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FINAL REPORT

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July 1987 - July 1990

Submitted to the office of:

The Science Advisor, U.S. Agency for International Development

Title of project:

IDENTIFICATION AND CHARACTERIZATION OF GENETIC STRAINS OF *BEMISIA TABACI* IN ISRAEL AND COLOMBIA

Principal investigators	Institution
Prof. David Wool	Tel Aviv University
Prof. Den Gerling	Tel Aviv University
Dr. Anthony C. Bellotti	CIAT, Colombia
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Project number: C7-077

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Project full title:

IDENTIFICATION AND CHARACTERIZATION OF GENETIC STRAINS AND SPECIES OF WHITEFLIES (ALEYRODIDAE, HOMOPTERA) IN ISRAEL AND COLOMBIA.

EXECUTIVE SUMMARY

In this study we obtained convincing evidence for genetic differentiation (measured by electrophoresis) among local (geographical) populations of the whitefly, *Bemisia tabaci*, in Colombia. These differences are undetectable by conventional taxonomic procedures, but their existence implies that *B. tabaci* is genetically differentiated and different variants may respond differently to control measures.

This finding is very important since the entire global population of *B. tabaci* is considered one and the same species. The presence of intraspecific differentiation should be taken seriously when quarantine measures are considered.

In Israel there was no indication of intraspecific differentiation according to host-plant or geography, but there was a clear difference in enzymatic pattern between samples from insecticide-treated commercial fields and samples from unsprayed control populations. Their difference may be used in monitoring the frequencies of resistant individuals in field populations.

In addition to the analysis of genetic variation within one species, we have developed electrophoretic markers which can be used to identify adults of different whitefly species in Israel and in Colombia. Whitefly taxonomy is based on morphological characters of the pupal case, and adults of many species of whiteflies are indistinguishable morphologically. The use of electrophoresis for this purpose can help field operators to correctly identify adult whiteflies which may endanger crops by transmission of viral diseases. (We also have preliminary indication that adults of different

species may be identified morphologically using the Scanning Electron Microscope (SEM), where and when this service is available).

The project was carried out in full cooperation between Israeli and Colombian (CIAT) scientists. The electrophoretic techniques developed in Israel were transferred to CIAT and used with skill by the local personnel. (The SEM study was done in Colombia only).

As can be seen from the results, to be reported below, this was a very fruitful cooperative study which laid the foundation for further cooperative work in the future.

FOREWARD

Research objectives

This report summarizes three years of cooperative research (1987-1990) on genetic differentiation of whitefly populations (*Bemisia tabaci* Genn.) in Israel and Colombia.

As we explain more fully below, whiteflies are identified by taxonomists using "pupal" morphological characters. Adults of different species are often difficult or impossible to tell apart. Adults are vectors of serious plant viral diseases and their correct identification is crucial for effective control. Moreover, different vector capacities of geographical populations of one species - *Bemisia tabaci* - raised the suspicion that this whitefly is genetically different in different localities and countries, although the taxonomist is unable to detect the differences.

The objectives of this research were to: (1) develop methods for simple identification of species, strains and varieties of *Bemisia tabaci* in Israel and Colombia; (2) search for genetic markers and obtain quantitative measures of genetic variation in whitefly populations on different host plants and from different regions of the two countries (using electrophoresis); (3) study the ability of these insects to colonize new hosts and their insecticide resistance.

Objectives (1) and (2) were followed in detail. Objective (3) was not pursued intensively, due to the heavy work load, but important information was collected during the 3-year study in Israel on the relationship of insecticide resistance with electrophoretic variation.

SCIENTIFIC BACKGROUND

Economic importance

B. tabaci has been known to entomologists for many years, but became a

primary pest of cotton relatively recently - first reported in the Sudan in the sixties, but running out of control in 1980-1981 (Dittrich, Hassan & Ernst 1985, 1986). At that time control problems were reported in California (Prabhaker, Coudriet & Meyerdirk 1985). In Israel *B. tabaci* was known from cucurbits and solanaceous crops, but became a serious pest of cotton in 1975 (Gerling, Motro & Horowitz 1980; Gerling 1984). Jayaraj et al. (1986) report that at about the same time, it became a major cotton pest in India. In all these cases, the use of pesticides to control other cotton pests is blamed for the outbreaks (Ahmed, Elhaj & Bashir 1987; Dittrich 1987; Dittrich & Ernst 1990; Prabhaker, Coudriet & Meyerdirk 1985; Jayaraj et al. 1986). *B. tabaci* is still a major threat to crops in all these areas, despite heavy use of pesticides and continuous efforts to use biological control (Gerling 1984, 1985; Byrne, Bellows & Parrella 1990; Onillon 1990; Dittrich & Ernst 1990).

Whitefly damage is caused by direct feeding of the multitude of immatures and adults on the plants and by contamination of the crop (especially lint) with sticky honeydew, a favorable substrate for molds (e.g., on cotton, melons, beans, tomatoes and peppers). The transmission of viral diseases by whiteflies, e.g., in tomatoes and cassava, is sometimes more costly and damaging than direct feeding or contamination of the crop. Other than commercial crops, *B. tabaci* lives on a wide range of ornamental plants and weeds (Cock 1986).

For a recent compilation of information on whiteflies see Gerling (1990).

Intra-specific differentiation

The taxonomy and identification of whiteflies is based on the morphology of the pupal case (Martin, 1987), although some studies have shown that pupal characters may vary within species among host plants and is affected by environmental factors (e.g., Mohanty & Basu 1986). Taxonomically, the world populations of *B. tabaci* from India, Africa, Europe and The Americas, are

considered one and the same species. However, the insects in different countries appear to differ in biological characteristics including fecundity, developmental rates and host range (Costa & Russell 1975; Gerling, Horowitz & Baumgartner 1986). Bird (1982 and earlier), described differences in virus transmission properties among *B. tabaci* populations in Latin America, and suggested that these populations may represent distinct "biotypes". Recently, Robertson (1987) reported that cassava (*Manihot esculenta*) varieties, imported from Brazil, were heavily infected with *Bemisia*-borne viruses in Kenya, resulting in severe yield losses. The same varieties are not infected in their native Brazil. This suggests that the difference in disease frequency is due to different properties of the vector, *B. tabaci*, in the two countries.

All these facts suggest that, worldwide, *B. tabaci* may, in fact, be an aggregate of strains or races, morphologically indistinguishable but genetically different from each other. This was our working hypothesis.

We suggested that, in the absence of morphological characters, we should use electrophoresis in search of genetic differentiation within *B. tabaci* in Israel and Colombia. These methods have been used extensively in the last 20 years for genetic surveys of natural populations and over 1,100 species of plants and animals have been analyzed (see reviews by Nevo 1978, 1983; Nevo, Beiles & Ben Shlomo 1984). In particular, we noted the potential of these methods for taxonomy (Berlocher 1979). "Cryptic" strains and species of insects were detected by electrophoresis, where morphology failed to detect them (e.g., in *Simulium*, Snyder 1982. In butterflies, Brittnacher, Sims & Ayala 1978, Geiger & Scholl 1985; In weevils, Hsiao & Stutz 1985; In bees, Snyder 1977; In aphids, Steiner *et al.* 1985, Loxdale, Castanera & Brooks 1983, Tomiuk, Woehrmann and Eggers-Schumacher 1979).

These considerations led us to the design of the work plan for the project.

PROJECT DESIGN

The project was designed so that work could be carried out in parallel in Israel and at CIAT, Colombia, using similar equipment and identical methods. In this way, the results would be comparable.

To coordinate the research the principal Israeli participant (Prof. David Wool) traveled twice to CIAT. The first visit, in the summer of 1987, before the work actually began, lasted a few days and was used for discussions of the research program with the CIAT collaborators (Dr. Bellotti, Dr. Morales, Dr. Nolt and the director of CIAT, Dr. Laing). Details of the electrophoretic techniques which were developed at TAU were passed on to the CIAT personnel for use in the program. In the year that followed, CIAT hired a qualified technician to do the work (Mr. L.M. Constantino) and the first electrophoretic results were sent over to Prof. Wool in Israel.

The second visit, in the summer of 1988, lasted 4 weeks and was used for two purposes: (1) to review the results with the CIAT technician and plan the next two years of research, (2) for collection trips to different parts of Colombia, to get samples of *B. tabaci* (and other whiteflies) from diverse localities and different host plants. All the samples were stored in deep-freeze and analyzed subsequently. Additional field trips were taken by the CIAT personnel as necessary.

Meanwhile in Israel, efforts concentrated on collecting and analyzing *B. tabaci* populations from different parts of the country and on many host plants. The samples were analyzed electrophoretically, in search for genetic differences at three levels (1) geographically - related differentiation, (2) host-plant-related differentiation within localities, (3) temporal variation within localities and hosts.

MATERIALS AND METHODS: GENERAL

Collection

Adult *B. tabaci* were aspirated off the host plants. In some cases, in Israel, leaves of host plants infested with pupae were collected and held in small plastic cages in the laboratory. Emerging adults were collected. Samples were deep-frozen at -20°C upon arrival in the laboratory. In Colombia, where distances are larger than in Israel, a 10-l liquid nitrogen container was purchased and carried along during collection trips. Collected whiteflies were immediately stored in the container.

Electrophoresis

Each adult was separately homogenized in 10 microliters of grinding solution (containing 10% sucrose and some bromophenol blue as front-running marker dye) and then inserted in the gel pockets.

Usually 8-10 individuals of a given sample were run on a single gel. Two central pockets were loaded with homogenates of individuals from a standard control population (see below) for comparison of the migration distances of isozymes.

Vertical Acrylamide slab-gel electrophoresis was used throughout the study. The same recipes and procedures were followed at TAU and at CIAT. The electrophoretic apparatus at CIAT was a Bio-Rad minigel set (Mini Protean II). In Israel we used a locally built apparatus, and purchased a Bio-Rad later in the study. Two running buffers were used: 0.155 M Borate buffer, pH 8.2, and 0.1 M Citrate buffer, pH 7.0 (Table 1). Gel and electrode buffers were identical.

Gels were generally run for 2 hours (Borate buffer) or 3-4 hours (Citrate buffer) using constant current of about 40mA per gel (voltage varied around 100V).

Table 1: Electrophoretic recipes (summarised)

	Running buffer	components	pH
Esterase	0.15 M Borate	Boric acid; NaOH	8.2
Dehydrogenases	0.1 M Citrate	Citric acid; NaOH	7.0
Staining buffers		**substrates/stains**	
Esterase :	0.1 M Phosphate		6.5
		α - Naphthyl Acetate	
		β - Naphthyl Acetate	
		Fast Blue R R	
α - Glycerophosphate dehydrogenase	0.1 M Tris-Citrate	α- Glycerophosphate/ NAD,MTT,PMS	8.0
Malate dehygrogenase	0.1 M Tris-Citrate	Sodium malate/ NAD,MTT,PMS	8.0
Malic Enzyme	0.1 Tris-citrate	Sodium malate/ NADP,MTT,PMS	8.0

Several enzyme systems were stained. Four systems gave good staining: Esterase (EST), α-Glycerophosphate dehydrogenase (α- GPDH), Malate dehydrogenase (MDH), and Malic enzyme (ME) (Table 1). Most of the effort was spent on analyzing EST; The other systems appeared mostly monomorphic within species and whatever variation was detected, turned out to be inconsistent and very difficult to analyze. However, α- GPDH was useful for between-species discrimination (see section IV in this report).

