BIOTYPE-HOST ASSOCIATION AND DISTRIBUTION OF ADULT *Bemisia tabaci* (GENN.) (HOMOPTERA: ALEYRODIDAE) ON DIFFERENT HOST PLANTS.

BY

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ABSTRACT

Bemisia tabaci is an important agricultural pest worldwide, which used to be a minor pest in Ghana but has become a major pest mainly due to the misuse of insecticides. The objectives of this thesis were to examine the occurrence of biotypes, within and between-plant distribution, dispersion and sampling methods for *B. tabaci* adults on cassava, tomato, okra, garden egg and coral plant.

Using cross-infestation, population trends and random amplified polymorphic DNA – polymerase chain reaction (RAPD-PCR) two biotypes were identified. A cassava biotype found on cassava that could survive on cassava, garden egg and eggplant. A non-cassava biotype found on tomato, garden egg and coral plant that survived on all the host plants tested except cassava. The insecticide test did not reveal any differences in population characteristics.

Majority of *B. tabaci* adults were found on upper leaves (1st and 2nd) and bottom leaves (leaves 7th – 9th) on cassava. They were also found on the upper half of the tomato plant and on young and middle aged leaves on garden egg and okra. The insects were aggregated within-plants on tomato, garden egg and okra and between-plants on tomato and okra. The correlation coefficients for insect counts of different sampling units and whole plant counts and sample sizes at particular error levels are presented.

DECLARATION

I hereby declare that except for references to work of other researchers, which have been duly cited, this thesis consists entirely of my original research work conducted at the University of Ghana, no part of it has been presented for another degree elsewhere.

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CHAPTER ONE

INTRODUCTION

Bemisia tabaci is a small piercing and sap sucking insect belonging to the family Aleyrodidae in the order Homoptera. It is widely distributed throughout the warm parts of the world. It feeds on a wide range of plants (300 – 400 spp.), often herbaceous representing at least 65 74 families (Mound and Halsey, 1978; Greathead, 1986; Martin, 1987). *B. tabaci* is a pest of some important crops such as cotton, tomato, cassava, pepper, garden egg, okra, cowpea, cucurbits, sweet potato etc.

B. tabaci causes damage to the crop directly by sucking the plant sap and causing phytotoxic disorders and/or indirectly by viral disease transmission and honeydew secretions(Venugopal *et al*, 1989; Varma, 1962; Costa, 1969; McKinlay *et al*., 1992). In cotton crop, at the peak infestation level, honeydew secretion leads to sooty-mold development and affects the ginning quality of cotton lint. Depending on the variety, *B. tabaci* can cause losses varying from 21.7 to 31.8% in boll numbers, 10.3 to 23.7% in boll weight, 25.9 to 48.9% in seed cotton yield, 12.4 to 15.3% in seed oil and 29.4 and 66.5% in seed germination (Venugopal *et al*, 1989).

B. tabaci is responsible for transmitting a large number of viruses in many parts of the world (Varma, 1962; Costa, 1969). It is a vector for destructive viral diseases

such as African cassava mosaic virus (ACMV), which can cause losses of up to 95% in sub-Saharan Africa (Abisgold and Fishpool, 1990) and tomato yellow leaf curl virus (TYLCV). TYLCV is destructive when it infects young plants, causing them to show poor vigour and produce very few marketable fruits (McKinlay *et al.*, 1992).

Since the 1980s, there has been an increased problem of *B. tabaci* as an insect pest in many areas on different crops. This has been attributed to monocultures, misuse of insecticides and the devastating effect of destructive biotypes. Millions of dollars have been lost because of direct feeding damage and plant diseases caused by whitefly transmitted (WFT) Gemini viruses (Brown, 1992; Brown *et al.*, 1991; Cohen *et al.*, 1992; Costa and Brown, 1990; Costa and Brown, 1991; Costa *et al.*, 1993; Yokomi *et al.*, 1990). *B. tabaci* was responsible for damage in excess of USD 200 millions to US agricultural crops in 1992 (Faust, 1992). In the southwestern USA, where *B. tabaci* has occurred since the late 1920s without assuming a major pest status, populations have risen sharply, causing severe problems to cotton, sugar beet and various vegetable crops (Duffus and Flock, 1982). The explosion of *B. tabaci* populations in California, USA, and similar phenomena in Thailand and Sudan, were related to reductions in parasitoid (*Encarsia formosa* Gahan) numbers because of the applications of pyrethroid-based insecticides that were directed against other insect pests (Johnson *et al.*, 1982).

In Ghana *B. tabaci*, used to be a minor pest of vegetables but has become important because of the overuse and misuse of pesticides (Critchley, 1995). In whitefly surveys conducted in cassava fields in Ghana, it was observed that whitefly populations consisted mainly of *B. tabaci*. *B. afer* (Priesner & Hosney) was poorly distributed and less abundant than *B. tabaci* in cassava fields (Sotomey *et al.*, 1995). Parasitoids, other natural enemies, genetic heterogeneity in *Bemisia* populations and identity of cassava viruses/viral strains are yet to be determined in the country (A. R. Cudjoe, personal communication).

The presence of host races or biotypes of whitefly *B. tabaci* was recognized as early as 1957 (Bird, 1957). It has been observed that morphologically similar populations of *B. tabaci* exhibited measurably different biological traits, with respect to host range, host-plant adaptability and plant virus-transmission capabilities (Bird, 1957; Bird and Maramorosch, 1978; Costa and Russell, 1975). In the mid 1980s, a new whitefly was found causing severe damage to ornamental plant species in the southwestern US. Based on morphological similarities with *B. tabaci*, it was identified as a new strain or biotype of this species (Florida strain, poinsettia strain, B-strain, biotype B)(Bharathan *et al.*, 1990; Bethke *et al.*, 1991; Costa and Brown, 1991; Perring *et al.*, 1991). This strain was different from the existing strain or biotype (California strain, cotton strain, A-strain, biotype A). A- and B-biotypes do not breed successfully due to absence of female offspring (Costa *et al.*, 1993; Perring *et al.*, 1992). As a result, of widespread dispersal of the B-biotype, *B. tabaci* is now distributed nearly worldwide (Brown, 1994; Costa

et al., 1993). Recently, it has been reported to be devastating in areas where it was previously unimportant (Bedford *et al.*, 1992a; Brown *et al.*, 1995a; Costa *et al.*, 1993).

The presence of biotypes was confirmed by studies using allozyme markers (Perring *et al.*, 1992) and randomly amplified polymorphic DNA (RAPD) profiles (Gawell and Bartlett, 1993). Gawell and Bartlett (1993) conducted a detailed study using 20 RAPD primers in comparing A- and B-biotypes with *Parabemisia myricae* (Bayberry whitefly), *Trialeurodes abutilonea* (banded-winged whitefly). These whitefly species were more similar (0.165 similarity coefficient) than biotype A and B (0.059 similarity coefficient). They concluded that the RAPD technique was useful in distinguishing closely related organisms but was not convenient for elucidating taxonomic status in this case.

Population characterization is necessary, since specific phenotypes of whitefly and the interactions with their host plant(s) directly influence both pest status and the dynamics of the virus-vector/host interaction. The outcome of those interactions dictates whether successful colonization will occur, whether infestation are likely to reach or surpass economic thresholds, and whether vector populations will disperse and mediate the transmission of plant viruses (Brown *et al.*, 1995a).

An area of importance is the distribution of adult whitefly within- and between-

plants. Studies have revealed that the eggs are usually laid on the lower surface of tender leaves of the plant (Hussain, 1931; King, 1932; Elkhidir, 1965; David and Jesudasan, 1986). Ohnesorge *et al.* (1980) investigated the spatial distribution of *B. tabaci* on various food plants including tomato, potato and garden egg in Jordan. They observed that the final instar larvae occurred only on the older leaves and young leaves were generally preferred for oviposition. Consequently sampling plans have been developed that reduce sampling effort by concentrating on specific leaf positions that are most likely to harbor the stage of the insect of interest (vonArx *et al.*, 1984; Naranjo and Flint, 1994).

Knowledge about the spatial distribution of adult *B. tabaci* helps to define the optimal sample units, and the development of sampling plans for the precise estimation of the density of adult whiteflies. Sampling efficiency is a critical consideration because the time that can be devoted to sampling by researchers and pest managers is often limited. These sampling plans attempt to minimize cost (time) for estimating density with an acceptable level of statistical precision.

The objectives of this study were the characterization of whitefly populations associated with four important host plants (cassava, tomato, garden egg and coral plant) and study the within and between plant distribution of *B. tabaci*, as a step towards selecting appropriate sampling units in four crops (tomato, cassava, garden egg and okra).

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy:

Whiteflies belong to the order Homoptera in the family Aleyrodidae. There are about 1156 known whitefly species worldwide (Muniyappa, 1980). The adult whitefly offers so few characters to aid species identification, partly because of the difficulty of making good slide mounts, that the early workers turned to the final (fourth) instar larvae (Martin, 1987). Aleyrodid pupal cases (exuvia) provide many characters that are used to assist in the identification of species and most of these have been defined and discussed by Russel (1943, 1948).

The genus, *Bemisia*, includes two species, *B. tabaci* and *B. afer*; *B. hancocki* is a synonym of *B. afer*. Russel (1957) concluded that *B. inconspicua* Russel, *B. costa* Limai, *B. signata*; *B. bahiana*, *B. longispina*, *B. gossypiperda* var. *mosaicivectura*, *B. goldingi*, *B. rhodesiaensis*, *B. hibisci and B. nigeriensis* were synonyms of *B. tabaci* (Genn.). Hill (1969) conducted a comparative study in which all the different stages of *B. tabaci* and *Trialeurodes vaporariorum* were compared to enhance their recognition on tobacco plants in South Africa.

An outstanding work in the taxonomy of whiteflies is a key presented by Martin (1987) to aid the identification of pupal cases of 46 species of whitefly often found

infesting economic plants around the world. In this taxonomic key two sub-families are classified, Aleurodicinae and Aleyrodinae, which includes the genus *Bemisia*.

The physical characteristics of the host plant can substantially affect the appearance of the puparium of some species of whiteflies. This can result in mis-identification when dealing with populations from different host plants (Avidov, 1956; Azab *et al.*, 1969; David and Ananthakrishnan, 1976; Ghong, 1969; Lopez-Avila, 1986; Mohanty and Basu, 1986; Mound, 1963; Russel, 1957).

B. tabaci and *T. vaporariorum,* both of which occur on greenhouse crops, were differentiated on the basis of their surface lipid components. Post eclosion long-chain aldehydes and long-chain alcohol's were the dominant surface lipid components, C34 was found in *B. tabaci* and C32 in *T. vaporariorum*. The major wax esters were C46 on *B. tabaci* and C42 on *T. vaporariorum* (Nelson *et al.*, 1994).

