.

Strong rejection of the two populations of each other's main host has been consistently demonstrated, confirming the observations of Burban *et al.* (1992) and Gadelseed (2000) using host transfer experiments, allozyme markers and PCR. These hosts can, therefore, serve as useful discriminant hosts in behavioural characterisation and screening of populations involving the two biotypes. Consequently, the ability to colonise the two hosts is proposed as a diagnostic assay analogous to the squash silver leaf bioassay which is widely used to detect the presence of the B biotype (Costa *et al.*, 1993; Guirao *et al.*, 1997; Moya *et al.*, 2001).

Potentially informative inferences on the whitefly bionomics were made. The most significant mortality factor acted at the first nymphal instar in both biotypes, confirming the findings of Drost *et al.* (1998) and Thompson (2000). This is the first feeding instar and the only most mobile, hence environmental and host suitability effects are likely to eliminate less viable individuals leading to reduced mortality in the subsequent stages. Mobility increases spatial exposure to risk factors (Guershon and Gerling, 2001) and coupled with physiological transformation is likely to reduce survival at this stadium.

Mortality at the last nymphal instar was more significant in the okra biotype. Mummification and mouldiness of the pupae were observed, perhaps caused by some pathogen. Black and white moulds were also observed in the cross mating tests at this stage. Further, because parasitoids are most likely to emerge at this instar, it is possible that one or a combination of these factors is more active in the okra populations. It would therefore be useful to investigate their role in whitefly population dynamics and as a potential for some control mechanism.

Pepper and cabbage were only marginal rearing hosts of the okra whitefly, but heavy infestation by adult whiteflies has been observed in the field. This discrepancy may be indicative of the cost of host switching from okra to cabbage. Subsequent acclimatisation or specialisation of a sub-group may make it a competent host (Bernays, 1999) Alternatively, the population may be a new biotype not attacking cassava or okra at the moment. The latter hypothesis perhaps explains the observation of very high populations of *B. tabaci* on an ornamental plant, *Jatropha gossypifolia* at Pokuase, adjacent to an okra garden that was hardly infested at all. Gadelseed (2000) concluded that the *Jatropha* population belongs to the non-cassava biotype. However, a nearly monophagous *Jatropha* biotype has been described from Benin using allozyme electrophoresis (Costa *et al.,* 1993; Brown *et al.,* 1995). Infestation of non-regular hosts has been the first indication of invasion by a new biotype worldwide (Perring *et al.,* 1993; Bellows *et al.,* 1994; De Barro *et al.*, 1998).

All three solanaceous plants used in this study were satisfactory hosts of the two biotypes as was cowpea. Solanaceous weeds have been implicated as alternative hosts of *B. tabaci* and viral inoculum reservoir (Muniz *et al.,* 2000). The role of this plant family in the *B.*

tabaci host-virus complex should therefore be investigated further. Weeds like *Amaranthus* spp. and *Commelina* sp. harboured all stages of *B. tabaci* adjacent to both cassava and okra fields. It would be interesting to investigate the role of weeds as alternate hosts for the two biotypes.

Response to insecticides

The toxicological tests revealed clear difference between the two host populations, although great variation within similar populations was also observed in different locations. The okra biotype was significantly more tolerant to the two toxicants in two locations, but several times more tolerant in all locations. Whiteflies from Sinna's garden were the most tolerant. This garden has a history of continuous cropping with rotation of various whitefly hosts (garden egg, tomato, cassava, okra, cabbage, cucumber, Chinese cabbage and lettuce) with heavy insecticide use. Cymethoate (pyrethroid and organophosphate cocktail), cyhalothrin, chlorpyrifos, cypermethrin, and *Bacillus thurigiensis* are most frequently used. Spraying pressure reaches fortnightly depending on the value of the crop.

Continuous breeding of a resident population and heavy insecticide use predispose the population to great resistance development (Cheng, 1981; Tabashnik *et al.,* 1987), which could be worsened by limited introgression from non-resistant populations (Caprio and Tabashnik, 1992). As cassava and okra gardens were within flying reach of whiteflies, possibility of gene flow or adult migration between plots is likely to result in elevated rate of insecticide resistance in the untreated crop, and a reduction in the treated population Mohan and Gujar (2003). The populations are, however, quite heterogeneous as indicated by the low slopes of the dose response curves. Perhaps, heterogeneity is maintained by

immigration from other untreated hosts such as adjacent weeds and exposure of the cassava biotype to toxicants on shared vegetable hosts.

Pesticide isolation in different host populations, associated with host specialisation of a polyphagous species may set such populations in different evolutionary courses under continuous cropping systems (Owusu *et al.,* 1996). Distinct toxicological differences may illustrate ecological and genetic isolation of the two biotypes, leading to a likelihood of different rates of development of resistance. Since pesticide response is likely to shift in a cropping season, continuous surveillance might be necessary to eliminate seasonal effects of population dynamics on the response seen.

Mating interactions

The results of the mating experiments and inference of gene flow from molecular data support the view that some biological isolation is operating between different biotypes of *B. tabaci.* Burban *et al.* (1992) and Gadelseed (2000) hypothesised that hybridisation could be possible between the okra and cassava biotypes on shared hosts. Apparently, adult males and females from the two biotypes did not recognise the difference and entered into courtship resulting in successful copulation and fertilisation. Interbreeding has been observed between the B and Q biotypes (Ronda *et al.,* 1999, 2000) and between B and Australian biotypes (De Barro and Hart, 2000).