EST gels were fixed after staining, using a 1:5:5 mixture of Acetic acid, Methanol and water. Fixed gels were sealed in plastic bags. Dehydrogenase gels were not fixed because the blue stain fades in the fixative. The gels were read on an illuminated table, and the frequencies of the different isozymes recorded. The data were then stored on diskettes for analysis with an IBM-PC computer.

RESULTS

PART 1. ESTERASE VARIATION IN ISRAEL

More than 700 samples were collected in Israel during the 3 years of this project. About 15,000 individual whiteflies were analyzed by electrophoresis. A detailed list of sampling sites and host plants is given in the Appendix (Table A1).

We chose the population collected on *Lantana* in Ramat Hasharon as the control for the Israeli samples. This population was chosen because it is homogenous at the EST system, with most individuals FF homozygotes (see below). Two adults of this population were run on each gel.

a. Description of the EST isozyme pattern in Israeli *B. tabaci*

Fig. 1 illustrates the pattern of EST isozymes in Israeli *B. tabaci*. The pattern consists of a major isozyme system, which seems to be controlled by a single genetic locus with two alleles - fast (F) and slow (S), forming 3 genotypes: FF (a single fast-migrating band), SS (a single slow-migrating

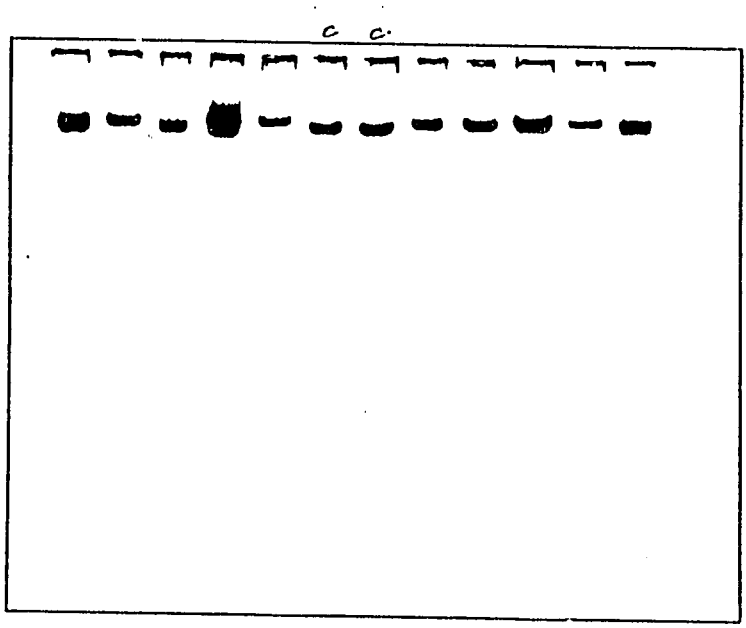

Fig. 1. Esterase pattern of Israeli *B. tabaci*: cells 1-5, 8-10 cotton, Beit Guvrin, 1987; cells 6-7, Lantana, Ramat Hasharon (control). From left: FS, S, FS, FS, S, F, F, S, S, FS, S, FS.

band), and FS (one band of each type). The difference in migration distance between F and S bands was small and sometimes difficult to observe in FS heterozygotes (Fig. 1). In addition, other isozymes were sometimes observed. A very fast (ff) band was weak and observed only on very good gels. It appears to be controlled by a variable locus with at least 2 alleles, but due to its weak staining we do not have proof for this. Other bands did occur at low frequency (weak slow [ws], very slow [vs], weak fast [wf]). The present analysis deals with allele frequencies at the major locus only.

Substrate affinity of FF and SS. When both α- and β- Naphthyl Acetate were used in the staining for EST, at a ratio of $\beta:\alpha$ = 1:9, the fast (F) band usually stained red (i.e., preferred the β- configuration substrate) and the slow (S) band stained black (preferred the α- configuration).

b. Host-plant differentiation in Israel

B. tabaci was collected from many host plants during the 3 years of study. Some hosts were infested by immatures as well as adults and supported breeding populations. On other hosts only adults were found at the time of sampling, and these could have been transient populations (Table 2).

Table 3 summarizes the frequencies of the three genotypes SS, FS and FF, on different host plants in Israel, and the gene frequency of F. Host plants from which only small samples were collected are not listed. The Solanaceae and Cucurbitaceae, from each of which several agricultural crops were sampled occasionally, are pooled and presented as groups. Figure 2 is a graphic summary of the Table.

Figure 2 is plotted in triangular coordinates (a De Finetti diagram), in which the perpendicular distances from each point to the labeled sides of the equilateral triangle are proportional to the frequencies of the three genotypes FF, FS, and SS (the sum of the 3 frequencies equals 1.00, the height of the triangle). The assemblages of points representing expected equilibrium

Table 2. Host plants for *B. tabaci* in the present study in Israel. Wild plants are listed by their generic Latin names. Commercial plants are listed by common names.

* Indicates a breeding population (presence of immatures of *B. tabaci* on that host).

Family	Host Plants
Malvaceae	Cotton*; *Malva*; *Tespesia*
Euphorbiaceae	*Poinsettia**
Labiatae	*Mentha*
Amaranthaceae	*Amaranthus*
Compositae	Sunflower; *Sonchus**; *Inula**
Cucurbitaceae	Melon*; Squash
Solanaceae	*Solanum*; Eggplant*; Tomato
	Pepper; Potato; *Datura*; *Abutilon*
Convulvulaceae	*Ipomoea*
Verbenaceae	*Lantana**; *Vitex*

frequencies (Hardy-Weinberg equilibrium) form a parabola (Figure 2).

Scanning Table 3, it becomes apparent that samples from agricultural crops (marked * in the Table) contain lower frequencies of FF individuals and higher frequencies of SS than samples from other host plants. This is expressed also in the allele frequencies of F. (We shall deal with the relationship of allele frequencies and insecticide treatment of the agricultural crops in the Discussion). The laboratory cotton population, and samples from *Lantana*, *Tespesia*, and *Poinsettia* contain the highest frequency of F.

The distributions of individuals in the three genotypic classes was very heterogeneous among host plants. This was tested for significance using G-tests for heterogeneity (Sokal & Rohlf 1981). For the 14 host plants listed in Table 3, G = 2720.86 (26 df, P << 0.001). When the five non-commercial host-plants were tested separately, G = 246.16 (8 df, P << 0.001). Even the two hosts which are located close together on Fig. 2 - *Poinsettia* and laboratory cotton - were significantly different from each other (G = 23.76, 2 df, P < 0.001) probably because sample sizes are very large. The 9 remaining samples were also very heterogeneous (G = 333.84, 16 df, P << 0.001).

Of particular importance is the fact that field samples from cotton (no. 4 in Table 3 and Fig. 2) and laboratory cultures reared on cotton (no. 14) were very highly significantly different in genotype frequencies (G = 1129.60, 2 df; P <<< 0.001). These data indicate that the heterogeneity is <u>not</u> due to the host-plant characteristics.

In Table 3, and in Fig. 2, the frequencies of the genotypes were pooled by host plant from different localities and collection dates. Spacial and temporal differences in allele frequencies could, therefore, be confounded with the differences due to the host plant. In order to focus on differentiation according to host plant, we concentrated on a single locality

Table 3. Frequencies of genotypes at the major EST locus. Host-plant differentiation (all localities and dates pooled). Only plants with sufficient sample sizes are listed. (All Cucurbitaceae, Labiatae and most Solanaceae were pooled in one category).

* commercial fields only ° control areas only

Host plant	Total individuals	inactive	FF	FS	SS	Freq.F
1. *Malva*°	123	8	.552	.191	.278	.618
2. Sunflower*	303	13	.197	.145	.648	.270
3. Solanaceae*	453	57	.361	.106	.532	.414
4. Cotton (field)	2454	183	.319	.094	.583	.366
5. *Lantana*°	3531	195	.788	.081	.132	.828
6. *Inula*	169	51	.347	.136	.508	.415
7. *Ipomoea*°	171	9	.580	.086	.340	.623
8. *Poinsettia*°	582	29	.962	.007	.034	.966
9. *Amaranthus*	106	17	.416	.135	.438	.483
10. Cucurbitaceae*	818	89	.514	.111	.373	.570
11. *Datura*°	297	5	.716	.062	.216	.747
12. *Tespesia*°	76	0	.789	.040	.171	.809
13. Labiatae	86	10	.303	.224	.421	.415
14. Cotton (laboratory)	1104	50	.898	.043	.055	.920

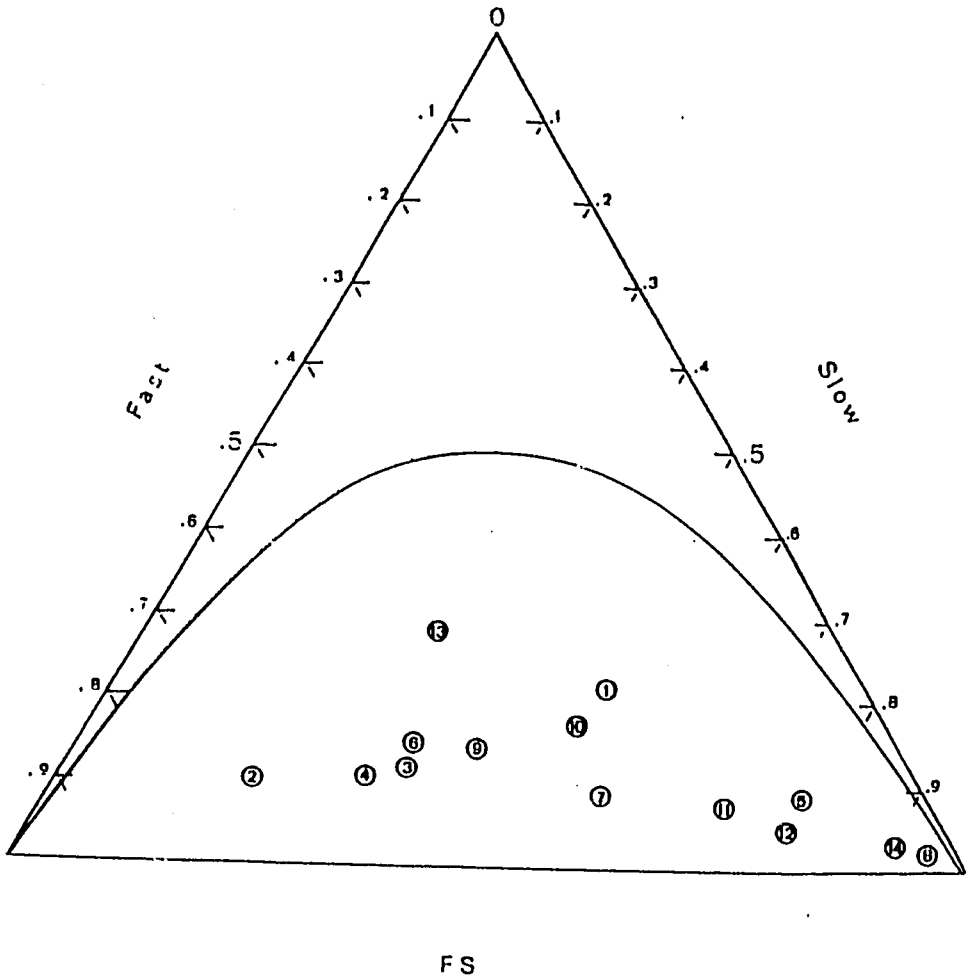

Fig. 2. Frequencies of EST genotypes of *B. tabaci* on different host plants in Israel, plotted in triangular coordinates. Data from Table 3. The perpendicular distances of a point from the sides labeled fast, slow, and FS are proportional to the frequencies of these genotypes in the sample. Sample sizes are indicated in Table 3. 1, *Malva*; 2, sunflower; 3, Solanaceae; 4, cotton (field); 5, *Lantana*; 6, *Inula*; 7, *Ipomaea*; 8, *Poinsettia*; 9, *Amaranthus*; 10, Cucurbitaceae; 11, *Datura*; 12, *Tespesia*; 13, Labiatae; 14, cotton (laboratory).