In the female abdomen, the morphology of the cement gland and setal pattern of the gonapophyses can easily be examined. These characters (mainly those concerning the cement gland) are consistent enough to allow the identification of some whitefly pest species, such as, *Aleurothrixus floccosus*, *Aleyrodes proletella*, *B. tabaci, Trialeurodes vaporariorum* and *Dialeurodes citri* (Guimaraes, 1996)

The use of electrophoretic technique enabled the differentiation between species of whitefly (Jansen and Oudman, 1994; Oliverira and Lima, 1997). Electrophoretic

analysis of six species of whitefly, including *B. tabaci* and *T. vaporariorum*, in Israel and Columbia indicated that, the patterns of esterase and glycerophosphate dehydrogenase isoenzymes were species specific and can be used to identify species that are difficult or impossible to be distinguished on their morphological characters (Wool *et al.*, 1989).

2.2 The Biotypes or Host-Races Concept and *B. tabaci*:

The presence of races in *B. tabaci* was recognised by Bird (1957), and Bird and Sanchez (1971). Flores and Silberschmidt (1958) called these races ecological biotypes. Differentiation of biotypes on the basis of host associations, revealed the presence of polyphagous and monophagous populations, and it was observed that the monophagous populations transmitted a limited number of plant viruses. *B. tabaci* populations in Puerto Rico (Sida race) could colonize numerous plant species (okra, beans, tobacco, many weed species), it was also an excellent vector of whitefly transmitted (WFT) Gemini viruses found in bean, tobacco and several indigenous weeds (Bird and Maramorosch, 1975, 1978). The Jatropha race (N-biotype) (Brown *et al.*, 1995a), a nearly monophagous population, colonized *Jatropha gossypifolia* and occasionally *Croton lobatus* (Bird, 1957; Brown and Bird, 1992), and transmitted the *Jatropha* mosaic virus (JMV) exclusively to *J. gossypifolia* (Bird, 1957).

B. tabaci population from Brazil had a wide host range but could not colonize cassava (Costa and Russell, 1975). Subsequent studies revealed a number

of biotypes. The *Asystasia* race (E-biotype) found in Benin colonizes only *Asystasia* species and exclusively transmits the *Asystasia* golden mosaic virus (AGMV) (Bedford *et al.*, 1994). The Nigerian sweet potato race (H-biotype) (Bedford *et al.*, 1994), has a narrow host range, transmits TYLCV (Yemen strain), but does not serve as a vector for other WFT Gemini viruses (Brown *et al.*, 1995a).

Molecular studies on host plant and geographical area associated differences between populations of *B. tabaci* confirmed the presence of biotypes. Burban *et al.* (1992) identified two biotypes of *B. tabaci* by the use of isoenzyme electrophoresis and experimental host range studies. One of them, which, was a cassava biotype was found only associated with cassava, aubergines, and transmitted ACMV; the other biotype (okra biotype) was polyphagous but did not infest cassava. Bedford *et al.* (1992), using non-specific esterase patterns characterized several populations of *B. tabaci* derived from different host plants and geographical locations. They found that some populations had a wide host range and induced phytotoxic symptoms such as, silverleaf on plants. These populations had large reproductive capabilities and were efficient vectors of fifteen geminiviruses tested and were referred to as the B- biotype. They concluded that this biotype originated from the Middle East, and can be found throughout the Mediterranean, South Africa, the Caribbean and Central and North America.

It is difficult to differentiate between the two biotypes (B-biotype and non-B-

biotype) morphologically but some differences in adult body lengths have been observed. The ability to induce, phytotoxic disorders by B-biotype in certain plant species were found between the two biotypes (Bedford *et al.*, 1994). Examination of esterase profiles of over 40 populations of *B. tabaci* from native and cultivated plants from different parts of the world, revealed the presence of 12 unique electromorphs, which were designated as separate biotypes. One of these biotypes (type B) has recently been proposed as a separate species (Brown *et al.*, 1995).

Based on the results of a three-year collaborative study among scientists in Israel and Columbia, a detailed study of EST (esterase) allele frequencies showed significant heterogeneity that was related to neither host plant nor geography but was related to exposure to insecticides. (Wool *et al.*, 1993).

Based on results of cross infestations studies, isoelectric focusing techniques, polymerase chain reaction (PCR) and genomic analysis, some authors have considered the B-biotype of *B. tabaci* as a new species (Perring *et al*, 1993). Subsequently due to morphological and allozyme pattern differences with *B. tabaci;* Bellows *et al.* (1994) described and named the new species *B. argentifolii* Bellows&Perring. However there is still much debate on this classification (Campbell *et al.*, 1993, Brown *et al.,* 1995, Markham *et al.*, 1995). Menozzi (1997) stated that, *B. argentifolii* is the most aggressive biotype (B-biotype) of *B. tabaci* but some biological characteristics have led some entomologists to consider this

biotype as a species. Rossell *et al.* (1997) found that *B. tabaci* and *B. argentifolii* represent a complex comprising highly cryptic species.

2.3 Ecology:

Cotton whitefly, *B. tabaci;* occurs throughout most tropical and sub-tropical regions of the world. Very widely distributed throughout warm parts of the world, it occurs naturally in a band around the world approximately 30-35 degress latitude (McKinlay *et al.*, 1992). Distribution out of this band is limited by low winter temperatures. The adults can fly for only short distances, but may be dispersed over large areas by wind (Blackmer and Byrne, 1993). Humans also transport immature and adult stages on plants (Joyce, 1981; Mound, 1983).

The inadvertent transport of the B-biotype on ornamental plants beginning in 1985 – 1986 established *B. tabaci* throughout Europe, the Mediterranean Basin, Africa, Asia, Central America, North America (Mexico and US), South America (Argentina, Brazil, Colombia, and Venezuela), and the Caribbean Basin (Bedford *et al.*, 1994; Brown *et al.*, 1995a; Costa *et al.*, 1993). It is well adapted to greenhouse environment and has become sporadic pest of green house crops in Europe and U.S.A. It feeds on a very wide range of plants (300 – 420 spp.) (Often herbaceous) representing at least 65 74 families (Mound and Halsey, 1978; Greathead, 1986; Martin, 1987). *B. tabaci* is an important pest of cotton, tomato, chillies, okra, garden egg, cassava, legume crops and cucurbits. It also colonizes a number of weed plants.

B. tabaci is found in large numbers on the lower surfaces of leaves and stems of infested plants and the adults fly away when disturbed (Boorman, 1981). The developmental stages of *B. tabaci* are aggregated within and between plants. Regardless of relative height of the leaves on poinsettia plants, the adults, eggs and the first instar nymphs occur on young leaves. The second and the third instar nymphs occur on middle-aged leaves and most of the pupae and empty pupal cases occur on middle-aged and older leaves (Liu *et al.*, 1993).

On cassava plants, the majority of nymphs were found on leaves 7^{th} - 20^{th}, with the majority of them at leaf positions 12^{th} - 14^{th} and then decreasing rapidly from leaves 15^{th} – 20^{th} (Abisgold and Fishpool, 1990). On cotton, the youngest leaves, irrespective of branch position were found to be the most preferred site for oviposition. The highest nymphal and pupal populations are noticed on third, second, fourth and first leaf of the fourth and fifth branches of the plant. The distribution of the adults on different leaves followed a similar trend as that of the eggs (Krishna and Lingappa, 1992).

In some cases, *B. tabaci* population collected from a particular host may not establish on a different host plant. Bird (1957), Bird and Sanchez (1971) noticed that *B. tabaci* reared on *Jatropha gossypifolia* could not feed or breed on *Sida carpinifolia* and vice versa. Flores and Silberschmidt (1958) called these races ecological boitypes. *B. tabaci* adults taken from cotton and sweet potato did not colonize cassava; none survived for longer than two days and nymphs emerging

from eggs laid on cassava died. In contrast cassava *B. tabaci* showed limited colonization on cotton and sweet potato (Legg, 1996).

The two species of whitefly, *B. tabaci* and *T. vaporariorum*, on green house crops do not coexist on the same leaf for longer than two generations (50 – 60 days). Liu *et al.* (1994) found that *T. vaporarioum* was the dominant species on green bean and *B. tabaci* was the dominant species on poinsettia. The dominance of *T. vaporariorum* on green bean and the dominance of *B. tabaci* on poinsettia are attributed to less feeding, ovipositional activity by the adults, reduced growth and possible survival of immature stages of the competitor.

2.4 Biology:

Insects in the family Aleyrodidae display simple metamorphosis. Typically, insects with simple metamorphosis have immature stages that closely resemble the adult. However, aleyrodids are unusual (resembling complete metamorphosis), in that the nymphs appear scale like and the last nymphal instar is quiescent and pupa-like (McKlinay *et al.*, 1992). The adult *B. tabaci* is a minute insect (2-3mm long), and the body is covered with white waxy powder, which repels water, and this makes it difficult to be killed by spraying with water-based insecticides (Boorman, 1981).

Females of *B. tabaci* usually lay their first eggs on the lower surface of the leaf on which they emerged, but soon move upwards to young leaves on the same plant. *B. tabaci* is multivoltine, producing 11 – 15 generations/year under conducive

tropical, subtropical and temperate conditions (Avidov, 1956; Azab *et al.*, 1971, Butler *et al.*, 1983; Husain and Trehan, 1933). The eggs are inserted vertically into the leaf tissue and water can pass from the plant tissue into the egg. Plants with high numbers of eggs may become water-stressed (Gameel, 1974). Eggs are laid singly at the initial stages but later in-groups (Gerling and Or, 1984). The average daily egg-laying capacity is two eggs/female, and the capacity may be 160 eggs/female (Gameel, 1974). Pear-shaped eggs are about 0.2mm long and hatch in about 7 days (McKinlay *et al.*, 1992). The last nymphal instar is about 0.7mm long and the red coloured eyes of the adult can often be seen through the larval integument. Nymphs complete three moults before pupation and emergence as adults (McKinlay *et al.*, 1992).

2.5-Control:

The control of *B. tabaci* varies with the type of crop and the growing conditions (greenhouse or outdoors). However, chemical, cultural and biological controls are commonly used.

The integration of cultural practices and insecticides to prevent the spread of TYLCV by *B. tabaci* has achieved some success. Straw mulch placed around germinating plants is more attractive than tomato leaves to *B. tabaci* (Cohen *et al.*, 1974). A combined treatment of yellow polyethylene, which attracts whiteflies and azinphos-methyl sprays effectively, prevented the spread of TYLCV (Cohen and Melamed-Madjar, 1978). Antignus *et al.* (1996) found that there was a significant

reduction in *B. tabaci* infestation in vegetable crops grown under UV-absorbent covered plastic tunnels when compared with non-UV-absorbent plastic.