Inter-biotype mating did not affect the fecundity of adult females, hatching rate or total survival of the progeny (Table 26). This could be interpreted by two hypotheses. First, it is possible that mating was normal so the adults did not spend extra time courting. As courting females neither feed nor oviposit (De Barro and Hart, 2000), absence of the male

distraction enabled normal oviposition. Alternatively, as sex ratios in *B. tabaci* may be influenced by the frequency of mating (Horowitz and Gerling, 1992), reduced mating between the two biotypes may have resulted in limited gamete transfer in the first place. On the other hand, courting and mating though complete may have resulted in unsuccessful fertilisation of a proportion of the ova rather than the production of non viable zygotes. This is a theoretical possibility and has not been reported in this species.

Fewer progeny from cross-mated okra whitefly females survived compared to the pure bred progeny (Table 26). This was not observed in the cassava biotype, although the females laid fewer eggs than their unmated or line mated counterparts. The reduced survival of crosses of the okra females suggests reduced fitness of hybrid individuals, which though hatched, could not develop into adults. There was also lower viability of the progeny of unmated okra biotype females and this could mean that greater death of male progeny during development might have occurred. In cassava biotype female crosses, this isolation could be operating at pre-oviposition stage resulting in reduced oviposition and hence hatching of principally male individuals. With fewer eggs, a reduction in larval survival may not be easily detectable.

B. tabaci is arrhenotokous hence the presence of female progeny in both reciprocal crosses and the absence of females in the progeny of virgin females is evidence of inter biotype fertilisation taking place (Costa *et al.,* 1993; De Barro and Hart, 2000). That these females were of normal size and shape of abdomen; and were capable of oviposition points to the comparable fitness of these individuals and perhaps fertility. Even so, a reduction in the proportion of females observed in inter-biotype progeny is indicative of some degree of reproductive isolation possibly at either or both pre-mating and post-mating stages. Pre-

mating isolation resulting in reduced transfer of sperm invariably leads to lower levels of fertilisation. Post mating isolation by genetic incompatibility of a high proportion of ova and sperm (possibly due to incompatibility of alleles or some mutual recognition factor) is likely to lead to the same effect. In the former case, fertilised eggs are likely to be normal if genetic isolation does not result in inferior individuals.

A possible cause of the interaction observed might be cytoplasmic incompatibility caused by infections by endosymbiotic proteobacteria like *Wolbachia* (De Barro and Hart, 2000). These manipulate arthropod host reproduction causing partial or complete reproductive isolation between species and populations. They may also cause selective death of male embryos, thelytokous parthenogenesis induction and feminisation (De Barro and Hart, 2000; Zchori-Fein *et al.*, 2001; Beitia *et al.*, 2003). Zchori-Fein and Brown, (2002) and Brown *et al.* (2003) have revealed, by PCR and sequencing of *Wolbachia* –specific 16S rDNA, that at least one third of populations of *B. tabaci* from all over the world harbour *Wolbachia.* Beitia *et al.* (2003) isolated *Wolbachia* from the S biotype and implicated it in the mating incompatibility between single crosses of S, Q and B biotypes. Mating between incompatible individuals in this case would lead to the production of males.

If this mechanism is operating between these populations, then it is bi-directional and partial hence the reduction in female progeny in both reciprocal crosses. Since the F_1 females were presumably fertile, such incompatibility seems to occur without concomitant genetic incompatibility between these biotypes. Possibly, the mechanism is a recent development and reinforced by ecological specialisation.

De Barro and Hart (2000) reported production of hybrids (about 30% females) between two Australasian biotypes, as in this study, but only 15% females in crosses between the B biotype and the Australian biotypes. Before then, no report of successful inter-biotype hybridisation had been made despite several attempts (Perring *et al.,* 1993; Bellows *et al.,* 1994; Ronda *et al.,* 2000). However, no study known to date has produced potentially laying F_1 females in cross biotype mating of *B. tabaci*. Yet, the ability of the F_1 females to oviposit does not necessarily imply the ability to give rise to viable adults. Therefore, production of a bisexual F_2 population from backcrosses of the two populations is necessary to ascertain sustainable compatibility (Maruthi, 2001).

The existence of crosses of these biotypes in the field had been suspected by Gadelseed (2000), who noted two intermediate RAPD patterns of whiteflies from garden egg. This study confirms this possibility, but cannot ascertain whether crossing occurs in the field. In fact, the estimate of gene flow based on Nei (1978) index was much higher among host types within the same location (Nm = 23.318) than between host races (Nm = 1.342) or between spatially isolated populations of the same host race (Nm = 2.5426). Similarly, biological isolation has been demonstrated between several populations of *B. tabaci* (Costa *et al.,* 1993; Perring *et al.,* 1993; De Barro and Hart, 2000).

Clear ecological isolation by contrasting host preference may preclude cross biotype mating and buttress other isolating mechanisms. Further, the cage mating performed here had the dual effect of increasing proximity of potential mates and minimising competition, as opposed to field situations. These may further reduce the possibility of cross biotype mating. In fact, a small number of crosses has been produced in laboratory conditions between B and Q biotypes (Ronda *et al.,* 1999, 2000) and between A and B biotypes

(Costa *et al.,* 1993) while molecular evidence showed otherwise. The occurrence of such crossing in the field should therefore be investigated. Although F_1 females of both reciprocal crosses oviposited, total fertility through egg hatchability and survival of progeny needs to be studied further.