- Ramat Hasharon - in which several host plants were sampled (Table 4). In this locality, no insecticides were applied to any of the host plants during the study period, and the physical environmental conditions were the same for all hosts (located within a 1-Km^2 area). And in fact, genotype frequencies on different hosts are much more similar to each other than in the previous Table (Fig. 3 illustrates these relationships in triangular coordinates). However, the frequencies of the genotypes on different hosts are still significantly different ($G = 141.34$, 10 df; $P < 0.001$). In particular, *Poinsettia* stands out with a very high frequency of F. *Poinsettia* is an ornamental plant, and is the only representative of the Euphorbiaceae sampled in Israel.

Removal of *Poinsettia* from the analysis still left the other five hosts significantly different from each other ($G = 53.48$, 8 df; $P < 0.001$).

Another fact which becomes apparent from Figures 2 and 3 is the scarcity of heterozygotes FS in the samples, compared with Hardy-Weinberg expectations (parabola in the figures). The breeding system of the Aleyrodidae is haplo-diploid, i.e. only females can be heterozygotes. But even when this is taken into account and assuming a 1:1 sex ratio (lower parabola), the frequencies of FS are still lower than expected.

Despite the highly significant differences in genotype frequencies among host plants, these data show no indication of host-race formation. Populations on different hosts differed in genotype frequencies, but in no case was an allele fixed on any host plant (the only exception may be *Poinsettia*, where the F allele is close to fixation). This conclusion agrees with our interim report (Wool 1990) which was based on smaller samples.

c. Geographical differentiation of B. tabaci populations in Israel

A list of the collection sites in Israel, and the host plants sampled at each site, is given in the Appendix (Table A1). To avoid excessive detail on the accompanying map (Fig. 4), the collection sites were grouped by

Table 4 Frequencies of isozymes at the major EST locus: Ramat Hasharon data only. Arranged by host plant.

Host plant	Total	Inactive	Frequencies FF	FS	SS	Freq. F
1. *Tespesia*	169	7	.784	.062	.154	.815
2. *Poinsettia*	527	28	.950	.008	.046	.954
3. *Malva*	76	7	.768	.072	.145	.804
4. *Lantana*	2864	137	.813	.079	.110	.852
5. *Ipomoea*	121	7	.666	.026	.298	.679
6. *Datura*	297	5	.716	.062	.216	.747

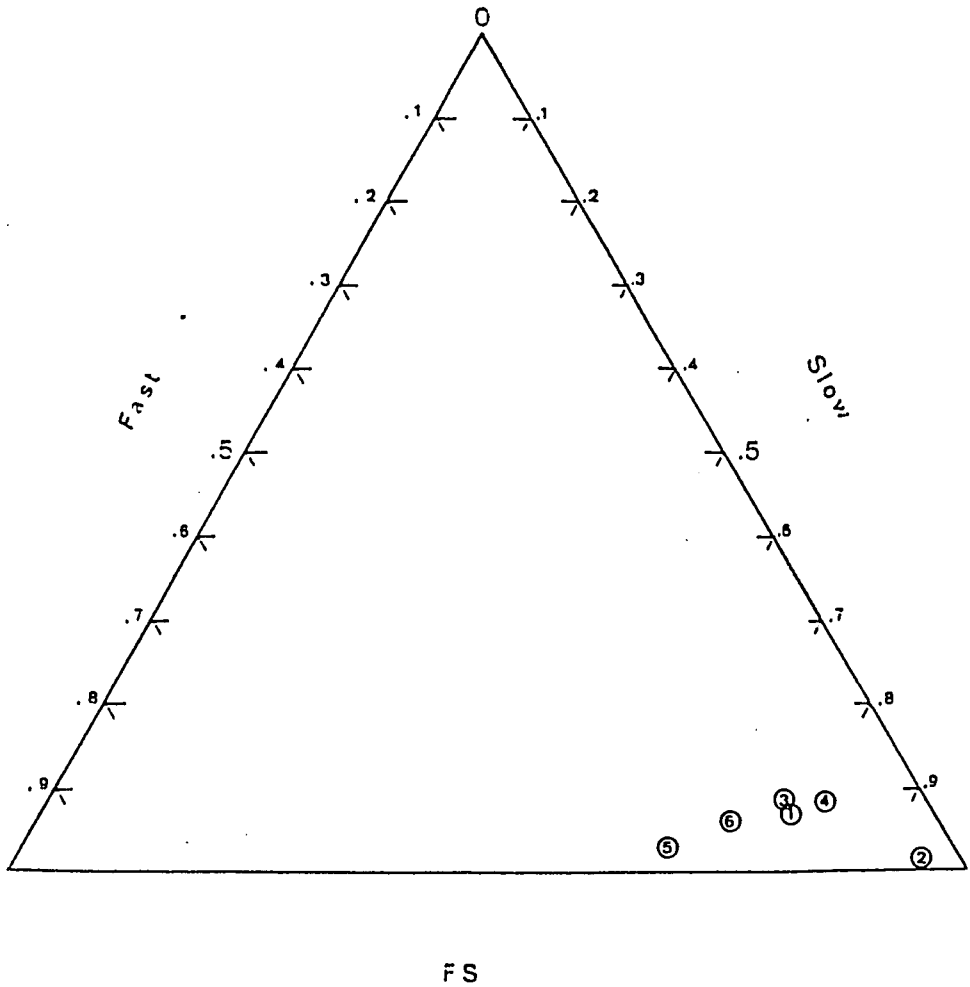

Fig. 3. Frequencies of EST genotypes on different host plants within one locality (Ramat Hasharon). Data from Table 4, plotted in triangular coordinates as in Fig. 2.

geographical proximity into 9 regions, which are marked on the map by Roman numerals. Only 2 sites, from which large numbers of whiteflies were sampled, are marked individually: Ramat Hasharon (X), a control population, and Tzora (XI), the site of intensive sampling from commercial cotton fields (see also Wool & Greenberg 1990).

Table 5 summarizes the genotype frequencies at the major EST locus in some sites from which reasonably large samples were analyzed. All but one of the sites are located in agricultural areas (the exception being Ramat Hasharon, where *B. tabaci* was collected from *Lantana* hedges surrounding ornamental gardens). All host plants were pooled within sites. In some regions, several small samples were pooled together.

There were considerable differences in genotype frequencies among sites. The frequency of F varied from a low of .174 to a high of .767 in agricultural fields, and of .850 in Ramat Hasharon. The distributions of the 3 genotypes were very heterogeneous among sites (G = 2205.69, 38 df; P <<< 0.001), and even when Ramat Hasharon was not included, the heterogeneity was still highly significant (G = 418.32, 36 df; P <<< 0.001). Generally the frequencies of F were quite low in the agricultural areas. The slow (SS) genotype clearly predominated there (Fig. 5).

There was some geographical trend in the frequencies of the genotypes: for example, samples from sites 6, 11-15, and 7 are arranged "linearly" in Fig. 5. The Upper Galilee sample (6) was geographically the farthest to the north, the Arava samples (7) the farthest to the south, and samples (10-14) are located geographically between them. However, if we ignore sample 7 and add the rest - all of which are located to the north of (10-14) and south of (6) (Fig. 4a) the pattern is no longer apparent and high and low frequencies of F are scattered with no obvious relationship to geography.

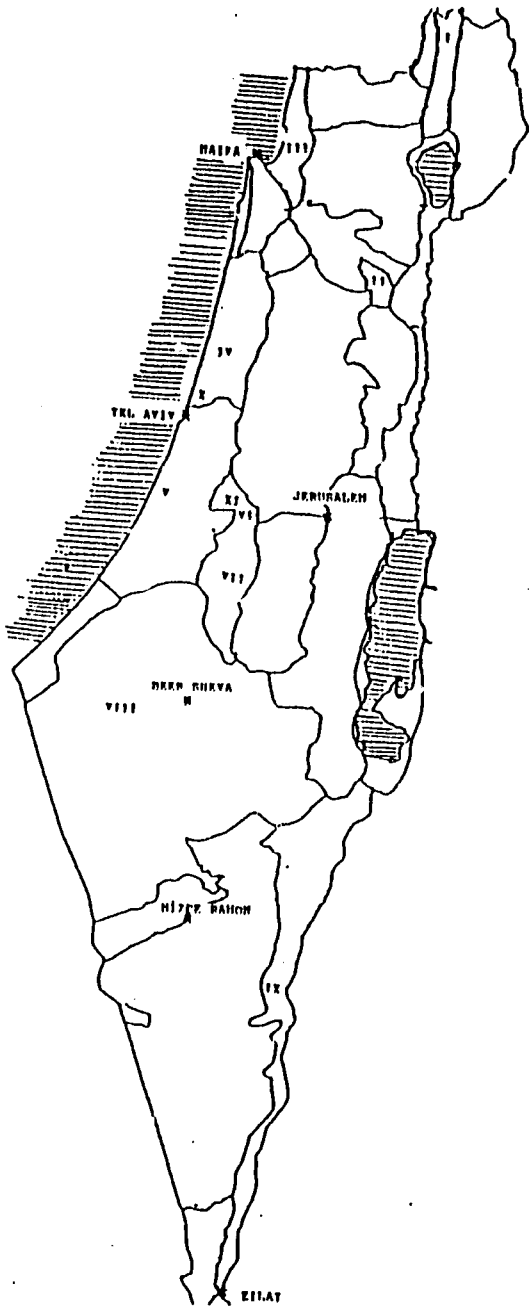

Fig. 4. Map of Israel showing the collection areas. Roman numerals correspond to regions indicated in the Appendix (Table 1A), where a detailed list of collection sites and host plants collected from is given.