Cultural control alone may also be effective. Drip irrigation appears to be useful in reducing adult whitefly population; it promotes the growth of shorter plants with fewer leaves (Sharaf *et al.*, 1984). On cotton, if irrigation is stopped earlier than usual time, the growing season is reduced. This restricts the late season development of whiteflies and their numbers can be reduced considerably before the start of the next wet season (Gahukar, 1991).

Biological control of *B. tabaci* by means of parasitoid releases or through management of natural parasitoids populations has been fully exploited under field conditions. The currently used and potential natural enemies of *B. tabaci* include: Parasitoids; *Encarsia Formosa* (Baraja *et al.*, 1996; Stenseth, 1993; Steiner, 1993), *E. lutea* (Kirk *et al.*, 1993) and *Eretmocerus mundus* (Manzaroli *et al.*, 1997). Predators, Mirid bugs, *Dicyphus tamaninii* and *Macrolophus caliginosus* (Barnadas *et al.*, 1998). Pathogens, *Verticilium lecanii* (Stirmanova, 1994; Masuda, 1993; Nier *et al.*, 1991; Meade and Byrne, 1991) and *Beauveria bassiana* (Stirmanova, 1994) achieved some success in controlling *B. tabaci*.

The control of *B. tabaci* throughout the world is generally achieved by the use of insecticides. Foliar sprays of pyrethroids, cypermethrin and fenpropathrin appear to be generally more effective than similar sprays of other classes of insecticides in controlling *B. tabaci* (Berlinger *et al.*, 1986; Mishra, 1986).

CHAPTER THREE

BIOTYPE – HOST ASSOCIATIONS

3.1 Introduction:

The characterization of biotypes plays a significant role in insect pest control. Knowledge about the host range guides the design of crop rotation programme in order to break the life cycle of biotype(s). Breeding plants resistant to insects is affected by insect biotype, that is why, field resistance, which often involves resistance against all locally occurring biotypes, is preferred to laboratory or greenhouse resistance (Horber, 1980).

Although Stiling (1993) had found that 11.5% of the classical biological control programmes failed because the wrong strain of the enemies were used, no differences in the biological responses of parasitoids to different whitefly biotypes are known (Brown *et al.*1995a). Associations of insecticide resistance with whitefly populations were reported by Prabhaker *et al.* (1985), Abdeldaffie *et al.* (1987) and Dittrich *et al.* (1990).

The objectives of this study were to use cross-infestations (insect survival on six crops: cassava, tomato, garden egg, coral plant, cotton and eggplant), sensitivity to three insecticides {Karate (25 gm – Lambda – cyhalothrin/L), Actellic (80 gms pirimiphos – methyl – 50 gms Permethrin/L), Perfekthion (Dimethoate = 400 g/L)}, the population dynamics and randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) to study population differences.

3.2 Materials and Methods:

3.2.1 Insects:

Three crops, tomato (*Lycopersicon esculentum*), garden egg (wild eggplant) (*Solanum gilo*) and cassava (*Manihot esculenta*) were established between November 5, 1999 –January 16, 2000 in the University Farm, Legon about 3 km from the main university campus. It has Guinea Savannah vegetation with mean annual rainfall of 112 mm and a temperature of 32 ^{0}C. The fourth crop, coral plant (*Jatropha multifida)* was established as an ornamental plant at Pokuasi (Accra). All the crops were exposed to natural infestations of whiteflies. Recommended agronomic and cultural practices were applied to each crop plant.

3.2.2 Cross – infestations:

3.2.2.1 Test plants:

Six test plants, cotton (*Gossypium sp.*), eggplant (*S. melongena*), tomato (*L. esculentum*), garden egg (*S. gilo*), cassava (*M. esculenta*) and coral plant (*J. multifida*) were used to characterize biotypes on the basis of host plant – affiliations (means survival).

3.2.2.2 Collection of the insects:

Twenty adult *B. tabaci* were aspirated from each of the four crops into a plastic tube, and the tube was inverted (upside down). After the insects had moved up, the aspirator system was removed (to be fixed on another tube) and the tube with insects was closed with a cork.

The aspiration of the insects was done in the morning when the temperatures were low and this prevented the insects from dying before clipping on test leaves. This also allowed enough time during the day for clipping them in cages.

3.2.2.3 Clipping of the insects:

The insects were chilled on ice; by immersing the lower half of the plastic collecting tube in ice cubes for 1 min. Twenty chilled insects in each tube were transferred to a clip cage. The cage was then clipped onto the lower surface of the young leaf of the appropriate crop plant, which was grown singly in a pot. The transfer and clipping of the insects was done as quickly as possible before the insects became active.

Plastic petri dishes (41 mm in dia.) were used as the cages. A hole of 20 mm diameter was made in the base of the petri dish (using a heated metal pipe) for ventilation. The hole was covered with one layer muslin cloth, which was attached to the petri dish with chloroform. The dish was held in place onto the leaf using

modified paper clips (4 paper clips/petri dish). A poster paper was cut into pieces of 45 x 45 mm; one or two pieces were placed on the upper leaf surface to strengthen it against the paper clip pressure (Figure 1).

A sample of each whitefly population was clipped onto four leaves (2 leaves/plant) from each of the six crops in a completely randomized design (CRD).

3.2.2.4 Data collection and analysis:

Seventy-two hours after clipping, the following data were collected with the aid of hand lens: number of surviving insects out of 20/clip cage. After the calculation of the percentage survival (Numbers survived/20) the data were arcsine transformed and the analysis of variance (ANOVA) (GENSTAT, 1995) was performed on the data. The means were separated by LSD ($p \leq 0.05$).

3.2.3 Insecticides test:

Three insecticides, Karate (25 gm – Lambda – cyhalothrin/L), Actellic (80 gms pirimiphos – methyl – 50 gms Permethrin/L), Perfekthion (Dimethoate = 400 g/L) and tap water (control) were used. The procedure that was followed, was a modification of that used by Ahmed *et al.* (1987).

Figure 1: Clip cages on the leaves of garden egg (bottom left) and coral plant leaves (top and bottom right).

Figure 2: *B. tabaci* on coral plant leaf (Pokuasi near Accra).

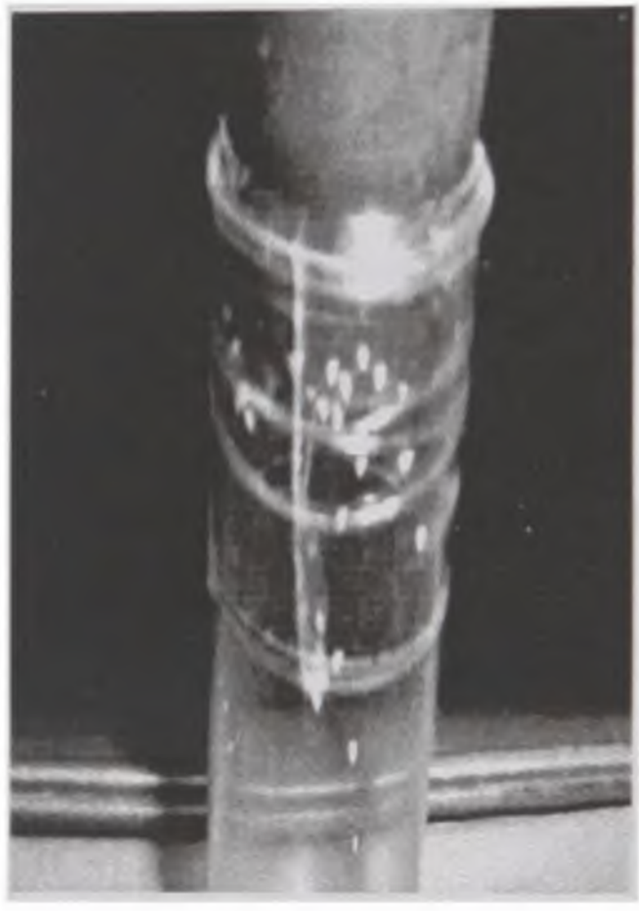

Figure 3: Aspirator showing *B. tabaci* adults aggregated on the top.

3.2.3.1 Collection of the insects:

The insects were collected by aspiration as reported earlier for the experiment on cross – infestations.

3.2.3.2 Preparation of dip solutions:

Fresh solutions of commercial formulations of the above mentioned insecticides were prepared at 0.1% concentrations (V/V). These doses were chosen based on preliminary tests (0.5% and 0.3% concentrations caused 100% mortality on all the populations). Tap water was used as a diluent. Usually 200 mls of the required insecticide was prepared in 400 ml glass conical flask.

3.2.3.3 Test procedure:

All tests were performed on garden-egg plants, in order to avoid variations in insecticide retention by leaves of different crop. Furthermore the results of cross – infestation tests, showed that all the four-whitefly populations could survive on garden egg plants.

Two young leaves on each plant were dipped into the appropriate insecticide solution for about 10 seconds with slight agitation and the excess solution was drained off from the leaves by shaking. Two plants (2 leaves/plant) grown singly in

pots were replicates for each whitefly population for each insecticide in a CRD. The control leaves were dipped in tap water. Twenty adult insects were clipped against each of the treated leaves in the same way as in the cross – infestations.

3.2.3.4 Data collection and analysis:

The mortality counts (no. of insects dead) were taken 24 hours after applying the treatment. ANOVA (GENSTAT, 1995) was used for the analysis of the data. The means were separated by LSD ($p \leq 0.05$).

3.2.4 Population dynamics:

This investigation was carried out on three whitefly populations infesting cassava, tomato and garden egg. The numbers of adult whiteflies were counted every week for seven weeks (05/12/99 – 16/01/00), starting from one month after transplanting the host plant.

The insects were counted after gently turning the leaves, during the cool hours of the day. Six leaves (during the first two weeks all the leaves) were taken/plant (2 old, 2 middle aged and 2 young), 5 plants/plot (diagonally and randomly selected), 20 plants/crop, was the sample size every week.

The data were taken as mean number of insects/plant/week, and the difference in trends were examined to differentiate among the populations.

3.2.5 Random amplified polymorphic DNA – polymerase chain reaction (RAPD – PCR):

3.2.5.1 DNA –extraction:

This experiment was done according to protocols described by Gawel and Bartlett (1993), Cenis *et al.* (1993) and Black *et al.* (1992) with modifications.

Adult insects were collected from the four-whitefly populations (cassva, tomato, garden egg and coral plant) and preserved over calcium chloride. Single insects were ground with a micro pestle in a 1.5 ml microcentrifuge tube containing 20 ul of the lysis buffer [200 mM Tris. HCl pH 8.5, 250 mM NaCl, 25 mM EDTA 0.5% SDS]. The volume was made to 200 ul with lysis buffer and 100 ul of 3M sodium acetate, pH 5.2 was added. The mixture was kept at -20 ^{0}C for 10 minutes, centrifuged for 5 minutes at 13000 rpm and the supernatant transferred to other tube. An equal volume (300 ul) of isopropanol was added. After 15 minutes at –20 ^{0}C, the precipitated DNA was pelleted by centrifugation at 13000 rpm for 20 minutes.