5.2 CONCLUSION

These results confirm the existence of two biotypes of *B. tabaci* infesting cassava, garden egg, okra and tomato. The two biotypes vary significantly in their host selection behaviour and host preference, and are at least reproductively isolated. But they are not readily morphologically distinguishable as nymphs and as adults. Random primers (OPA 02, OPB 08, OPC 06, OPD 16 and OPI 16) can be used to readily distinguish the two biotypes using RAPD PCR.

The etymology of these biotypes presently in use is contestable. The cassava –loving biotype has been called the cassava biotype (Burban *et al.*, 1992; Gadelseed, 2000) monophagous biotype (Cenis, personal communication), while the okra-loving biotype has been called okra biotype (Burban *et al.*, 1992), non-cassava biotype (Gadelseed, 2000) or the polyphagous biotype (Cenis, personal communication). A connotation of monophagy implied by the names may be misleading, as the host range has not been fully studied. The name non-cassava biotype (Gadelseed, 2000) is much more descriptive, but duplicates a similar name used to refer to a polyphagous biotype in Brazil that does not infest cassava. Similarly, names used for the other biotype are not informative enough. Because consistent differences have been observed using a variety of approaches, and the said hosts could perhaps support other biotypes not yet studied or introduced locally, it is suggested

that these biotypes be identified properly as the other biotypes worldwide perhaps with alphabetical letter names.

One *Bemisia* - like whitefly was observed on cassava in Pokuase and Sinna's garden. It is a little larger than *B. tabaci* and orients its wings roof-like over the body. A similar whitefly was observed in very low numbers on okra at Sinna's garden. It was a little larger than *B. tabaci* but had a brownish thorax but the nymphs were not seen. The first was described as *Bemisia* near *afer,* based on the pupal morphology, but the second was not studied. The whiteflies need to be identified and described properly and their ecological interactions ascertained.

Also, the emergence of whitefly infestation on cabbage should be investigated. Fungal mould observed at the pupal stage associated with high mortality is perhaps a potential biological control agent. Further, the role of solanaceaeous plants in carry over infestation of *B. tabaci* between seasons needs to be investigated, or their role in possible resistance spill over to untreated hosts like cassava.

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APPENDICES

APPENDIX I: PREPARATION OF STOCK SOLUTIONS

The following stock solutions were prepared according to protocols in Sambrook *et al* (1993), Cenis *et al*, (1993). Sterile distilled de-ionised water MilliQ water was used as the solvent or diluent of all aqueous solutions. Where appropriate, solutions were autoclaved at 121lb/sq in for 20 minutes in autoclave.

0.05 M EDTA pH 8.0).

To 186 g of disodium ethylenedianinetetraacetic acid (EDTA) (FW. 372.2), 800 ml f water was added and the mixture stirred vigorously. The pH was adjusted to 8.0 with NaOH. The volume was to 1 litre using milli-Q water and autoclaved.

70% ethanol.

To 700 ml absolute ethanol, 300 ml double distilled water was added and mixed thoroughly. This was used without autoclaving.

Ethidium bromide (10 mg/l)

To 1 g Ethidium bromide, 100 ml of milli-Q water was added and stirred vigorously till the dye dissolved. The solution was stored in a lightproof container at 4 °C.

Sodium acetate (pH 5.2)

In 800 ml of milliQ water, 408.1 g of sodium acetate . $3H_2O$. The pH was adjusted to 5.2 with glacial acetic acid, and the volume adjusted to 1 litre with milliQ water. The solution was sterilised by autoclaving.

Sodium dodecyl sulphate (SDS) (or sodium lauryl sulphate)

In 900 ml of milliQ water, 100 g of electrophoresis grade SDS. The mixture was stirred on a hot plate at 68oC to dissolve, and the pH adjusted to 7.2 using concentrated HCl. The volume was adjusted to 1 litre with milliQ water, and autoclaved.

50 x TAE buffer.

To half a litre of milliQ water, 242 g of Tris base (FW 121.1) 57.1 ml glacial acetic acid and 100 ml of 0.5 M EDTA (pH 8). The solution was stirred well till all the Tris base had dissolved, the topped up to 1itre by milliQ water. It was autoclave before storage. It was used at 1 x strength for electrophoresis.

To constitute 1 x TAE buffer, one part of 50 X TAE buffer was mixed with 49 parts of milliQ water and mixed thoroughly.

6 x Bromophenol blue

0.25% bromophenol blue was added to 40% sucrose in water and stored at 4°C.