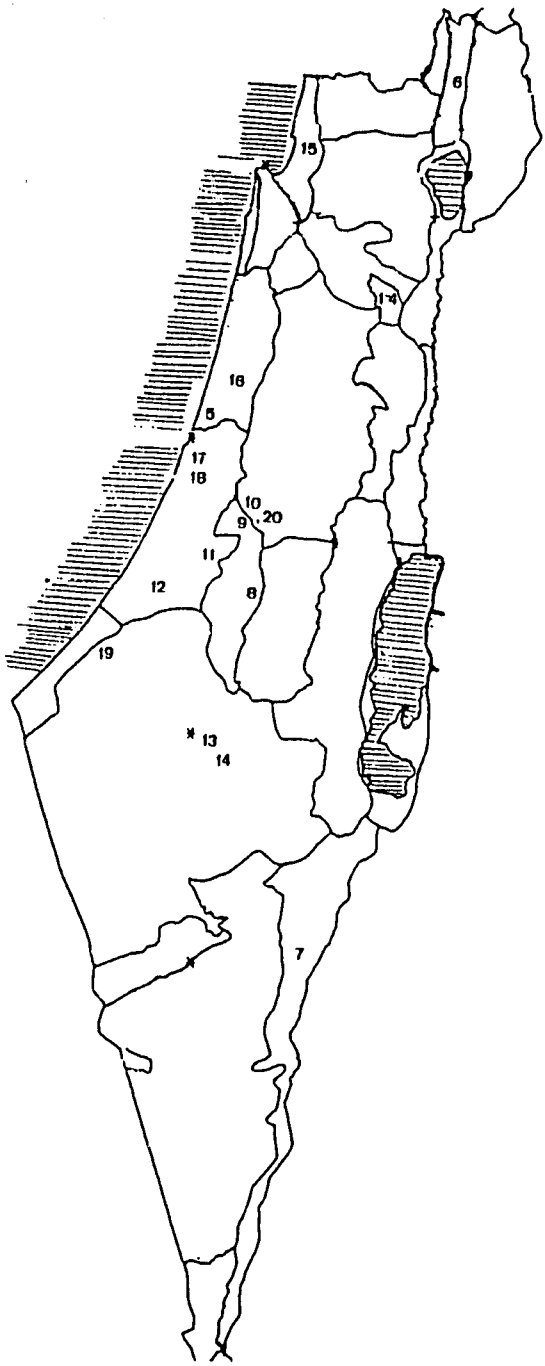

Fig. 4A. Map of Israel showing the location of 20 collection sites, listed in Table 5. 1, Tirat Zvi; 2, Sde Eliyahu; 3, Eden; 4, Reshafim; 5, Ramat Hasharon; 6, Upper Galilee; 7, Arava valley; 8, Beit Guvrin; 9, Tzora; 10, Ayalon valley; 11, Masmia; 12, Gvar Am; 13, Beer Sheva; 14, Nevatim; 15, Western Galilee; 16, Sharon; 17, Beit Hanan; 18, Givat Brener; 19, Kfar Aza; 20, Kfar Uriah.

Table 5: Frequencies of isozymes at the major EST locus - arranged by sites (all host plants and dates pooled). Not all sites are listed.
* commercial fields. ° control.

Locality	Total	Inactive	Frequencies FF	FS	SS	freq. F
1. Tirat Zvi*	61	18	.744	.046	.209	.767
2. Sde Eliyahu*	365	41	.460	.043	.503	.482
3. Eden*	805	46	.548	.112	.327	.604
4. Reshafim*	93	5	.489	.102	.409	.540
5. Ramat Hasharon°	4160	217	.817	.065	.118	.850
6. Galilee (upper)*	64	15	.102	.143	.755	.174
7. Arava valley* (all sites)	688	41	.379	.176	.439	.467
8. Beit Guvrin*	160	24	.353	.184	.456	.445
9. Tzora*	993	64	.350	.075	.575	.388
10. Ayalon (Nachshon Gezer, Barechia,Latrun,Azaria)*	355	30	.185	.114	.701	.242
11. Masmia, Kiriat Gat* Ashdod, Sde Yoav	198	13	.221	.097	.665	.270
12. Gvar Am*,Keremya	105	12	.247	.118	.634	.306
13. Beer Sheva	199	18	.249	.116	.624	.307
14. Nevatim	60	7	.302	.075	.623	.340
15. Western Galilee* (all sites)	151	9	.218	.085	.697	.260
16. Sharon (all sites)	310	27	.523	.113	.360	.580
17. Beit Hanan,Beit Oved	32	7	.480	.200	.320	.580
18. Givat Brenner	36	3	.333	.242	.424	.454
19. Kfar Aza	153	26	.441	.024	.535	.453
20. Kfar Uriah	100	12	.330	.045	.625	.352

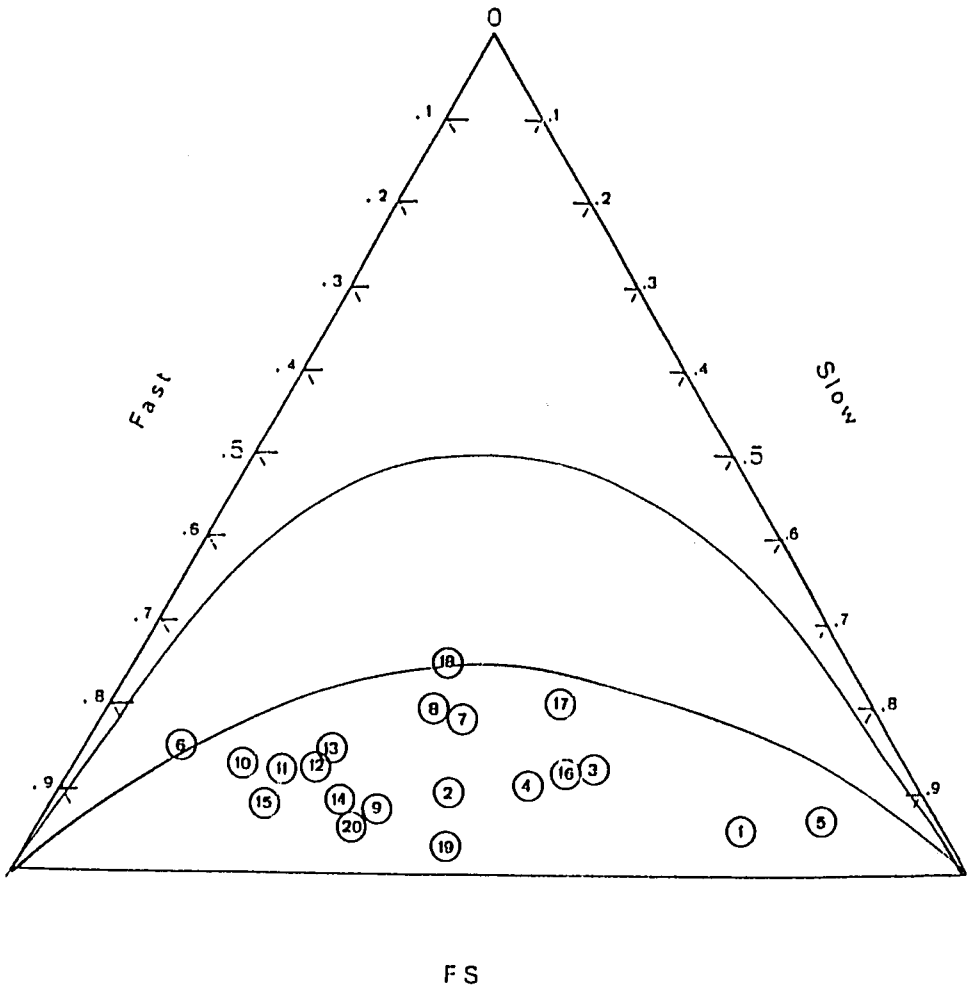

Fig. 5. Frequencies of EST genotypes in samples from 20 sites in Israel. (Data from Table 5). Plotted in triangular coordinates as in Fig. 2. For location of the collection sites see Fig. 4A.

There was no indication of geographical race formation. The differences in genotype frequencies among sites were quantitative, not qualitative. Both alleles were present, at all sites, and no fixation of alternative alleles was detected at any site.

Frequencies of EST genotypes on cotton, cucurbits and *lantana* at different sites

The pooled frequencies of genotypes calculated in samples from different sites (in the preceding section) could be biased because different host plants were sampled. We now analyze samples taken from the same host plant at several sites.

(1) Cotton (Table 6). As before, the samples from control cotton populations have a very high frequency of F. All but one of the samples taken from commercial field populations (No. 6 - Poleg) have considerably lower frequency of F (all are located at the left end of the triangle in fig. 6). Fig. 6 illustrates that samples from geographically close localities on cotton are sometimes also similar in genotype frequencies, perhaps due to similarity of pesticide application rate, but this is certainly not the rule. When all localities were considered, as before, the genotype frequencies were very different among localities ($G = 1454.24$, 36 df; $P \lll 0.001$). This was still true when control populations were excluded from the analysis. (It is interesting to note that the two controls, (1) and (2), in Table 6, were significantly different from each other in genotype frequencies ($G = 11.80$, 2 df; $P < 0.01$).

(2) Cucurbits (melon and squash) (Table 7) were sampled from eleven populations. The variation in frequencies of the EST alleles is illustrated in Fig. 7. As before, the frequencies of the EST genotypes were very heterogeneous among sampling sites ($G = 119.72$, 20 df; $P < 0.001$). There was no relation between geographical proximity and genotype frequencies on

Table 6: Frequencies of EST genotypes on cotton at different localities (sites with sample sizes <20 were omitted).

Locality	Total	Inactive	Frequencies FF	FS	SS	Freq. F
1. TAU	947	46	.897	.049	.049	.922
2. Volcani	157	4	.902	.007	.092	.906
3. Tzora	672	49	.154	.093	.754	.200
4. Sde Eliyahu	250	29	.434	.032	.543	.450
5. Reshafim	93	5	.489	.102	.409	.540
6. Poleg	39	3	.778	.056	.167	.806
7. Nachshon	51	12	.205	.051	.744	.230
8. Masmia	72	10	.161	.016	.823	.169
9. Masarik	29	2	.259	.185	.556	.352
10. Kiryat Gat & Keremya	49	6	.256	.047	.698	.280
11. Hagash	25	1	.042	.292	.667	.188
12. Kfar Am	48	2	.283	.087	.630	.326
13. Gezer	38	1	.270	.081	.649	.310
14. Eden	715	46	.490	.129	.366	.554
15. Beer Sheva	39	1	.263	.053	.684	.290
16. Bet Haemeq	47	1	.217	.109	.674	.272
17. Berechia	27	2	.240	.120	.640	.300
18. Ayalon & Latrun	39	1	.079	.158	.763	.158
19. Kfar Aza	78	6	.208	.028	.764	.222

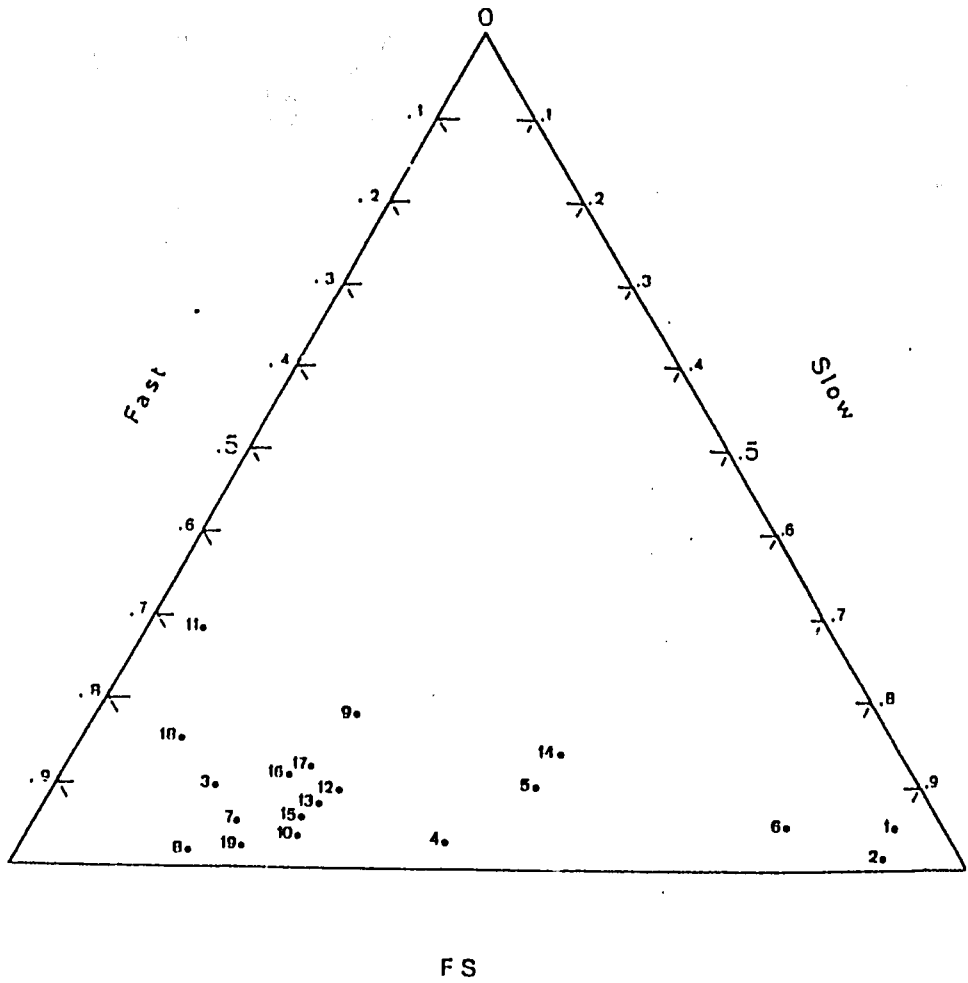

Fig. 6. Frequencies of EST genotypes in 19 samples from cotton in Israel. Data of Table 6, plotted in triangular coordinates as in Fig. 2. 1, Tel Aviv University; 2, Volcani Center for Agricultural Research (control population); 3, Tzora; 4, Sde Eliyahu; 5, Reshafim; 6, Poleg; 7, Nachshon; 8, Masmia; 9, Masarik; 10, Kiryat Gat; 11, Hagash; 12, Gvar Am; 13, Gezer; 14, Eden; 15, Beer Sheva; 16, Beit Haemeq; 17, Berechia; 18, Ayalon; 19, Kfar Aza.