The DNA was washed with 70% ethanol, the pellet was vaccum dried and resuspended in 50 ul of TE buffer (10 mM Tris, 0.1 mM EDTA pH 8.0).

3.2.5.2 PCR reactions:

The primers used were the ten-mer oligonucleotides of arbitrary sequence (OPA – 04, 17, -05 and 15) supplied by Operon Technologies (Alameda, CA). Fifteen

insects of each population were analyzed using each primer.

PCR reactions were performed in 25 ul pre-mixed, pre-dispensed reactions [Ready To Go PCR Beads (Amersham Pharmacia Biotech)] containing ~1.5 units of Taq DNA polymerase, 10 mM Tris – HCl, (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$ (adjusted to 3.5 mM), 200 uM of dNTP and stabilizers, including BSA. 1 ul of previously prepared template DNA solution was added to this reaction mixture. A drop of mineral oil was added to the reaction mixture. The reaction was run for 45 cycles in thermal cycler (progene) under the following conditions: denaturation at 94 ^{0}C for 1 min., annealing at 35 ^{0}C for 1 min., extension at 72 ^{0}C for 1 min.

3.2.5.3 Agarose gel electrophoresis:

Ten ul of the product DNA were loaded on a 2.0% TAE (0.04 M Tris-acetate, 0.001 M EDTA, pH 8.0) agarose gels and electrophoresed for 4 hrs at 50 V in IX TAE buffer. DNA bands were visualized under UV light after staining the gel with ethidium bromide and photographed on a 365 nm UV transilluminator.

Intra- and inter-population variations were determined by calculating the similarities indices within and among the populations.

3.3 Results:

3.3.1 Cross-infestation:

The mean number of insects that survived from the four *B. tabaci* populations (cassava, tomato, garden egg and coral plant) on the six crops (cassava, tomato, garden egg, cotton, eggplant, and coral plant) is presented in Table 1. Cassava *B. tabaci* population survived on cassava, garden egg and eggplant (range of means survival 19 - 14.75 insects out of 20) but could not survive on tomato, coral plant and cotton (range of means survival 0 - 4.5 insects out of 20). Tomato *B. tabaci* population survived on all test plants used (range of means survival 16 - 19.75 insects out of 20) except cassava (mean survival = 1.25 insects out of 20) and coral plant (mean survival = 9.25 insects out of 20). Coral plant *B. tabaci* population survived on all test plants used (range of means survival 17.5 19.5 insects out of 20) except cassava (mean survival = 0 insects out of 20). Garden egg *B. tabaci* population survived on all test plants used (range of means survival 16 - 18.75 insects out of 20) except cassava (mean survival = 0 insects out of 20) and coral plant (mean survival = 5 insects out of 20). Analysis of variance tables are in Appendices 1 - 6.

On garden egg, eggplant and coral plant, although there was a significant difference between the mean numbers of insects that survived (Appendices 4, 5 and 3), there was no distinct grouping of the *B. tabaci* populations based on the means separations using LSD ($p \leq 0.05$).

On cassava, cotton and tomato there was a significant difference (Appendices 1, 6 and 2) and the populations of *B. tabaci* were characterized (categorized) into two groups. Tomato, garden egg and coral plant whitefly populations associated in one group and cassava whitefly population in a different group.

3.3.2 Insecticides test:

The results (mortality %) are summarized in Table 2 and Appendices 7 - 10. Perfekthion treatment showed no significant difference between the populations (range of mean percentages mortality 96.2 97.5) (Appendix 10). Actellic treatment (Appendix 8) categorized the populations into two groups based on mean percentages mortality, cassava and coral plant in one group, tomato and garden egg in a different group. Karate treatment (Appendix 9) showed interaction grouping, cassava and garden egg populations in one category and coral plant population in a different one, tomato populations associated with both of them.

3.3.3 Population dynamics:

The population fluctuations of the three-whitefly populations on cassava, tomato and garden egg are presented in Figure 4 and Appendix 11.

The trends of tomato and garden egg (Solanaceous crops) *B. tabaci* populations were highly synchronized. With the exception of the 7th and 8th weeks their population's densities followed a similar trend throughout the period. In contrast,

the trend of cassava *B. tabaci* population did not show any relation to any trend of the two other populations.

3.3.4 RAPD - PCR:

The four primers used in this test produced RAPD patterns that distinguished the four-whitefly populations into two groups. Figure 5 and 6 showing amplification products resulted from primer OPA-17 and OPA-04 respectively. The average number of RAPD bands/primer was 5.5. Distinct differences between the populations were evident. *B. tabaci* populations from tomato, garden egg and coral plant (Lanes 0 – 14 and 0 – 13; Figure 5 and 6 respectively) could be distinguished as a group from the cassava population (lanes 15 – 19 and 14 – 18 Figure 5 and 6 respectively).

Inter- and intra- population similarities were estimated based on RAPD bands scores. RAPD bands of equal molecular weight but different intensities were given the same scores, only if phenotypically distinguishable for each insect. Only the reproducible bands in three attempts were scored. The scored band for each population were analyzed using a Numerical Taxonomic System (NTSYS) (NTSYS pc, version 2.0), the results are presented in a dendogram (Figure 7).

The highest intra-population similarities coefficient was 1.00 and the lowest was 0.89 with exception of samples 6 and 8 (garden egg populations). Cassava *B. tabaci* (Samples 1 - 5, Figure 5 & 6) were found in the same cluster with 0.89

intra-population similarity coefficient. With exception of samples 6 and 8 (Garden egg population), tomato, garden egg and coral plant populations (samples 6 - 20) were found in a single cluster having 0.89 inter-population similarity coefficient; implying that they belong to the same biotype.

Insect samples 6 and 8 were intermediate between the two described clusters. Sample 8 was closer to the cassava population (0.72 similarity coefficient); whereas sample 6 was closer to tomato, garden egg and coral plant populations (0.65 similarity coefficient). The cassava population in one group and tomato, garden egg and coral plant in an other group had 0.45 inter-population similarity coefficient.

Table 1: The mean* ± s.e. of adult insects which survived out of 20 insects from the four whitefly populations clipped on six crop plants.

Crop	*Bemisia tabaci* Population			
	Cassava	Coral plant	Tomato	Garden egg
Cassava	19.00 ^{a} ± 0.2	00.00 ^{b}	01.25 ^{b} ± 0.2	00.00 ^{b}
Coral plant	00.00 ^{a}	19.50 ^{b} ± 0.1	09.25 ^{c} ± 0.1	05.00 ^{d} ± 0.3
Tomato	00.75 ^{a} ± 0.1	17.75 ^{b} ± 0.1	17.75 ^{b} ± 0.2	16.00 ^{b} ± 0.2
Garden egg	16.75 ^{c} ± 0.2	17.50 ^{bc} ± 0.3	19.75 ^{a} ± 0.1	18.75 ^{ab} ± 0.2
Cotton	04.50 ^{b} ± 0.3	17.50 ^{a} ± 0.4	16.00 ^{a} ± 0.2	16.25 ^{a} ± 0.2
Egg plant	14.75 ^{b} ± 0.6	18.75 ^{a} ± 0.3	17.50 ^{ab} ±0.1	16.25 ^{b} ± 0.1

* Means within a row followed by the same letter are not significantly different (P ≤ 0.05)

Table 2: Mean* percentages mortality of whitefly populations due to Actellic, Karate and Perfekthion treatments.

Insecticide	*Bemisia tabaci* Population			
	Cassava	Coral plant	Tomato	Garden egg
Actellic 25 EC	100.00 ^{a}	100.00 ^{a}	96.20 ^{b}	94.90 ^{b}
Karate 25 EC	94.90 ^{a}	75.60 ^{b}	87.20 ^{ab}	92.40 ^{a}
Perfekthion 20 EC	96.20 ^{a}	96.20 ^{a}	96.80 ^{a}	97.50 ^{a}
Control	1.25 ^{a}	2.50 ^{a}	2.50 ^{a}	1.25 ^{a}

* Means within a row followed by the same letter are not significantly different (P ≤ 0.05).

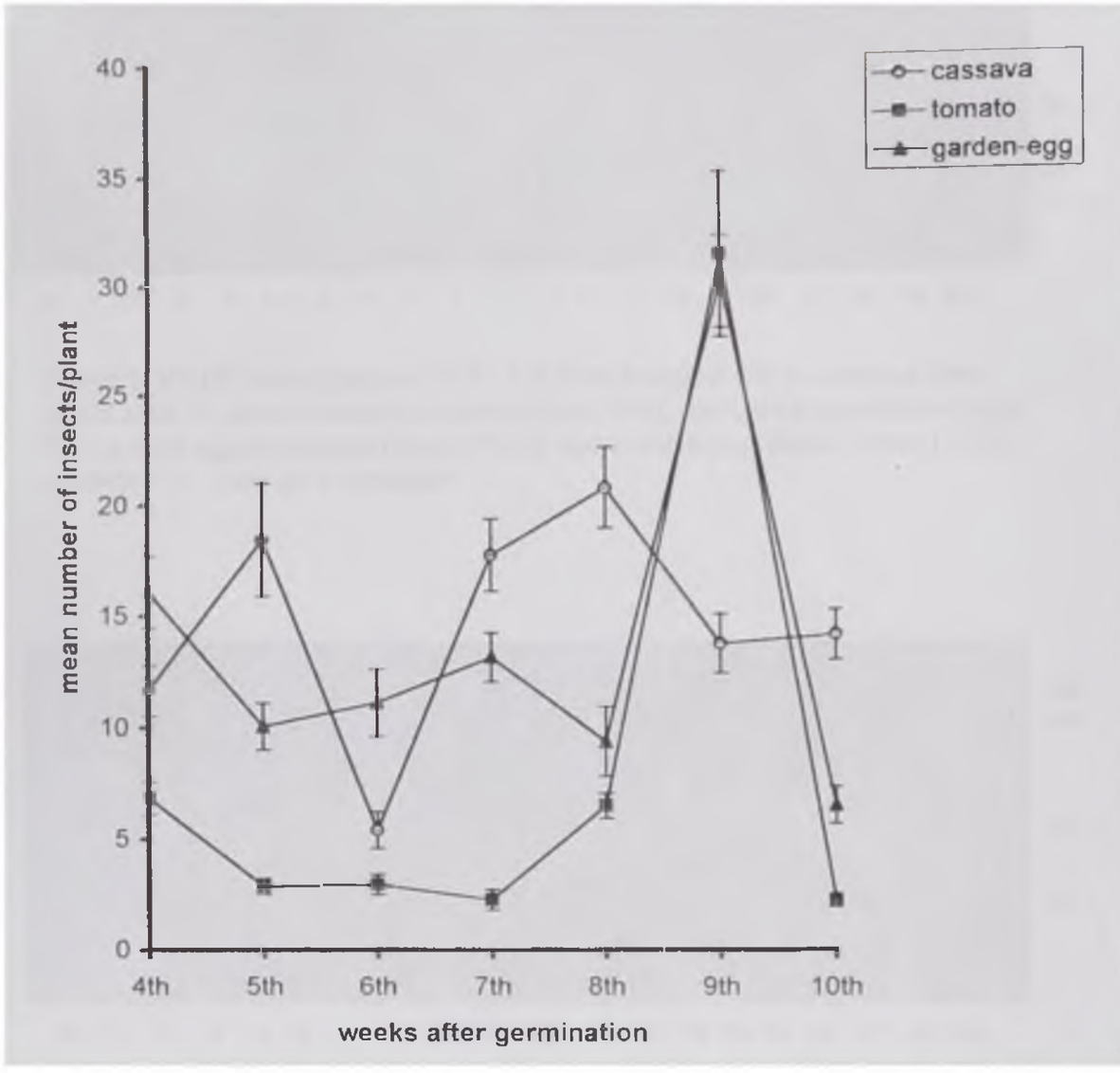

Figure 4: Population Dynamics of *Bemisia tabaci* from Cassava, Tomato and Garden-egg Populations