Variate: wing length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	25501	5100	3.78	0.006
Residual	43	58029	1350		
Total	48	81796			

Variate: Length of hind tibia

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	2177.5	435.5	3.76	0.006
Residual	44	5099.9	115.9		
Total	49	6996.6			

Subject Female okra whiteflies

Variate body length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	192157	38431	2.07	0.079
Residual	70	1297004	18529		
Total	75	1452486			

Variate: Wing length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	19618	3924	2.01	0.087
Residual	78	152590	1956		
Total	83	171952			

APPENDIX II: DESCRIPTIVE STATISTICS

Subject Male cassava whiteflies reared on five hosts

Variate: body length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	4	14638	3660	1.78	0.170
Residual	21	43154	2055		
Total	25	53493			

Variate: Wing length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	4	3359	840	.0.82	0.528
Residual	25	25755	1030		
Total	29	29084			

Variate: Length of hind tibia

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	4	12956.8	3239.2	7.08	< 0.001
Residual	26	11896.5	457.6		
Total	30	24853.1			

Subject: cassava whiteflies reared on sis hosts

Variate: body length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	52908	10582	1.73	0.152
Residual	37	226132	6112		
Total	42	258161			

Variate: Length of hind tibia

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	5796.8	1159	9.51	< 0.001
Residual	74	9023.6	121.9		
Total	79	14783.4			

Subject: Male okra whiteflies

Variate: Body length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	74600	14920	0.87	0.510
Residual	52	895753	17226		
Total	57	967731			

Variate: Wing length

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	39818	7964	2.80	0.024
Residual	63	179206	2845		
Total	68	214995			

Variate: Length of hind tibia:

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	1007.76	201.55	2.19	0.067
Residual	60	5531.47	92.19		
Total	65	6442.82			

Mating interactions: Analysis of variance

Variate: Eggs per female per day

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (cross)	5	162.57	32.51	2.49	0.042
Residual	56	731.50	13.06		
Total	61	894.06			

Variate: Egg hatchability

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	4351.3	870.03	2.89	0.022
Residual	53	15985.8	301.6		
Total	58	20012.2			

Variate: Larval survival

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	1877.6	375.5	2.02	0.090
Residual	56	10424.7	186.2		
Total	61	12302.3			

Variate: Total survival

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	5	5605.7	1121.1	3.24	0.012
Residual	56	19355.6	345.6		
Total	61	24961.3			

Variate: Length of hind tibia

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	3	2.55	0.85	0.05	0.984
Residual	37	608.81	16.45		
Total	40	611.37			

Variate Length of abdomen

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	3	607.2	202.4	0.60	0.621
Residual	37	12530.4	338.7		
Total	40	13137.6			

Width of abdomen

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	3	616.9	205.6	0.90	0.451
Residual	37	8471.5	229.0		
Total	40	9088.4			

Variate: Abdominal ratio (length/width)

Source of Variation	d.f.	ss	ms	v.r	F pr
Treatment (crops)	3	0.004029	0.001343	0.19	0.902
Residual	37	.0259591	0.007016		
Total	40	0.263620			

Analysis of variance: %Hatch (Transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Biotype	1	0.06214	0.06214	2.11	0.156
Host	7	0.41287	0.05898	2.00	0.086
Biotype x Host	7	0.67651	0.09664	3.28	0.010
Residual	32	0.94417	0.02951		
Total	47	2.09570			

Analysis of variance

Variate: Nymph1 survival (Transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Biotype	1	0.85465	0.85465	71.18	<.001
Host	7	5.08344	0.72621	60.48	<.001
Biotype x Host	7	5.14877	0.73554	61.26	<.001
Residual	32	0.38425	0.01201		
Total	47	11.47110			

Analysis of variance: Survival (arcsine transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Biotype	1	0.23295	0.23295	23.11	<.001
Host	7	2.62045	0.37435	37.14	<.001
Biotype.Host	7	2.54837	0.36405	36.12	<.001
Residual	32	0.32250	0.01008		
Total	47	5.72427			

Analysis of variance: Nymph 3 survival (arcsine transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Host	4	0.85257	0.21314	8.65	0.003
Residual	10	0.24636	0.02464		
Total	14	1.09893			

Analysis of variance: Eclosion rate (arcsine transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Host	4	0.02932	0.00733	0.13	0.970
Residual	10	0.58237	0.05824		
Total	14	0.61169			

Analysis of variance: Nymph 2 survival (arcsine transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Host	5	3.79466	0.75893	64.44	<.001
Residual	12	0.14132	0.01178		
Total	17	3.93598			

Analysis of variance: Nymph survival (arcsine transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Host	6	0.64372	0.10729	5.61	0.004
Residual	14	0.26752	0.01911		
Total	20	0.91124			

Analysis of variance: Nymph survival (arcsine transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Host	6	0.34805	0.05801	1.20	0.362
Residual	14	0.67670	0.04834		
Total	20	1.02475			

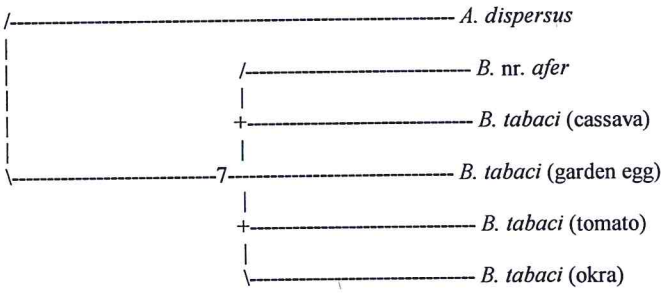
Analysis of variance: % Eclosion1 (Transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Host	6	1.35981	0.22664	8.58	<.001
Residual	14	0.36989	0.02642		
Total	20	1.72971			