Table 7: Frequencies of EST genotypes on Cucurbits at different localities.

Locality	Total	Inactive	Frequencies FF	FS	SS	Freq. F
1 TAU	70	8	.581	.145	.274	.654
2 Paran/Samar	115	2	.407	.150	.442	.482
3 Tzora	40	19	.238	.068	.667	.262
4 Hagash	39	14	.160	[illegible]	.840	.160
5 Ein Yahav	146	4	.620	.099	.282	.670
6 Eilot	107	2	.190	.238	.571	.309
7 Eden	117	2	.817	.061	.122	.848
8 Beit Guvrin	37	5	.500	.031	.469	.516
9 Almog	39	3	.639	.167	.194	.722
10 Ramat Hasharon	31	14	.647	.000	.353	.647
11 Kfar Uriah	66	12	.463	.019	.519	.472

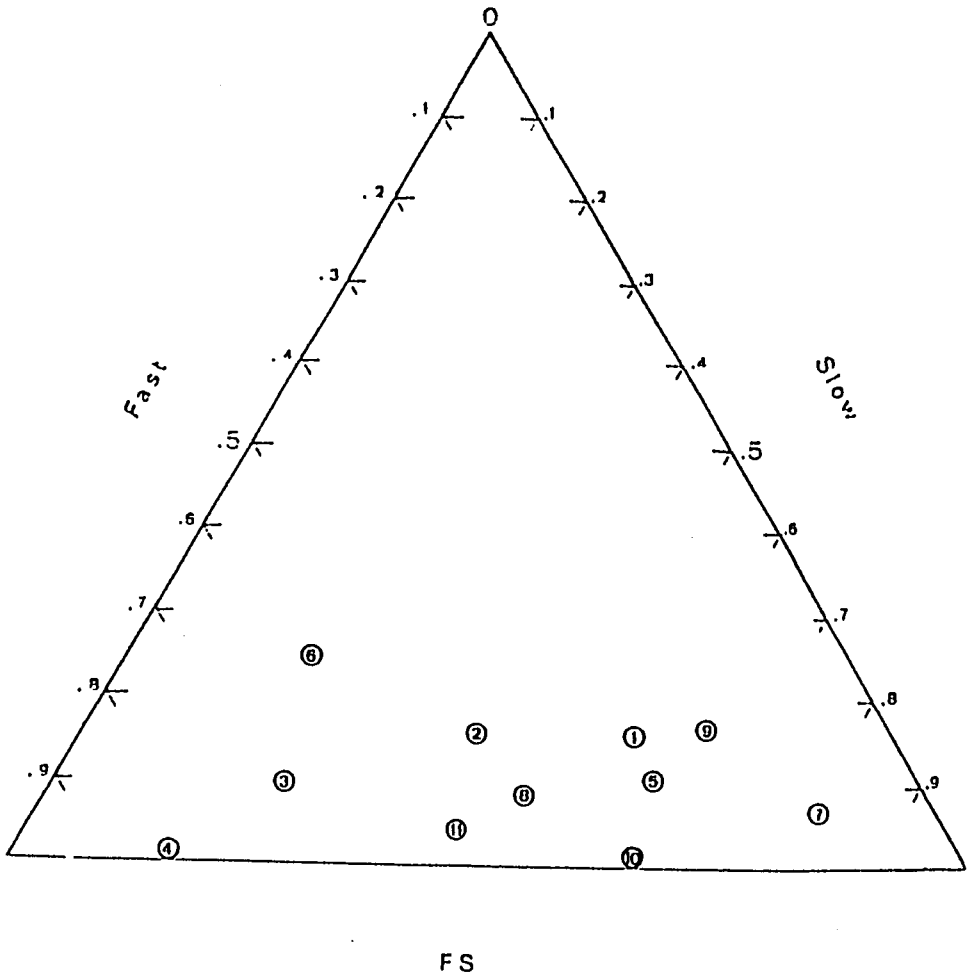

Fig. 7. Frequencies of EST genotypes in samples from Cucurbitaceae in Israel. Data of Table 7, plotted in triangular coordinates as in Fig. 2. 1, Tel Aviv University; 2, Paran/samar; 3, Tzora; 4, Hagash; 5, Ein Yahav; 6. Eilat; 7, Eden; 8, Beit Guvrin: 9, Almog; 10, Ramat Hasharon; 11, Kfar Uriah.

Table 8: Genotype frequencies on *Lantana* - arranged by localities.

Locality	Total	Inactive	Frequencies FF	FS	SS	Freq. F
1 Beit Hanan	41	4	.541	.351	.108	.716
2 Beer Sheva	78	5	.178	.123	.671	.240
3 Givat Brener	29	0	.310	.241	.448	.430
4 Kfar Sava	44	7	.459	.162	.378	.540
5 Ramat Hasharon	2846	137	.811	.080	.111	.851
6 Sde Eliyahu	115	12	.515	.068	.417	.549
7 Tel Aviv University	86	4	.927	.073	.000	.964
8 Tirat Zvi	61	18	.744	.047	.209	.768
9 Tzora	221	6	.953	.014	.028	.960

cucurbits: samples from the southernmost part of Israel, the Arava valley (2,5,6,9) are close geographically, but very dissimilar in frequencies (Fig. 7). Moreover, at Hagash and Eden (4 and 7 in Fig. 7), samples were available from cotton (11 and 14, in Table 6) as well as from cucurbits. The frequencies on the two crops were not the same. (Hagash: G = 7.59. 2 df, P < 001; Eden: G = 44.30, 2 df, P << 0.001). The two crops are planted during different parts of the year, which brings up the question of temporal variation in genotype frequencies (see below).

(3) *Lantana*. Samples on this host were available from nine sites, the largest sample being from the control population at Ramat Hasharon (Table 8). Again, a wide range of genotype frequencies among localities was apparent. Heterogeneity in genotype frequencies was again highly significant among sampling sites (G = 337.86, 16 df. P << 0.001). The highest frequency of F was at Tel Aviv University campus and at Ramat Hasharon, insecticide-free localities. The extent of insecticide treatment at other locations is unknown but it is unlikely that any insecticides were applied except perhaps as drift from nearby agricultural fields (*Lantana* is grown as hedges in ornamental gardens and along fences of orchards). The Tzora size is particularly interesting: it is located inside the kibbutz (village) perimeter, near homes, and is not treated with pesticides. The treated cotton fields surround the kibbutz. Genotype frequencies in samples from the fields (No. 3 in Table 6) and the *Lantana* site are radically different (G = 487.01, 2 df, P <<< 0.001) (compare point 9 in Fig. 8 with the asterisk [representing the field samples]). Climatic factors may also be involved in the regulation of the genotype frequencies: the two southernmost localities, Beer Sheva and Givat Brenner, had the lowest frequency of F (table 8).

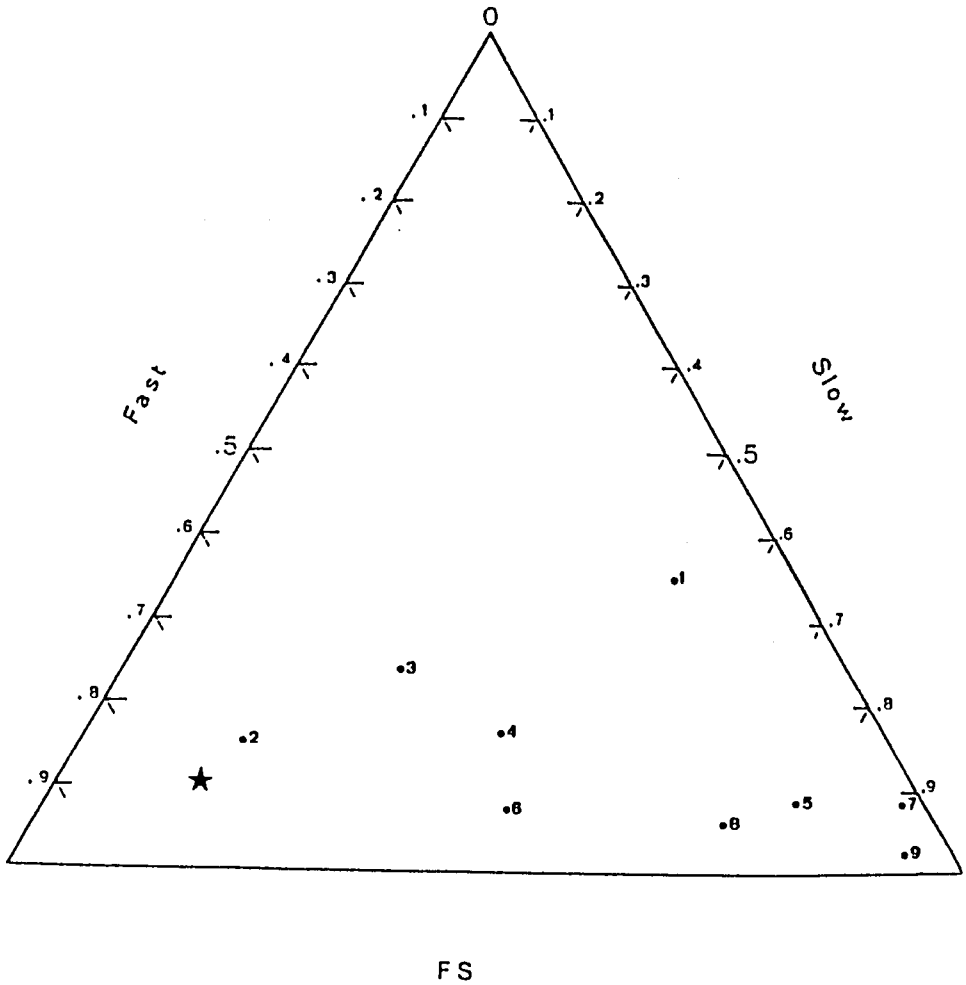

Fig. 8. Frequencies of EST genotypes in samples from *Lantana* in Israel. Data of Fig. 8, plotted in triangular coordinates as in Fig. 2. 1, Beit Hanan; 2, Beer Sheva; 3, Givat Brener; 4, Kfar Sava; 5, Ramat Hasharon; 6, Sde Eliyahu; 7, Tel Aviv University; 8, Tirat Zvi; 9, Tzora.

d. Temporal variation in the frequency of F

1. Ramat Hasharon

Fig. 9 illustrates the frequency of the F allele in the control population from Ramat Hasharon, from 1987 through July 1990 (a few samples were collected in October 1986 before the commencement of the project). Different symbols indicate samples from different host plants at the same site. With the exception of some samples in the summer of 1988, the frequency of F in the control population remained high (0.8-1.00) during the entire study period.