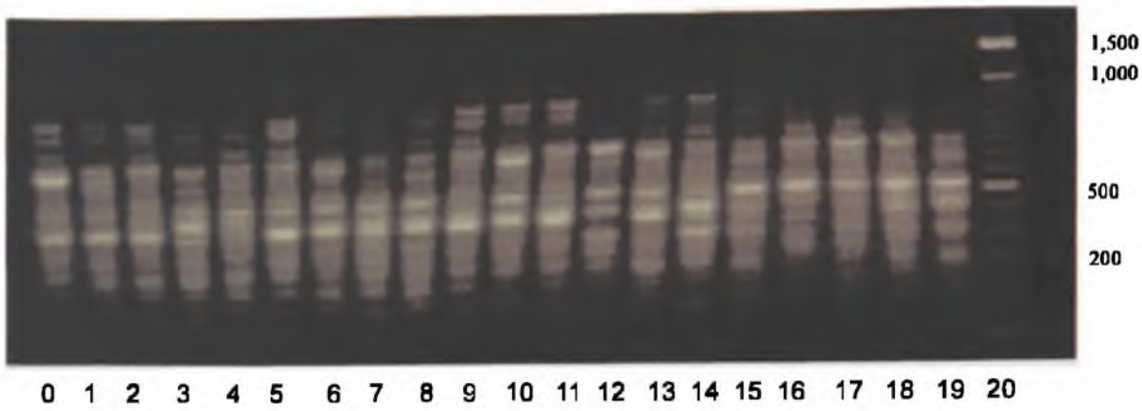

Figure 5: RAPD bands (primer OPA - 17) from template DNA extracted from single adult *B. tabaci* tomato population (lanes 0-4), coral plant population (lanes 5-9), garden egg population (lanes 10-14), and cassava population (lanes 15-19), respectively. Lane 20 is a marker

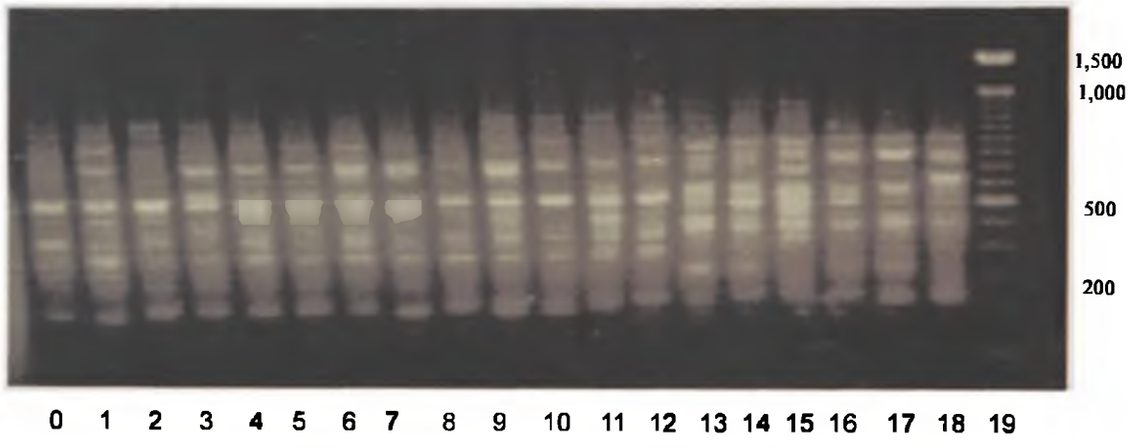

Figure 6: RAPD bands (primer OPA - 04) from template DNA extracted from single adult *B. tabaci* tomato population (lanes 0-3), coral plant population (lanes 4-8), garden egg population (lanes9-13), and cassava population (lanes 14-18) respectively. Lane 19 is a marker

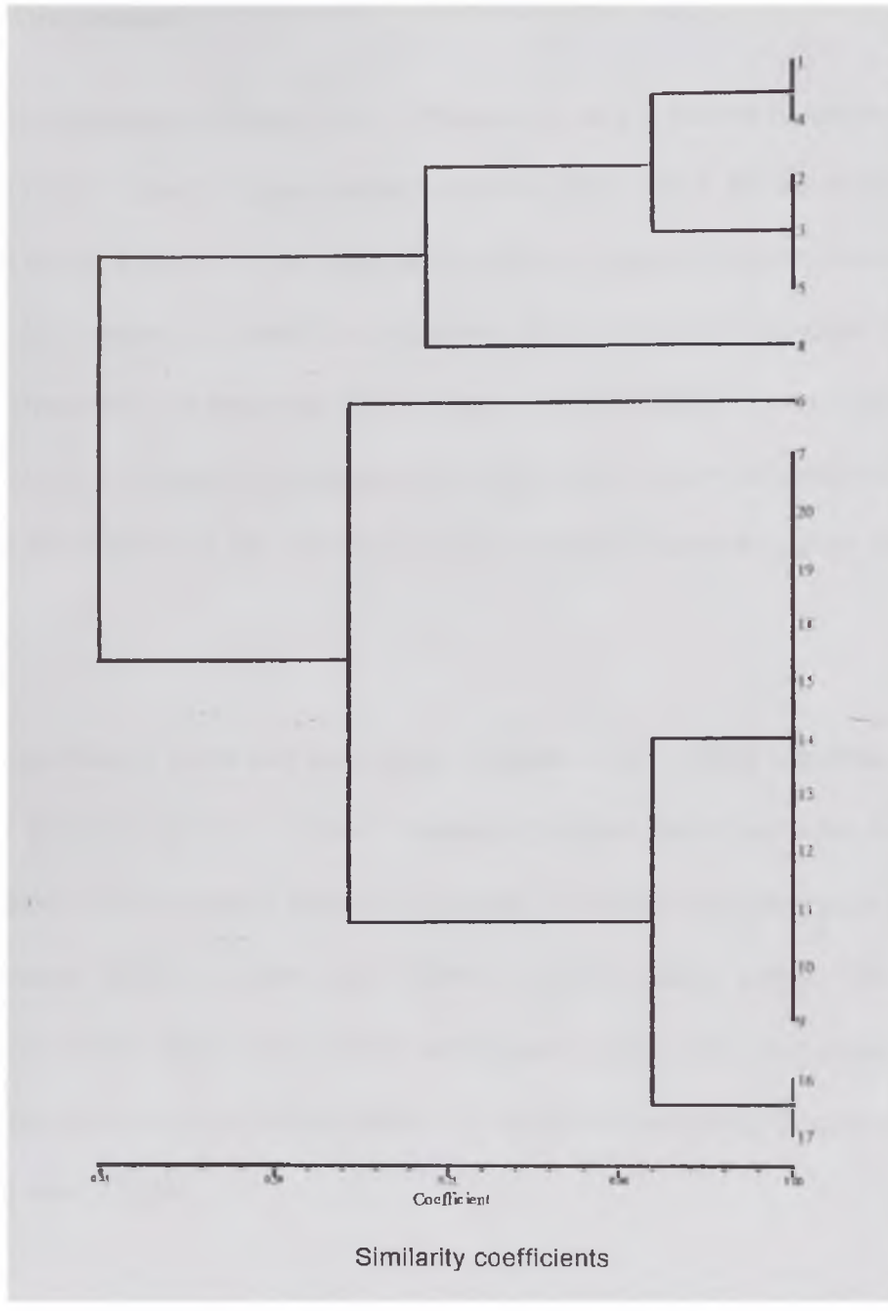

Figure 7: A dendogram showing genetic similarities among four populations of adult *B. tabaci*. Cassava population (samples 1 - 5), garden egg population (samples 6 - 10), coral plant population (samples 11 - 15) and tomato population (samples 16 - 20).

3.4 Discussion:

Cross-infestation (Host plant - affiliations) is the oldest technique that has been used in *B. tabaci* biotype characterization (Bird, 1957; Bird and Sanchez, 1971). In this study, based on the main *B. tabaci* host plants (Cotton, tomato and cassava) the technique revealed the presence of two distinct biotypes. Cassava biotype survived only on cassava, garden egg and eggplant. The tomato, garden egg and coral plant biotype (non cassava biotype) survived on all host plants used (range of means survival 16- 19.75 out of 20) except cassava (range of means survival 0.0 – 1.25).

These results were not surprising, Burban *et al.* (1992) reported the presence of two biotypes in Ivory Coast, cassava biotype found only on cassava and wild eggplant (garden egg) and okra biotype, which was polyphagous but did not infest cassava. Both biotypes were found on garden egg. Legg (1996) found that *B. tabaci* adults taken from cotton and sweet potato did not survive on cassava for longer than two days and cassava *B. tabaci* showed limited colonization on cotton and sweet potato.

The susceptibility of *B. tabaci* to insecticides in this study did not appear to be a criterion useful in population characterization. Exposure to Actellic placed cassava and coral plant populations, which do not usually receive insecticide treatment in Ghana, in one category. Tomato and garden egg populations, which are normally

treated with insecticides, were placed in a different category. The relatively low susceptibility of coral plant *B. tabaci* population to Karate (pyrethroid) may be attributed to the insecticidal characteristics of the coral plant which may have induced some resistance or tolerance to insecticides in the insects.