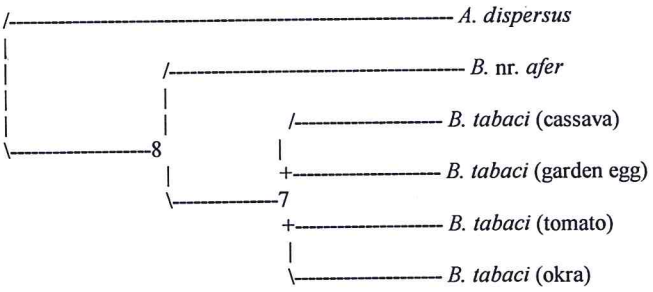
Analysis of variance: Sex ratio of crossbred progeny

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Crosses	5	3.380640	0.676128	104.29	<.001
Error	40	0.259327	0.006483		
Total	45	3.639967			

APPENDIX III: SOME NON CONSENSUS TREES



Phylogram based on unordered, unweighted characters of the five populations



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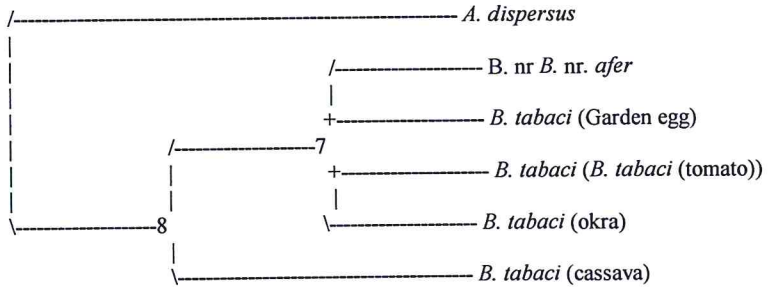
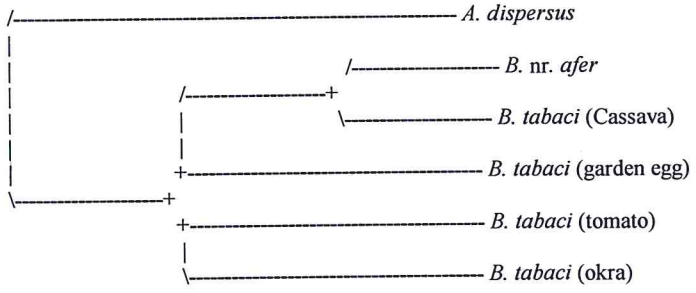
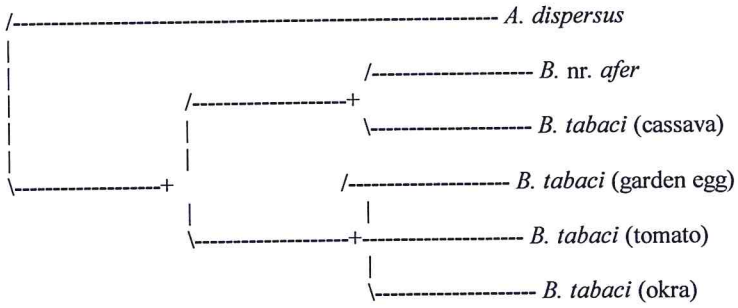


Tree number 3.



Tree number 1 (rooted using default outgroup)



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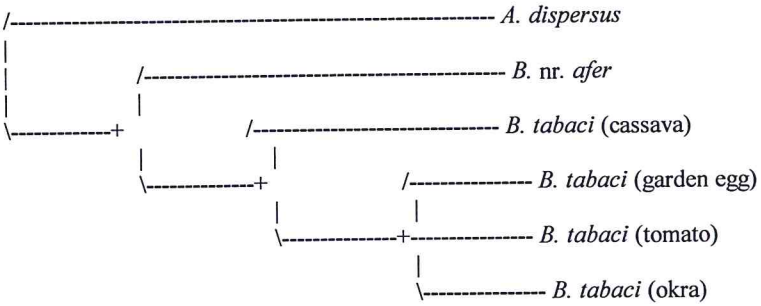

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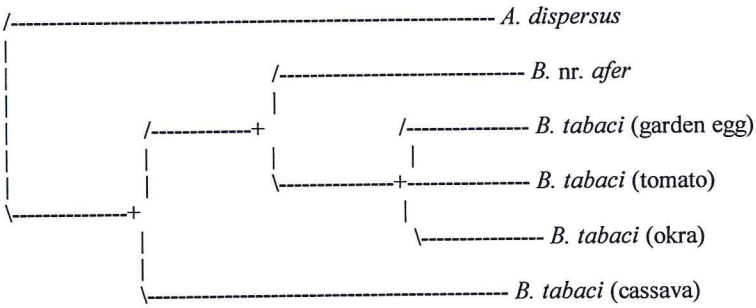

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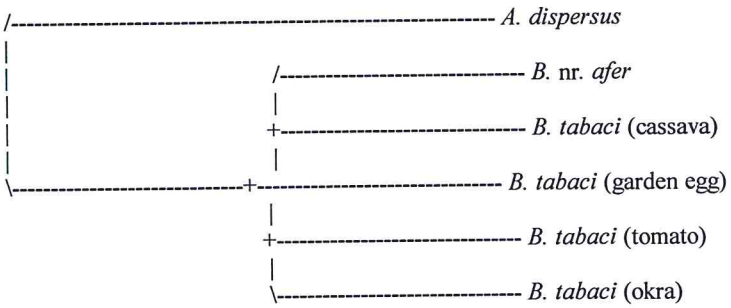