The reasons for the lower frequency of F in June-November, 1988, in some samples are unclear. It was first interpreted as the result of migration of individuals from commercial fields in the neighborhood, where the slow allele was predominant (Wool 1990). The pattern did not recur in 1989, and the explanation remains tentative.

2. Beit Shean (cotton-growing area)

Fig. 10 illustrates the temporal pattern of changes in the frequency of the F allele in the Beit Shean area, on cotton, in 1988-1989. Since cotton is a summer crop, no samples are available in winter months.

In 1988, the frequency of F was high in the early summer, but dropped to zero in June and July. This was interpreted as a result of the intensive insecticide application in the field during the cotton season (Wool, 1990). The pattern in the summer of 1989 was not the same (fig. 10), but the sampled sites and the insecticide treatment schedules were not the same in the two years, so that the explanation cannot be confirmed on the basis of the data from the two years.

3. Tzora (a cotton-growing area) in 1989-1990

Beginning in the summer of 1989, intensive sampling was carried out in the cotton fields of Kibbutz Tzora (Site X on the map, Fig. 2), where a

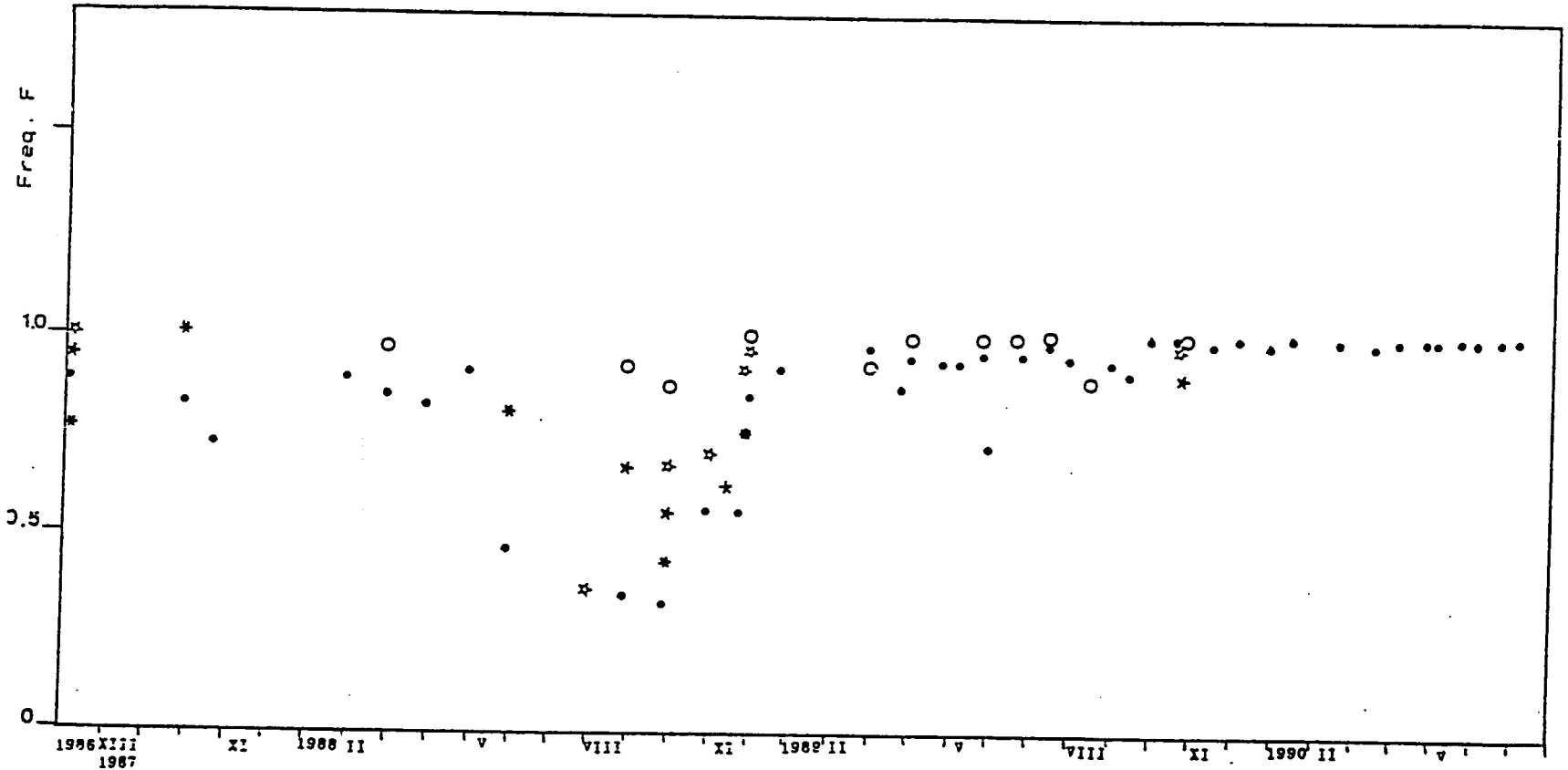

Fig. 9. Temporal changes in frequency of the fast (F) allele in samples from Ramat Hasharon, a control (unsprayed) site. Apart from some samples in the summer of 1988, the frequency of F remained high throughout the study period.

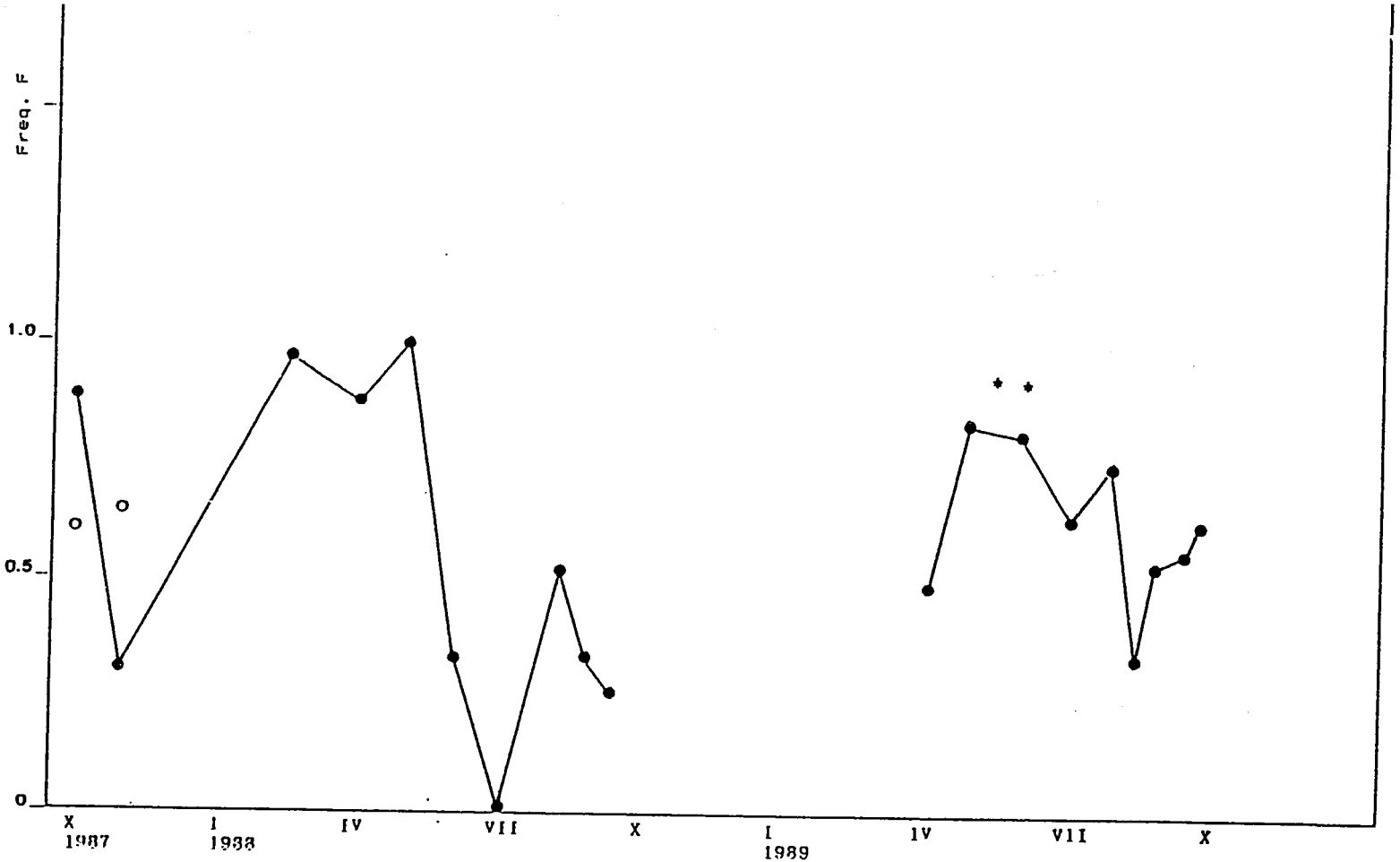

Fig. 10. Temporal changes in frequency of the fast (F) allele in samples from cotton at Beit Shean in 1988 and 1989.