The results of this test (susceptibility to insecticides) confirmed the results of the previous research (Wool and Greenberg, 1990 and Costa *et al.*, 1993), which concluded that the variations in insecticide resistance depends on whether the samples are collected from treated, or non-treated populations. Wool and Greenberg (1990) compared insecticide resistance in cotton field population that had been routinely treated with organophosphorus with untreated controls. Despite the fact that both populations were the B-biotype, field populations were three - five times more resistant than control populations. Laboratory colonies of A- and B-biotypes were found almost equally sensitive to an organophosphate, profenphos, while the B-biotype was considerably more resistant to permethrin (Costa *et al.*, 1993).

Despite the number of studies on *B. tabaci* biotypes this appears to be the first study, which has attempted characterization of *B. tabaci* biotypes using populations trends. The experiment confirmed the results of cross-infestation that the populations fall into two biotypes cassava biotype and non-cassava biotype. The study indicated that the apparently continuously distributed individuals also could be relatively distinct populations.

RAPD-PCR is one of the modern molecular techniques used in genetic variation studies, such as differentiation of insect populations (Gawel and Bartlett, 1993; Black *et al.*, 1992). The results have shown that RAPD can be used to characterize the insect biotypes.

RAPD results confirmed the data on cross-infestations and populations dynamic trends of the two biotypes. The cassava and non-cassava biotypes exhibited a high level of homogeneity (0.89 similarity coefficients). These levels of homogeneity are however less than the intra-population genetic similarities of biotype-A and -B (similarities of 0.999 and 0.991) respectively as observed by Gawel and Bartlett (1993) using laboratory raised colonies. This may be because laboratory populations are more homogenous than field populations.

Gawel and Bartlett (1993) estimated a similarity coefficient of 0.059 between biotypes A and B and 0.165 between some other whitefly species. However in the current study, a similarity coefficient of 0.45 was found among the cassava and non-cassava biotypes. Hence cassava biotype and non-cassava biotype are genetically closer than A- and B-biotypes. They are also closer than some whitefly species.

The two intermediate individuals found on garden egg, may either belong to different biotypes or may be hybrids of the two described biotypes (cassava and non-cassava). Further studies are required to ascertain interbreeding among these biotypes.

CHAPTER FOUR

DISTRIBUTION AND SAMPLING OF ADULT *BEMISIA TABACI* (GENN.) ON CASSAVA, TOMATO, GARDEN EGG AND OKRA

4.1 Introduction:

Monitoring is very important for timing and evaluation of pest control measures. It is a means for determining when pests enter the crops, when numbers have built up to sufficiently warrant control measures or to predict correct timing for such measures (Van-Emden, 1996). Chemical control of whiteflies must be based on knowledge of the life cycle and forecasts of population densities (Diana and Sannino, 1995). Central to any insect pest-monitoring programme is the sampling technique that is used to measure changes in insect abundance (Dent, 1991).

An understanding of the breeding habits and feeding sites of the pest is often necessary for selecting a sampling unit that is crucial for monitoring, forecasting and designing an IPM programme. The aims of this study were to examine the distribution of adult *B. tabaci* within- and between-plants. Information from this study could be utilized in developing efficient sampling methods and subsequently dependable monitoring tools.

4.2 Materials and Methods:

The investigations were undertaken between November 5, 1999 and January 16, 2000 in the University Farm, about 3 km from the center of the main university campus. It has Guinea Savannah vegetation with mean annual rainfall of 112 mm and a temperature of 32 ^{0}C.

The insect counts were made during the cooler hours of the day by gently turning over the leaves. The data were transformed to $\sqrt{(count + 0.5)}$ before the analysis (Steele and Torrie, 1960). Between-plant distribution patterns of whitefly were based on single plant counts, and within-plant distribution patterns on single leaf counts, which correspond to the "natural habitat units" for sampling as described by Patil and Stiteler (1974).

4.2.1 Within-plant distribution:

The counts were made from all leaves of 20 randomly selected plants (five weeks old) from each host. Counts of whitefly population on each leaf level were subjected to analysis of variance (ANOVA), and the means were separated using least significant difference test (LSD) at $P \leq 0.05$ (GENSTAT, 1995). Correlation analysis was used to select acceptable leaf-range as a sampling unit.

4.2.2 Between-plant distribution:

The counts were made from100 plants (started from 5 weeks old plants) of each host, randomly selected and arranged into 10 blocks (10 plants/block) of a 3 – day sequence.

4.2.2.1 Thc calculation of the sample size:

The number of plants needed to estimate the insect population from the sampling unit with a given level of error was calculated according to Southwood (1978) formula as follows:

$$n = [\, tS/DX]^2 \qquad (1)$$

Where n = number of plants required, t = student's t at $P < 0.05$, D = error as proportion of the mean, X = mean number of insects, and S = standard deviation of the sample.

4.2.3 Dispersion pattern analysis:

Taylor's power law method (Taylor, 1961) (2), (3) and Iwao's patchiness regression method (Iwao, 1968, 1977) (4) were used to examine within- and between- plant dispersion patterns,

$$S^2 = am^b \qquad (2)$$

$$\text{Log}_{10}(S^2) = \log(a) + b\log(m). \qquad (3)$$

Where S^2 is variance, m is mean number of each adult whitefly per leaf (within

plant) or per plant (between-plant), a, is largely a sampling factor related to sample unit size and b is a measure of aggregation. In Taylor's power law method, b >1, b = 1and b <1 was considered aggregated, random and uniform distribution respectively.

Iwao's patchiness regression method has shown that mean crowding (m*) is related to the mean (m) over a series of densities:

$$m^* = \alpha + \beta m \qquad (4)$$

In which m* is defined by Lloyd (1967) as:

$$m^* = m + (s^2/m - 1) \qquad (5)$$

Where s^2 = sample variance, m = sample mean. The intercept α is the index of basic contagion and β is the density contagiousness coefficient among sample units (Iwao, 1968). In Iwao's patchiness regression method, $\beta < 1$, $\beta = 1$ and $\beta > 1$ were considered aggregated, random and uniform distribution respectively.

4.3 Results:

4.3.1 Within-plant distribution:

The number and percentage of *B. tabaci* adults on each leaf position of cassava, tomato, garden egg and okra are presented in Table 3. The analysis of variance (ANOVA) tables for different leaf positions are presented in Appendices 12 - 16. On cassava the majority of adults were found on young, top (Leaves 1 2) and

upper, bottom (Leaves 7 - 9) leaves. On tomato, the majority of the insects were found on the upper half (Leaves 1 - 6) of the plant. On okra and the garden egg, the majority of the insects were limited to young and middle aged leaves (Leaves 3 - 5) and (Leaves 3 - 6) respectively.

Correlation coefficients between the counts of *B. tabaci* adults from selected leaf combinations with the whole plant counts on cassava, garden egg, tomato and okra are presented in Table 6.The results showed that the sampling units can be limited to the leaves that have the majority of the insects.

The indices of distribution patterns (Slope coefficients, b and β) of Taylor's and Iwao's methods and the linear coefficients of determination (r^2) of the regressions of within-plant distribution on cassava, tomato, garden egg and okra are presented in Table 4 and Appendices 16 - 23.

In Taylor's power law method, the slopes or the indices of aggregation (b) for *B. tabaci* adults ranged from 0.72 to 2.11. These were significantly greater than one on tomato and okra, but not significantly greater than one on garden egg (1.33) and not significantly less than one on cassava ($p \leq 0.05$ or $p \leq 0.01$, t-test at Ho: b = 1). Iwao's patchiness regression ranged from 0.59 to 1.76, which was significantly greater than one on garden egg (1.59) and on okra (1.76) ($p \leq 0.05$ or $p \leq 0.01$, t-test at Ho: $\beta = 1$). These results demonstrate that *B. tabaci* adults were aggregated within-plants on tomato, garden egg and okra and randomly distributed within cassava plants.

4.3.2 Between-plant distribution:

The distribution pattern indices for between plant distributions of *B. tabaci* adults on cassava; tomato, garden egg and okra are presented in Table 5 and Appendices 24 - 31. The linear coefficients of determination (r^2) ranged from 0.48 - 0.88 (Taylor's power method) and from 0.89 - 0.98 (Iwao's methods).

In Taylor's power law method the slopes (b) ranged from 0.867 to 2.56, which was only significantly greater than one ($p \leq 0.05$, t-test at Ho: $b = 1$) on okra (2.56). In Iwao's method the slopes (β) ranged from 0.943 – 1.54, which were significantly greater than one ($p \leq 0.05$, t-test at Ho: $\beta = 1$) on tomato and okra. These indicated that *B. tabaci* was aggregated between plants on okra and tomato.

4.3.2.1 The calculation of the sample size:

The sample sizes for estimating *B. tabaci* adult population on cassava, tomato, garden egg and okra; at different levels of error (0.25, 0.20, 0.15, 0.1 and 0.05) are presented on Table 7 Okra showed the largest sampling sizes (33 - 819) and cassava showed the least (10 - 157).

Table 3: Distribution of *B. tabaci* adults on cassava, tomato, garden egg and okra

	Crop							
	Cassava		Tomato		Garden egg		Okra	
Leaf level	Mean	%	Mean	%	Mean	%	Mean	%
1st	$7.2^{a} \pm 0.5$	44.0	$3.0^{c} \pm 0.7$	5.4	$1.0^{cd} \pm 0.3$	6.1	$0.3^{d} \pm 0.1$	0.5
2nd	$1.9^{b} \pm 0.3$	11.0	$8.0^{b} \pm 1.4$	15.0	$1.9^{bcd} \pm 0.6$	12.3	$6.0^{bc} \pm 2.6$	12.0
3rd	$0.7^{c} \pm 0.2$	4.0	$17.0^{a} \pm 3.0$	31.0	$2.5^{ab} \pm 0.7$	16.6	$8.0^{b} \pm 2.0$	16.0
4th	$1.0^{c} \pm 0.3$	5.8	$11.4^{a} \pm 2.0$	21.0	$2.7^{a} \pm 0.5$	17.6	$12.5^{a} \pm 3.0$	25.0
5th	$0.6^{c} \pm 0.2$	3.3	$5.5^{b} \pm 2.0$	10.0	$2.5^{ab} \pm 0.5$	16.2	$14.3^{a} \pm 2.0$	28.0
6th	$0.7^{c} \pm 0.2$	4.0	$3.5^{bc} \pm 0.6$	6.4	$1.7^{abc} \pm 0.4$	11.0	$6.3^{b} \pm 1.1$	12.0
7th	$1.1^{c} \pm 0.3$	6.4	$2.3^{c} \pm 0.7$	4.3	$2.0^{ab} \pm 0.6$	13.2	$3.0^{c} \pm 0.7$	6.5
8th	$1.1^{c} \pm 0.3$	6.7	$1.8^{c} \pm 0.5$	3.3	$1.1^{de} \pm 0.6$	7.0		
9th	$1.3^{cb} \pm 0.3$	7.6	$1.1^{c} \pm 0.4$	1.8	0.0^{e}	0.0	-	-
10th	$0.7^{c} \pm 0.3$	4.2	$1.1^{c} \pm 0.3$	1.8	-			-
11th	$0.5^{c} \pm 0.2$	3.0	-		-			-

* The means within a column followed by the same letter are not significantly different ($P \leq 0.05$, LSD).