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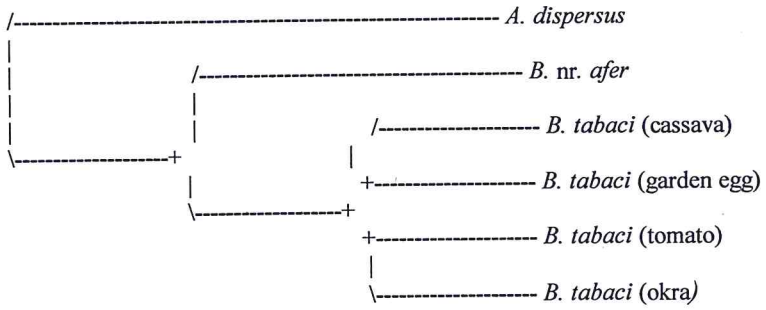

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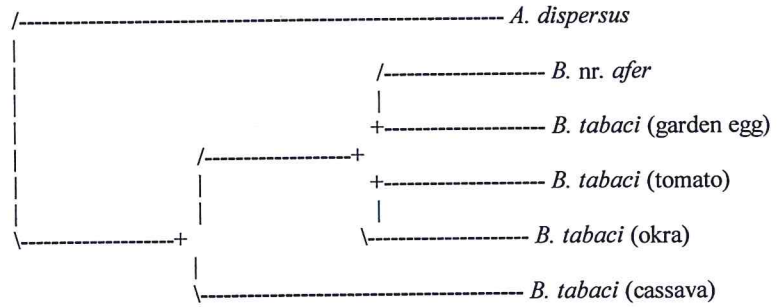
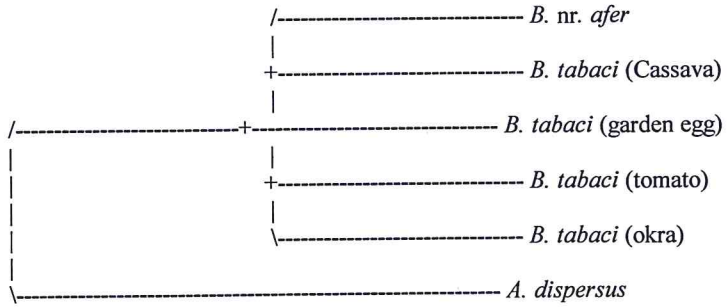
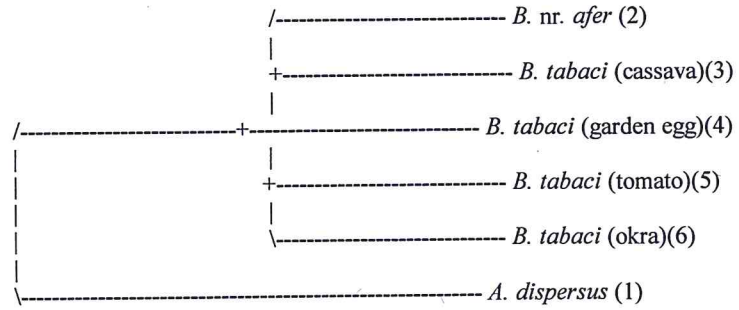


Tree number 7 (rooted using default outgroup)

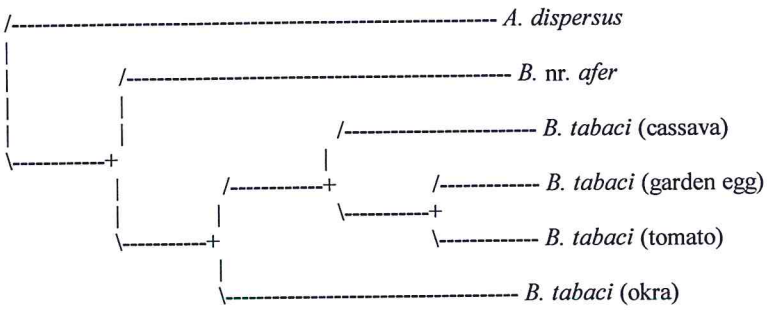


Strict consensus of 7 trees

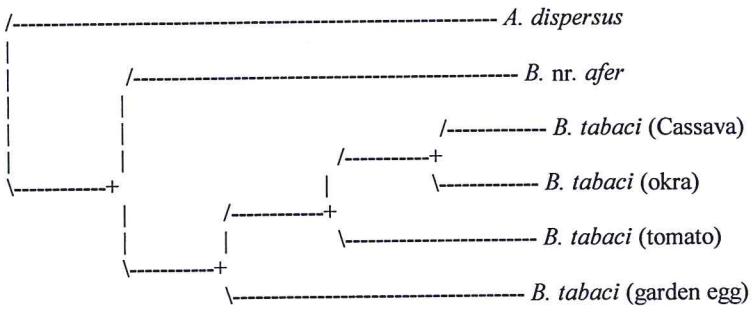


50% Majority-rule consensus of 7 trees

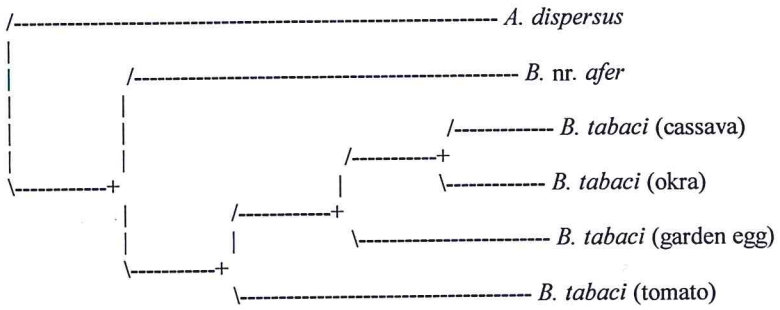
B: TREES FROM WEIGHTED AND ORDERED CHARACTER ANALYSIS.