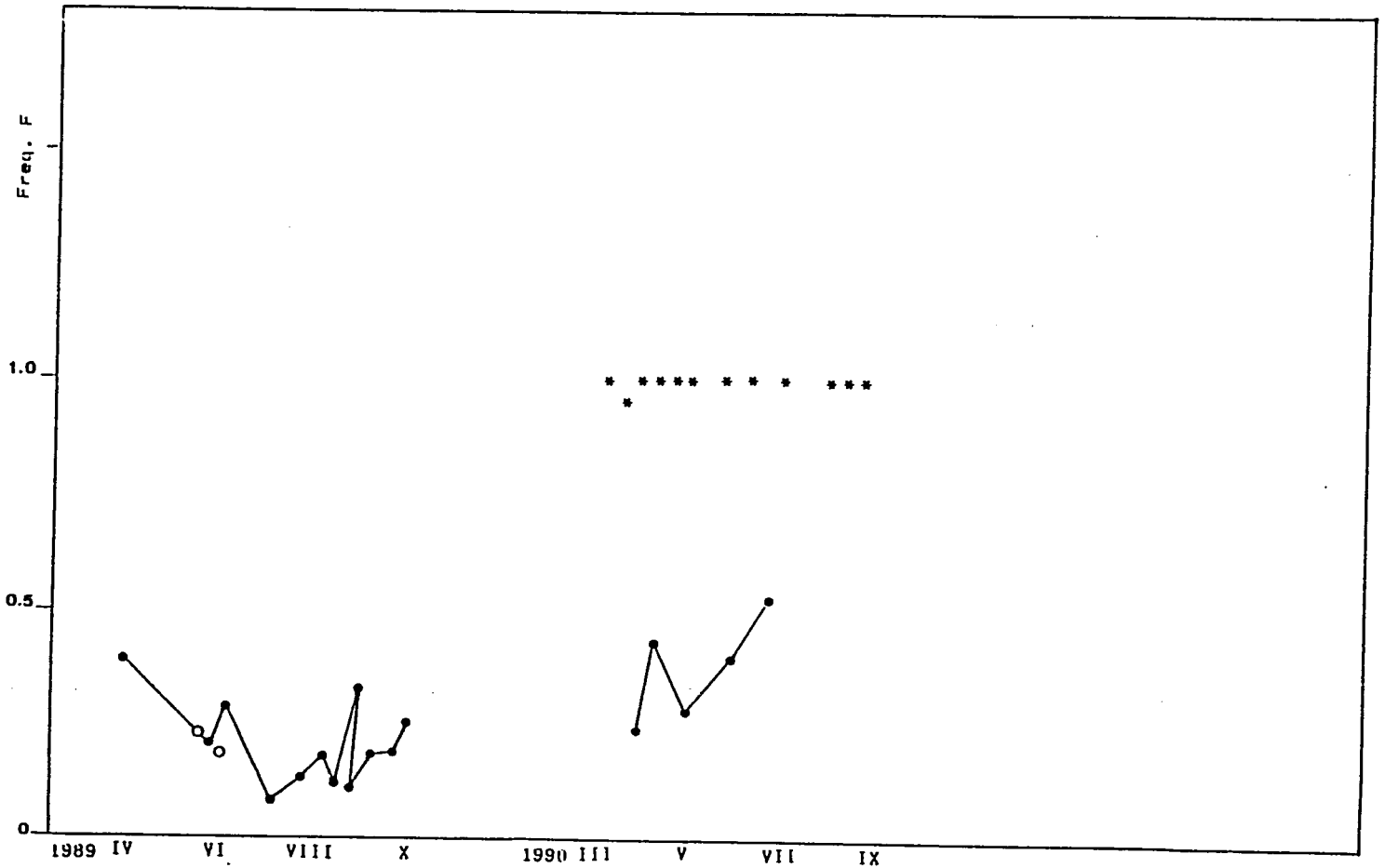

Fig. 11. Temporal changes in frequency of the fast (F) allele in samples from cotton at Tzora in 1989 and 1990.

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regular schedule of insecticide treatments was applied, including the application of Aldicarb (Temik) to the soil with drip irrigation and several aerial sprays against the whitefly. Samples of adult whiteflies from cotton were used for electrophoresis as before, as well as for estimation of the frequency of insecticide-resistant individuals in the sample (for this end we used a physiological-biochemical method based on esterase activity levels, Wool and Greenberg 1990). This activity also continued during the 1990 cotton season.

During the winter, we carried out a search in the vicinity of Tzora in an attempt to discover the site and host plants of overwintering *B. tabaci* populations, since cotton is an annual crop and is reinfested every summer. We discovered an overwintering breeding population on a *Lantana* hedge inside the residential area of Tzora. In March 1990, a few adults emerged and were analyzed. They carried the FF genotype. Larger samples were collected from April through July. All of the adults carried the FF genotype (a marker for insecticide susceptibility; Wool & Greenberg 1990).

In mid-April 1990, we were alerted by the cotton growers at Tzora to the presence of whiteflies on weeds in a field 3 km west of the village, which was being ploughed for cotton sowing. There were no immatures on these weeds (*Solanum*), indicating that this was a transient adult population, migrating from somewhere. Upon examination, these adults were mostly SS individuals, which therefore could not have come from the *Lantana* hedge (SS is associated with insecticide resistance; Wool & Greenberg 1990). A search for a possible source revealed vegetable fields with *B. tabaci* on squash at Kfar Uriah, a few km to the north-west. These vegetable crops, with regular insecticide treatment, were grown throughout the winter at Kfar Uriah (probably other villages as well). (It is likely that *B. tabaci* infesting cotton in the summer

at Tzora comes from the west because this is the direction of prevailing winds).

The temporal trends of F-allele frequencies at Tzora are illustrated in Fig. 11.

The frequency of F in the field remained low (most of the cases below 0.3). All the samples contained many insecticide-resistant individuals, as shown by our physiological assay of Esterase activity Wool and Greenberg 1990).

DISCUSSION / Israeli data

Using the EST system as a marker, we can deduce with confidence from the results that Israeli *B. tabaci* seem to form one panmictic population. We found no evidence of race differentiation by host plant, at least as reflected by the EST system, and there was no evidence for geographical differentiation other than due to selection by human application of pesticides in agricultural fields. In no case did we observe fixation of alternative EST alleles in different localities or appearance of unique EST genotypes other than near-fixation of S in insecticide-treated fields.

We found that samples taken from different host plants even at the same site, were genetically heterogeneous. But the differences were quantitative, not qualitative. The host plant, like any other environmental factor (as well as random effects) may have an effect on the genotype frequencies of the feeding and reproducing whiteflies, but these effects have not led to the formation of specific host-related races in Israel. This statement is supported by the fact that samples from the *same* host plant (cotton, *Lantana*, Cucurbits) taken in different localities, were very heterogeneous genetically.

However, there is one exception which deserves notice: the (near) fixation of the F allele in samples from *Poinsettia*. This is the only

representative of the Euphorbiaceae among the host plants in our survey. It is worthwhile to mention that cassava (*Manihot esculenta*) which belongs in that family, (an important human food item in South America and in Africa) is a host of *B. tabaci* in Africa (Robertson 1987). Research in the Ivory Coast (Burban, Fishpool and Abisgold 1989) discovered that *B. tabaci* on cassava were electrophoretically different from populations sampled from other host plants. (However, we found no consistent difference between populations on Euphorbia and on other hosts in Colombia - see below).

Samples from different localities were highly significantly heterogeneous in genotype frequencies. There was no clear geographical pattern in the frequencies of the genotypes. Apart from the difference between agricultural sites, and unsprayed, control sites with commercial crops (especially cotton), to be discussed shortly, heterogeneity was detected between samples from the same host plants when sampled at different sites. Some of this variation could be due to "founder effects" - the genotype distributions depending on the chance composition of a (usually) small group of adults infesting the field early in the season. Differences in climatic conditions at different localities, different agricultural practices or treatment-intensities in agricultural crops, and the fact that samples were not collected simultaneously at all localities (a technical impossibility), may be the reasons for the heterogeneity among samples.

An important aspect of the results from Israel is the relationship between the SS genotype frequency at the EST locus and insecticide treatment in field populations. The frequency of the S allele was very low - near zero - in control (unsprayed) populations and remained so during the three years of study. (The exceptional increase in frequency at Ramat Hasharon in the summer of 1988 is probably due to immigration from nearby agricultural fields). By contrast, in agricultural areas its frequency was high, sometimes an entire

sample contained only SS individuals. We now know from other work (Wool and Greenberg 1990) that SS individuals have low EST activity and they are also resistant to insecticides (we are studying now the physiological mechanism of resistance). The EST enzyme produced by SS and FF homozygotes differ in substrate affinity: SS preferentially cleaves the α-configuration, but FF cleaves the β-configuration preferentially. The electrophoretic SS-EST may therefore serve as a marker for identifying insecticide resistant individuals in field samples. In this way we identified the source of the first spring infestation of cotton by whiteflies in the cotton fields of Kibbutz Tzora. This may be a convenient method for monitoring resistance in field populations.

The deficiency of heterozygotes compared with Hardy-Weinberg expectations in Israeli samples remains unexplained. Even when we take into account the haplo-diploid mating systems of whiteflies (and assume a 1:1 sex ratio) most samples contained much fewer FS individuals than expected. It is unlikely that our samples included more males than females (actually, in samples which were sexed carefully, just the reverse was the case - an excess of females). There must be another reason for the heterozygote deficiency which we do not know at present.

PART II - ESTERASE VARIATION IN COLOMBIA

Introduction

The study of Colombian populations of *B. tabaci* began simultaneously with the work in Israel. After some preliminary attempts, the methods which were developed at TAU were routinely used at CIAT. Data sheets recording the results were periodically sent to TAU and stored in computer memory. We made every effort to standardize the method so that the data from the two countries will be comparable. However, we strictly avoided sending live whiteflies between countries. Therefore we could not run Israeli and Colombian whiteflies side by side on the same gel and had to judge the similarity of isozymes from their relative migration distance, when measured against a control.

Collection sites and principal host plants

Samples were collected in 13 Colombian states and in two neighboring countries - Ecuador to the south and Venezuela to the north-east (see Fig. 12). Table A2 in the Appendix summarizes the collection sites from which data are available, as well as the main host-plants and sample sizes.

RESULTS

A. The basic electrophoretic EST pattern in Colombian samples of *B. tabaci*: Comparison with the Israeli pattern.

The pattern of EST variation in Colombia turned out to be more complex than in Israel (see below). However, we could identify a major system which looks almost identical to the major Israeli system. It involves two bands behaving like two alleles (S and F) at a single locus (Fig. 13), with the appropriate double-banded heterozygote. The fastest band (ff) appeared on almost all gels from most of the states of Colombia (see below) and served as a useful marker in the interpretation of the various electrophoretic patterns. This band was also detected in Israeli *B. tabaci* (see in the description of

DISTRIBUCION GEOGRAFICA DE RAZAS DE *Bemisia tabaci* EN EL NORESTE DE SURAMERICA

Fig. 12. Map of Colombia, showing the locations of different EST variants. (see Legend in the Figure).

the major Israeli system above) but there it was rare and detectable only on good gels.

The behavior of the major system, differed in some aspects from the Israeli system. First, despite attempts to make the techniques identical, the Colombian F and S bands migrated further apart from each other and were more easily detectable (compare Fig. 13 with Fig. 1). Second, although the staining substrate was routinely a 9:1 mixture of and Napthylacelate, no preferential staining of F or S to different configurations of the substrate was detected.

Further complications arose when geographical variation in genotype frequencies was observed. Luckily, the first samples we analyzed came from the agricultural area around CIAT (State Valle), in which the EST pattern is that illustrated in Fig. 13. This pattern was also found in a laboratory colony of *B. tabaci* which had been maintained in cages on beans (*Phaseolus*) for several years. This population was, therefore, chosen to be our control population. Two or three individual adults from this population were run on every gel for comparison.

B. Genetic patterns. Interpretation of the Colombian EST.

The analysis of the EST banding patterns at other states of Colombia resulted in a change in the interpretation of the results. At first, the patterns were classified into 8 groups (Fig. 14), which were tentatively identified as different races. Their geographic distributions are illustrated in the map of Colombia (Fig. 12).