The leaf levels are in descending order from the youngest to the oldest.

Table 4: The estimated values of indices of within-plant distribution of Taylor's power law and Iwao's regression for adult *B. tabaci* on cassava, tomato, garden egg and okra.

	No of	Taylor's power law		Iwao's regression	
Crop	data sets	b	r^2	ß	r^2
Cassava	20	0.72	0.18	0 59	0 30
Tomato	13	2.10*	0.73	1.45	0.29
Garden egg	15	1.33	0.82	1.59*	0.78
Okra	15	2.11**	0.91	1.76**	0.89

* Significant at P ≤ 0.05; ** Significant at P ≤ 0.01 (Ho: b = 1, ß = 1, t-test)

Table 5: The estimated values of indices of between-plant distribution of Taylor's power law and Iwao's regression for adult *B. tabaci* on cassava, tomato, garden egg and okra.

	No. of	Taylor's power law		Iwao's regression	
Crop	data sets	b	r^2	ß	r^2
Cassava	10	1.31	0.66	1.15	0.92
Tomato	10	1.50	0 81	1.13*	0.98
Garden egg	10	0.87	0.48	0.94	0.96
Okra	10	2 56*	0.72	1.54*	0.89

* Significant at P ≤ 0.05 (Ho: b = 1, ß = 1, t-test).

Table 6: Correlation coefficients (r-values) between selected leaves of the plant with the plant counts of *B. tabaci* adults on cassava, garden egg, tomato and okra.

Crop							
Cassava		Garden egg		Tomato		Okra	
Leaves	r-values	**Leaves**	r-values	**Leaves**	r-values	**Leaves**	r-values
(1 - 2)	0.17	**(3- 4)**	0.78	**(3- 4)**	0 67	**(4- 5)**	0.89
(1 - 2, 9)	0.23	**(3- 5)**	0.79	**(2- 4)**	0.76	**(3- 5)**	0.90
(1 - 2,8 - 9)	0.58	**(2- 5)**	0.88	**(2- 5)**	0.89	**(2- 5)**	0.98
(1-2, 7 - 9)	0.36	**(2- 6)**	0.91	**(2- 6)**	0.91	**(3- 6)**	0.95
(1 - 2, 7 - 9, 4)	0.76	**(3- 7)**	0.93	**(1- 6)**	0.92	**(2- 6)**	1.00
(1 - 2, 4, 7-10)	0.85	**(2-7)**	0.97	**(1-7)**	0.94		
(1 - 4, 7 - 10)	0.90	**(2- 8)**	0.99	**(1- 8)**	0.97		
(1 - 4, 6 - 10)	0.94	**(1- 8)**	1.00				
(1 - 10)	0.95						

The leaf levels are as indicated in Table 3.

Table 7: Sample sizes for estimating *B. tabaci* adult populations at different error levels; on cassava, tomato, garden egg and okra.

Crop	Error level				
	25%	20%	15%	10%	5%
Cassava		10	18	39	157
Tomato	11	18	31	70	282
Garden egg	26	40	71	160	716
Okra	33	51	91	205	819

4.4 Discussion:

The present study quantified the within-plant and between-plant distribution patterns of *B. tabaci* adults on cassava, garden egg, tomato and okra. *B. tabaci* was not uniformly distributed on leaves of the four plant species. The adults occurred mostly on young and old leaves of cassava. This was not different from reports by Liu *et al.* (1993) on whitefly populations on poinsettia plants grown in the greenhouse. Most *B. tabaci* adults occurred on young leaves {top (31.7%) and top middle (24.3%)} and old leaves {middle bottom (16%) and bottom (14.3%)} and the lowest percentage (13.7%) occurred on the middle leaves of poinsettia. On cotton, the youngest leaves irrespective of branch position were found to be the most preferred site for the adults (Krishna and Lingapa, 1992). In contrast to the above reports, it was observed that most adult whiteflies occurred on young and middle aged leaves of tomato and middle-aged leaves of garden egg and

okra. This could be attributed to the difference in crop type, growing conditions and/or age of crop.

B. tabaci adults were significantly aggregated within-plants on tomato (b = 2.10), garden egg (β = 1.59) and okra (b = 2.11, β = 1.76). Liu *et al.* (1993) reported that *B. tabaci* adults were significantly aggregated on poinsettia grown in greenhouse (b = 1.89, β = 1.26). In contrast, in this study *B. tabaci* adults exhibited significantly random distribution within cassava plants (b = 0.72, β = 0.59).

B. tabaci adults were significantly aggregated between-plants on tomato (β = 1.13) and okra (b = 2.56, β = 1.54). Liu *et al.* (1993) observed aggregated distribution of B. tabaci adults on poinsettia grown in greenhouse (b = 1.99, β = 1.83). In contrast, some of the results in this experiment showed random distribution of *B. tabaci* adults between cassava plants (b = 1.31, β = 1.15) and garden egg (b = 0.867, β = 0.943). *B. tabaci* adults are more aggregated within-plants than between-plants.

Sampling is important in warning growers of the first infestation of the pest, and the presence of damaging population levels and so helps in making informed decisions in pest management practices. It is also used to estimate the influence of control measures, in the study of insect population dynamics, dispersal activity and in relation to disease epidemiology.

Many scientists concentrated on the most infested leaves and used whitefly counts from this area to estimate, the density (number/plant). Gerling *et al.* (1980) used the most infested leaf plus two from the older and two from the younger leaves in nymph counts on cotton. Melamed-Madjar *et al.* (1982) used only the most infested leaves on cotton.

Liu *et al.* (1993) concluded that the vertical distribution of sweet potato whitefly adults was affected by the extremely high population infestation on the plants. Under this condition, the adults tend to translocate to lower leaves for feeding and oviposition. In this study a particular range of leaves comprised the sampling unit on the different crops. Hence the sample area is wide enough to reduce the effect of changes in within-plant distribution of *B. tabaci* due to plant age or time of year. However, the selection of sampling unit is governed by many factors, such as time available, finance and required level of accuracy. Leaves 2 - 6 (r-value = 0.91) on tomato, leaves 3 - 7 (r-value = 0.93) on garden egg, leaves 2 - 5 (r-value = 0.98) on Okra and leaves 1 - 4 and 7 10 (r-value = 0.90) on cassava can be selected as sampling units, otherwise other choices are available from correlation coefficients in Table 6. It was observed that the aggregated distribution of adults *B. tabaci* within-plant resulted in smaller sampling units with high r-values (Okra, garden egg and tomato) but the aggregated distribution between-plants resulted in large sampling size (okra) Table 7

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

The experiments revealed the presence of two *B. tabaci* biotypes. The cassava biotype found only on cassava, which could survive on cassava, garden egg and eggplant. The other biotype is the non-cassava biotype found on tomato, garden egg and coral plant, which could survive on all host crops tested except cassava.

Host plant-affiliation experiments showed that this test can only be effective if main host plants or ones from which *B. tabaci* are collected are used. The results of insecticide test agreed with previous work that show that variation in insecticide resistance depend on whether the samples are collected from treated or non-treated populations regardless of host plants. The population trends indicated that the apparently continuously distributed individuals could be relatively distinct populations. RAPD-PCR analysis exhibited a high potential in characterizing biotypes and can be employed in the future studies.

Studies are needed on the capabilities of the two biotypes to transmit ACMV and TYLCV. In resistant varietal trials the associated *B. tabaci* population for the particular crop should be used. The results of the population dynamics imply that in mixed cropping systems the *B. tabaci* population density should be estimated for each crop. The recent out breaks of *B. tabaci* in the country and its association with biotypes and insecticide use needs to be investigated. When characterizing biotypes on the basis of host plant-affiliation the use of the major host plants are

recommended. Despite the fact that *B. tabaci* is an economic pest of cotton worldwide, cotton fields in Ghana do not seem to suffer any whitefly infestations and the causes of this situation should be scientifically investigated.

An area of importance is the distribution of adult whitefly within- and between-plants. On cassava the majority of insects were found on top leaves and also some of the bottom leaves. On tomato the majority of insects were limited to the upper half of the plant. On okra and garden egg the majority of the insects were found on the middle-aged leaves. The correlation analysis between *B. tabaci* counts on selected leaf ranges and the whole plant counts indicated that sampling area could be limited to the leaves having the majority of the insects.

The *B. tabaci* adults were significantly aggregated within- tomato, garden egg and okra plants and between- tomato and okra plants. The size of the sampling unit was found to be affected by the dispersion patterns within- and between-plants. The aggregation within-plants reduced the sampling unit and between-plants increased the sampling size.

The relationship between the sampling unit (leaf range) and the sampling size (no. of plants)) needs to be established to determine which should have priority; especially when the resources are limited. That is to say if a large number of leaves/plant (high r-value) and smaller number of plants (high error level) should be used or vice-versa.

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Appendices

Appendix 1.

Analysis of variance of *B. tabaci* adult survival on cassava.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.031452	0.010484	1.10	
Reps.*Units* stratum					
Cassava	3	4.913339	1.637780	172.13	<.001
Residual	9	0.085632	0.009515		
Total	15	5.030424			

*Appendix 2.

Analysis of variance of *B. tabaci* adults percents survival on tomato.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.02301	0.00767	0.62	
Reps.*Units* stratum					
Tomato	3	3.70946	1.23649	100.03	<.001
Residual	9	0.11125	0.01236		
Total	15		3.84372		

Appendix 3.

Analysis of variance *B. tabaci* adults percents survival on coral plant.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.02459	0.00820	0.78	
Reps.*Units* stratum					
Coral plant	3	4.55072	1.51691	143.80	<.001
Residual	9	0.09494	0.01055		
Total	15	4.67025			

Appendix 4.

Analysis of variance for *B. tabaci* populations percentages survival on garden egg.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.05530	0.01843	0.69	
Reps.*Units* stratum					
Garden egg	3	0.57826	0.19275	7.22	0.009
Residual	9	0.24032	0.02670		
Total	15	0.87388			

Appendix 5.

Analysis of variance for *B. tabaci* populations percentages survival on eggplant.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.13717	0.04572	1.69	
Reps.*Units* stratum					
Eggplant	3	0.50217	0.16739	6.20	0.014
Residual	9	0.24280	0.02698		
Total	15	0.88214			

Appendix 6.