Tree number 1 (rooted using default outgroup)



Tree number 2 (rooted using default outgroup)



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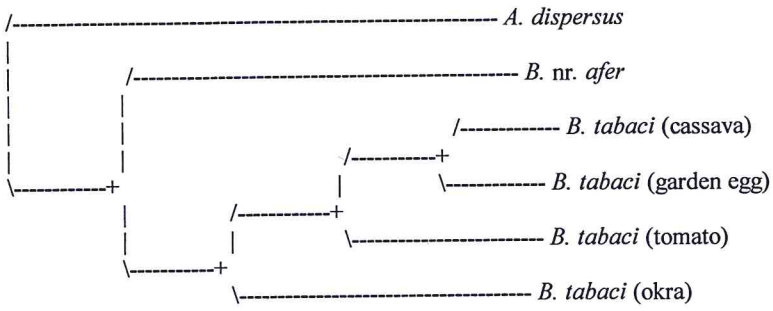
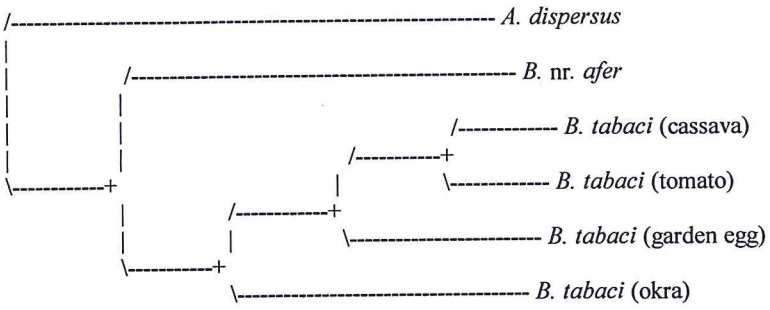


Tree number 4 (rooted using default outgroup)



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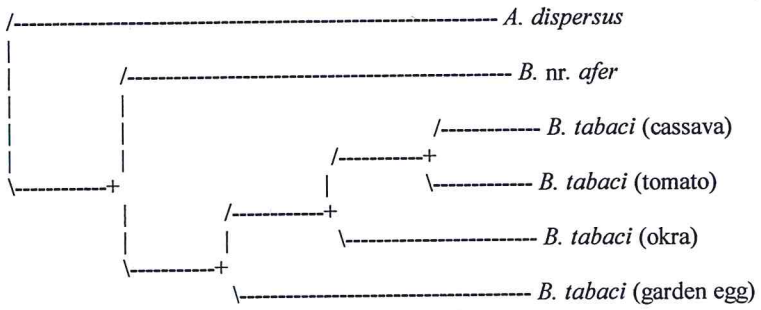

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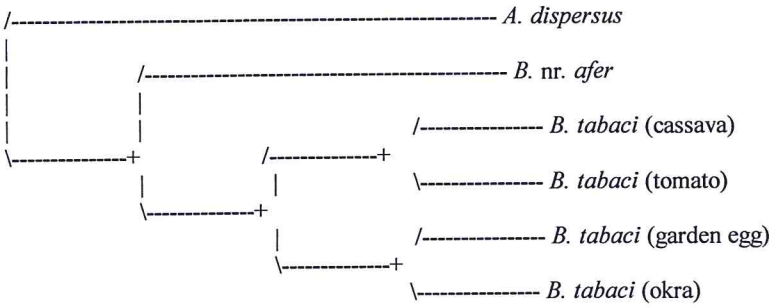

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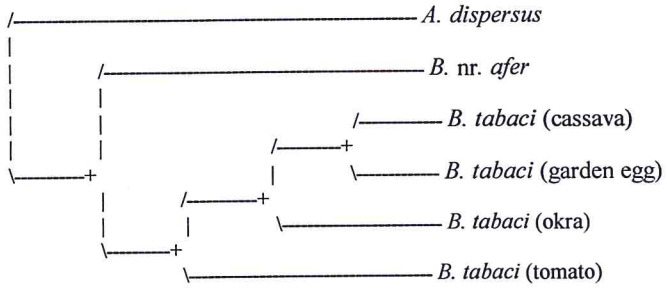

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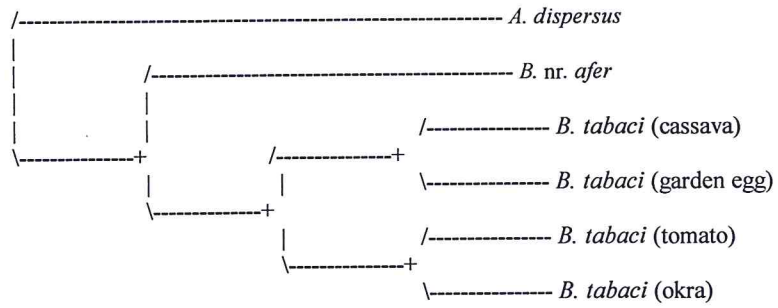

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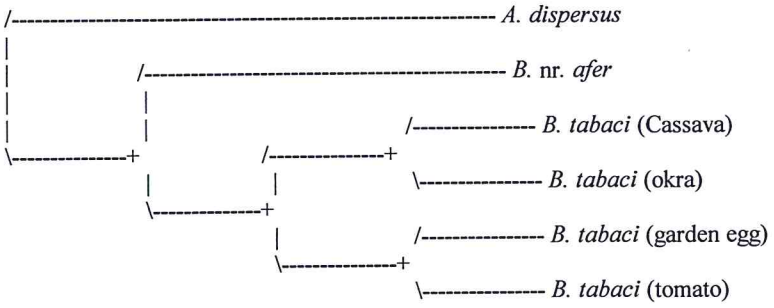

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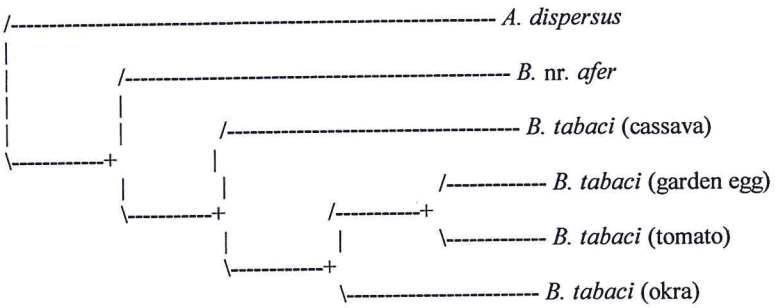

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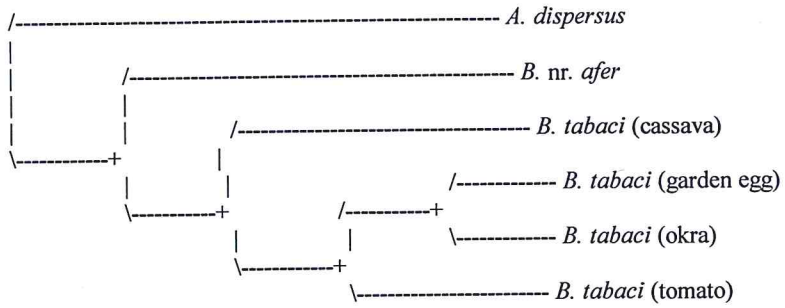

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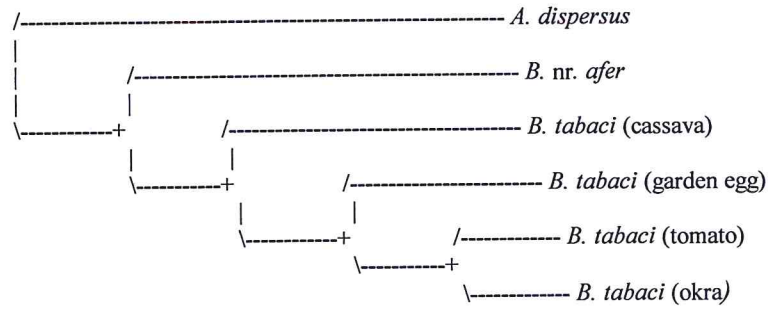

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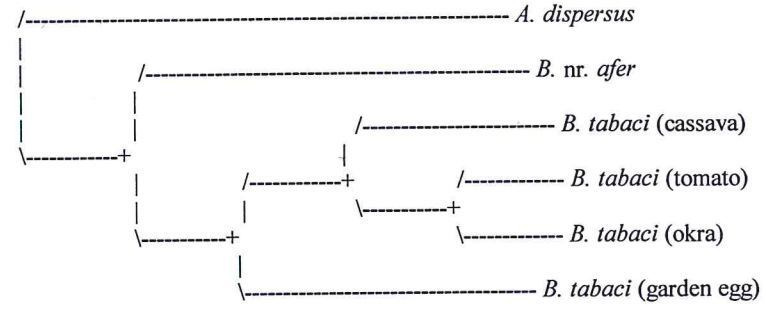

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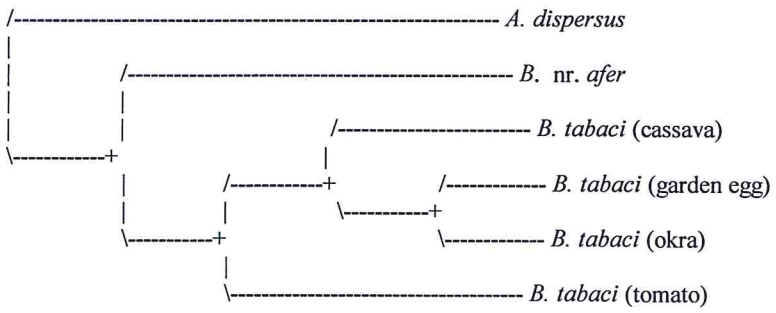

Tree number 15 (rooted using default outgroup)