Analysis of variance for *B. tabaci* populations' percentages survival on cotton.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.04302	0.01434	0.49	
Reps.*Units* stratum					
Cotton	3	1.91184	0.63728	21.62	<.001
Residual	9	0.26526	0.02947		
Total	15	2.22013			

Appendix 7.

Analysis of variance for *B. tabaci* populations mortality percent due to water (control).

Variate: control (Transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.0006255	0.0002085	0.23	
Reps.*Units* stratum					
Plant	3	0.0006255	0.0002085	0.23	0.873
Residual	9	0.0081318	0.0009035		
Total	15	0.0093828			

Appendix 8.

Analysis of variance for *B. tabaci* populations' mortality percents due to Actellic.

Variate: Actellic (Transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.03987	0.01329	0.80	
Reps.*Units* stratum					
Plant	3	0.26677	0.08892	5.36	0.022
Residual	9	0.14936	0.01660		
Total	15	0.45599			

Appendix 9.

Analysis of variance for *B. tabaci* populations mortality percents due to Karate.

Variate: Karate (Transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.02725	0.00908	0.34	
Reps.*Units* stratum					
Plant	3	0.40591	0.13530	5.13	0.024
Residual	9	0.23724	0.02636		
Total	15	0.67040			

Appendix 10

Analysis of variance for *B. tabaci* populations' mortality percents due to Perfekthion.

Variate: Perfekthion (Transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	0.06211	0.02070	0.41	
Reps.*Units* stratum					
Plant	3	0.03341	0.01114	0.22	0.881
Residual	9	0.45699	0.05078		
Total	15	0.55251			

Appendix 11.

Population dynamics of *B. tabaci* adults; cassava, tomato and garden egg populations.

Weeks after germination.	Mean ± s.e. of insects/plant		
	Cassava	Tomato	Garden egg
4th	11.65 ± 1.14	6.85 ± 0.74	16.1 ± 1.14
5th	18.45 ± 2.57	2.85 ± 0.37	10.05 ± 2.57
6th	5.40 ± 0.84	2.95 ± 0.45	11.1 ± 0.84
7th	17.7 ± 1.63	2.25 ± 0.45	13.10 ± 1.63
8th	20.73 ± 1.83	6.47 ± .55	9.33 ± 1.83
9th	13.67 ± 1.32	31.33 ± 3.74	30.13 ± 1.32
10th	14.07 ± 1.12	2.20 ± 0.30	6.47 ± 1.12

Appendix 12.

Analysis of variance for *B. tabaci* adults counts on different leaf position on okra.

Variate: Okra

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	14	50.2133	3.5867	4.80	
Reps.*Units* stratum					
Leaf-position	6	73.1632	12.1939	16.33	<.001
Residual	84	62.7353	0.7468		
Total	104	186.1118			

Appendix 13

Analysis of variance for *B. tabaci* adults counts on different leaf positions on cassava.

Variate: Cassava

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	19	6.6341	0.3492	1.71	
Reps.*Units* stratum					
Leaf position	9	51.3805	5.7089	27.88	<.001
Residual	171	35.0145	0.2048		
Total	199	93.0291			

Appendix 14.

Analysis of variance of *B. tabaci* adults counts on different leaf positions on tomato.

Variate: Tomato

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	12	17.4436	1.4536	2.35	
Reps.*Units* stratum					
Leaf position	9	93.7954	10.4217	16.85	<.001
Residual	108	66.8011	0.6185		
Total	129	178.0401			

Appendix 15.

Analysis of variance for *B. tabaci* adults counts on different leaf positions on garden egg.

Variate: Garden eggs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
reps stratum	14	12.5874	0.8991	3.77	
reps.*Units* stratum					
Leaf position	8	13.3797	1.6725	7.01	<.001
Residual	112	26.7293	0.2387		
Total	134	52.6965			

Appendix 16.

Regression analysis for within-plant distribution of *B. tabaci* adults on cassava (Taylor's power law method).

The regression equation is

LOG VAR = 0.562 + 0.722 LOG MEAN

Predictor	Coef	StDev	T	P
Constant	0.56230	0.07789	7.22	0.000
LOG MEAN	0.7225	0.3686	1.96	0.066

S = 0.2411 R-Sq = 17.6% R-Sq(adj) = 13.0%

Appendix 17.

Regression analysis for within-plant distribution of *B. tabaci* adults on cassava (Iwao's patchiness regression method).

The regression equation is

M* = 3.33 + 0.594 MEAN

Predictor	Coef	StDev	T	P
Constant	3.329	1.270	2.62	0.017
MEAN	0.5941	0.8140	0.73	0.475

S = 1.614 R-Sq = 2.9% R-Sq(adj) = 0.0%

Appendix 18.

Regression Analysis for within-plant distribution of B. tabaci adults on tomato (Taylor's power law method)

The regression equation is

LOG VAR = - 0.006 + 2.10 LOG.MEAN.

Predictor	Coef	StDev	T	P
Constant	-0.0056	0.2808	-0.02	0.985
LOG MEAN	2.1045	0.3861	5.45	0.000

S = 0.2412 R-Sq = 73.0% R-Sq(adj) = 70.5%

Appendix 19.

Regression analysis for within-plant distribution of *B. tabaci* adults on tomato (Iwao's patchiness regression method).

The regression equation is

M* = 0.04 + 1.45 MEAN.

Predictor	Coef	StDev	T	P
Constant	0.042	4.031	0.01	0.992
MEAN	1.4498	0.6855	2.12	0.058

S = 5.200 R-Sq = 28.9% R-Sq(adj) = 22.5%

Appendix 20.

Regression Analysis for within-plant distribution of adults *B. tabaci* on garden egg (Taylor's power law method).

The regression equation is

LOG VAR = 0.185 + 1.33 LOG MEAN.

Predictor	Coef	StDev	T	P
Constant	0.18531	0.06095	3.04	0.009
LOG MEAN	1.3294	0.1759	7.56	0.000

S = 0.2192 R-Sq = 81.5% R-Sq(adj) = 80.0%

Appendix 21.

Regression analysis for within-plant distribution of *B. tabaci* adults on garden egg (Iwao's patchiness regression method).

The regression equation is

M* = - 0.056 + 1.59 MEAN.

Predictor	Coef	StDev	T	P
Constant	-0.0558	0.4543	-0.12	0.904
MEAN	1.5860	0.2335	6.79	0.000

S = 0.8780 R-Sq = 78.0% R-Sq(adj) = 76.3%

Appendix 22.

Regression Analysis for within-plant distribution of adults *B. tabaci* on okra (Taylor's power law method).

The regression equation is

LOG VAR = - 0.172 + 2.11 LOG MEAN.

Predictor	Coef	StDev	T	P
Constant	-0.1719	0.1524	-1.13	0.280
LOG MEAN	2.1132	0.1818	11.63	0.000

S = 0.1724 R-Sq = 91.2% R-Sq(adj) = 90.6%

Appendix 23.

Regression Analysis for within -plant distribution of B. tabaci adults on okra (Iwao's patchiness regression method).

The regression equation is

M* = 0.02 + 1.76 MEAN.

Predictor	Coef	StDev	T	P
Constant	0.020	1.480	0.01	0.989
MEAN	1.7587	0.1690	10.41	0.000

S = 3.001 R-Sq = 89.3% R-Sq(adj) = 88.5%

Appendix 24.

Regression analysis for between-plant distribution of *B. tabaci* adults on cassava (Taylor's power law methed).

The regression equation is

LOG VAR. = 0.083 + 1.31 LOG MEAN.

Predictor	Coef	StDev	T	P
Constant	0.0828	0.3705	0.22	0.829
LOG MEAN	1.3146	0.3335	3.94	0.004

S = 0.2297 R-Sq = 66.0% R-Sq(adj) = 61.8%

Appendix 25.

Regression analysis for between-plant distribution of B tabaci adults on cassava (Iwao's patchiness regression method)

The regression equation is

M* = 1.29 + 1.15 MEAN.

Predictor	Coef	StDev	T	P
Constant	1.289	1.879	0.69	0.512
MEAN	1.1541	0.1296	8.90	0.000

S = 2.072 R-Sq = 90.8% R-Sq(adj) = 89.7%

Appendix 26.

Regression analysis for between-plant distribution of *B. tabaci* adults on okra (Taylor's power law method).

The regression equation is

LOG VAR. = - 1.34 + 2.56 LOG MEAN.

Predictor	Coef	StDev	T	P
Constant	-1.3357	0.8494	-1.57	0.154
LOG MEAN	2.5592	0.5618	4.56	0.000

S = 0.3030 R-Sq = 72.2% R-Sq(adj) = 68.7%

Appendix 27.

Regression Analysis of between-plant distribution of *B. tabaci* adults on okra (Iwao's patchiness regression method)

The regression equation is

M* = 3.76 + 1.54 MEAN.

Predictor	Coef	StDev	T	P
Constant	-3.755	7.176	-0.52	0.615
MEAN	1.5392	0.1928	7.98	0.000

S = 8.668 R-Sq = 88.9% R-Sq(adj) = 87.5%

Appendix 28.

Regression analysis for between-plant distribution for *B. tabaci* adults on tomato (Taylor's power law method)

The regression equation is

LOG VAR. = - 0.113 + 1.50 LOG MEAN.

Predictor	Coef	StDev	T	P
Constant	-0.1128	0.1801	-0.63	0.548
LOG MEAN	1.4972	0.2563	5.84	0.000

S = 0.2916 R-Sq = 81.0% R-Sq(adj) = 78.6%

Appendix 29.

Regression analysis for between-plant distribution of B. tabaci adults on tomato (Iwao's patchiness regression method).

The regression equation is

M* = 2.02 + 1.13 MEAN.

Predictor	Coef	StDev	T	P
Constant	2.0161	0.5846	3.45	0.009
M(Tomato	1.12552	0.05065	22.22	0.000

S = 1.514 R-Sq = 98.4% R-Sq(adj) = 98.2%

Appendix 30.

Regression analysis for between-plant distribution of B. tabaci adults on garden egg (Taylor's power law method).

The regression equation is

LOG VAR. = 0.550 + 0.867 LOG MEAN.

Predictor	Coef	StDev	T	P
Constant	0.5500	0.3499	1.57	0.155
Log MEAN	0.8670	0.3206	2.70	0.027

S = 0.2018 R-Sq = 47.8% R-Sq(adj) = 41.2%

Appendix 31.

Regression analysis for between-plant distribution of *B. tabaci* adults on garden egg (Iwao's patchiness regression method).

The regression equation is

M* = 3.86 + 0.943 MEAN.

Predictor	Coef	StDev	T	P
Constant	3.864	1.010	3.82	0.005
M(Gar Eg	0.94261	0.06616	14.25	0.000

S = 1.561 R-Sq = 96.2% R-Sq(adj) = 95.7%