However, an examination of the 8 patterns revealed many similarities among them. The Valle pattern (1) looks identical with the Israeli pattern, and will serve as a basis for further comparisons: (1A) is the heterozygote FS, (1B) is the homozygote FF, and (1C) is the homozygote SS. The thin fast band (ff) appeared in all Valle individuals including the CIAT control (on

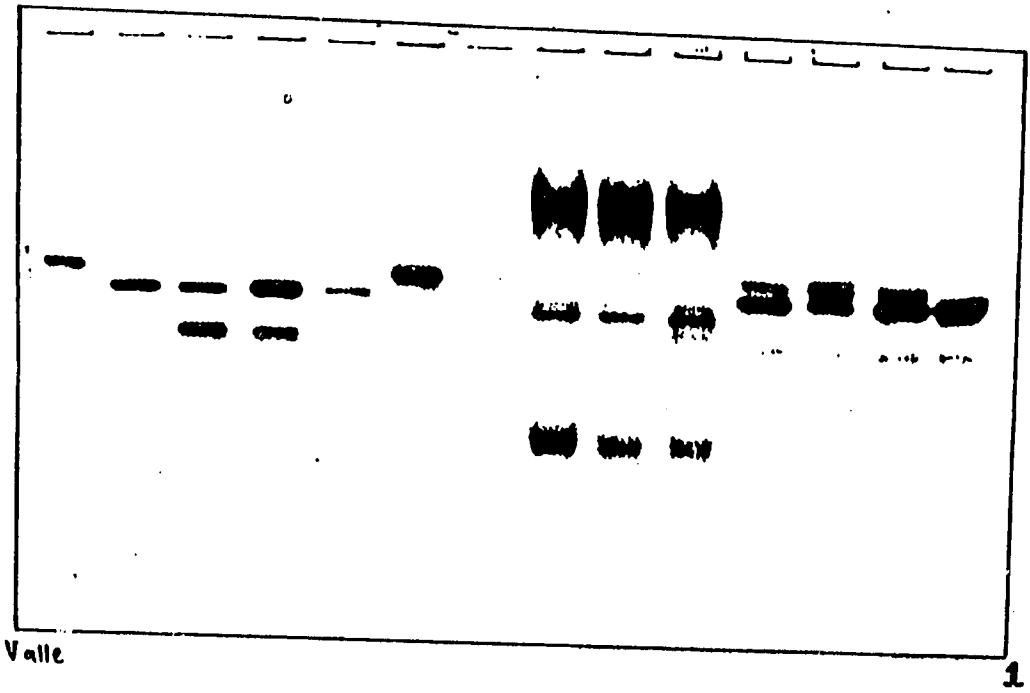
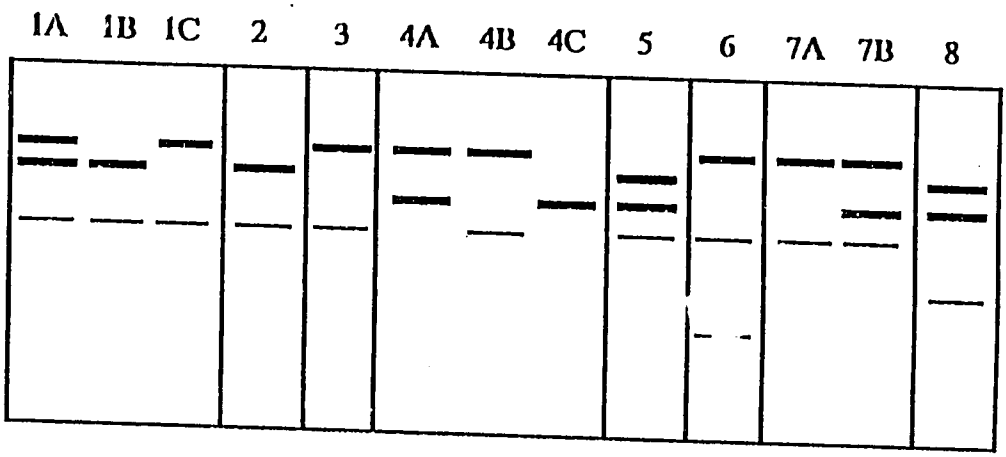


Fig. 13. The major EST pattern in samples from Valle, Colombia. Cells 8-10 from left are individuals of *Bemisia tuberculata*. The others are *B. tabaci*. From left: S, F, F, F, FS, S, S; *B. tuberculata*; FS, FS, FS, F. Notice the faster isozyme ff, which is present in all *B. tabaci* individuals (see text).

Race specific isozyme patterns of the whitefly Bemisia tabaci



Esterase isozyme banding patterns of eight Bemisia tabaci races from Colombia, Ecuador, and Venezuela. The numbers correspond to the following geographic regions: 1. Valle 2. Cucuta 3. Cauca 4. Tolima 5. Caribe 6. Villavicencio 7. Manabi 8. Maracaibo. Three regions appear to contain heterogenous populations of B. tabaci as marked by the letters. The races tend to occur in areas that are geographically isolated by mountain ranges.

Fig. 14. Race-specific isozyme patterns of Colombian *B. tabaci*. (Full legend in Figure).

beans).

Patterns (2) and (3) seem to be part of the same EST system, except that (2) is fixed for the F allele and (3) is fixed for the S allele. This fixation may be due to small sample sizes or to biological factors (see discussion).

There is a second group of patterns, involving patterns (4), (5), and (7). The major characteristic of this group is a strong, additional, fast band which we shall call (tentatively) VF. It alternates with the S band in 4B, 4C, or co-occurs with it as in (4A) and (7B), creating the impression that VF might be an additional allele at the major system. This idea is supported by pattern (8), where VF is associated with the F allele. Pattern (6), like (4B) and (3), seems to be fixed for the S allele. [There is also an occasional appearance of other very fast bands (patterns (6) and (8)].

The simplest interpretation of the major Colombian banding patterns is that the major system is composed of 3 alleles, at a single locus, (S,F and VF), with the appropriate three possible two-banded heterozygotes, SF, FVF, and SVF. A problem with this interpretation is presented by pattern (8), a sample from Maracaibo (Venezuela) in which, according to the figure, only heterozygotes were recorded. This is unlikely to happen for a sexually breeding population.

If this interpretation is correct, six genotypes therefore result: S, F, VF, SF, SVF and FVF. All six were in fact identified (at varying frequencies) in the samples, and only 28 of nearly 3000 individuals could not be assigned unambiguously to a genotype. This model seems to fit the data well.

C. Host-plant differentiation of EST genotype frequencies in Colombian samples.

Table 9 lists the host plants from which *B. tabaci* was collected in Colombia. In contrast with Israel, where most samples were collected from agricultural crops (especially cotton), most Colombian samples came from weeds

(especially species of *Euphorbia*). This important point is taken up in the Discussion.

Table 10 is a partial list of the frequencies of the 3 genotypes on different host plants in Colombia, which is comparable to the Israeli system. (Our initial interpretation of the data, in particular from Valle, was that the systems in Israel and Colombia may have the same underlying genetic structure, of one locus with 2 alleles). The data are illustrated in triangular coordinates in Figure 15.

The Colombian samples, especially on beans and on *Euphorbia* (which provided the most whiteflies) contain much higher frequencies of heterozygotes FS than Israeli *B. tabaci* (compare Fig. 15 with Fig. 2). The control population at CIAT, which was the source of the samples from beans, and also the samples from *Euphorbia*, were much closer to Hardy-Weinberg equilibrium frequencies than any samples from Israel (but still significantly different from expectation: for beans, $X^2 = 16.6$, 2 df, $P < 0.01$; for *Euphorbia*, $X^2 = 56.4$, 2 df, $P << 0.001$).

All samples except beans (control) show predominance of the S allele (Fig. 15).

A more complete and updated list of Colombian data by host-plants is given in Table 11, which is arranged with the new interpretation in mind (one locus with 3 alleles). But since different host plants were sampled at different localities, quantitative comparisons will be deferred until the differences among localities are discussed.

D. Geographical differentiation

Samples from different Colombian states, and from the two neighboring countries, Venezuela and Ecuador, yielded different electrophoretic patterns, which are illustrated below.

Interpreted as one locus with 3 alleles, the frequencies of 7 genotypes

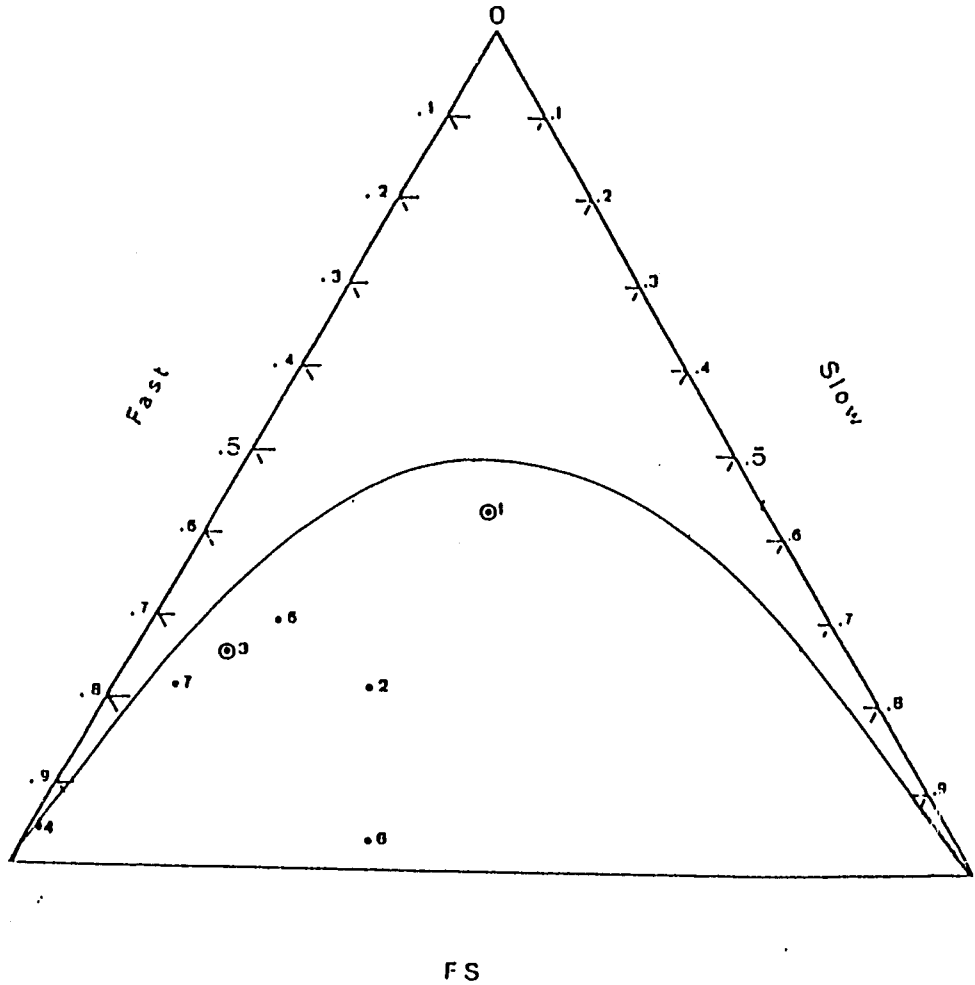


Fig. 15. Frequencies of EST genotypes at the major locus, in samples from different host plants, in Colombia. Data of Table 10, plotted in triangular coordinates as in Fig. 2. 1, beans (control); 2, cotton; 3, *Euphorbia*; 4, *Merremia*; 5, *Rhyncosia*; 6, *Sida*; 7, *Wedelia*.

Table 9: List of host plants from which *B. tabaci* was collected in Colombia. Wild plants are listed by their scientific names; commercial crops by their common names.

Family	Species
Euphorbiaceae	*Euphorbia hypercifolia; E. hirta; E. heterophylla; E. pruniflora; Croton* sp.
Leguminosae	Beans; cowpeas; *Cajanus cajan; Rhyncosia minima*
Solanaceae	Tomato
Malvaceae	Cotton; *Sida acuta*
Cucurbitaceae	Squash
Convulvulaceae	*Merremia* sp.
Compositae	*Wedelia latifolia*

Table 10: Frequencies of alleles at the major EST system, listed by host plant. Colombian data (early interpretation)

	Plant	# individual	# inactive	Frequency of FF	FS	SS	Freq. F
1.	Beans	650	4	.283	.415	.298	.491
2.	Cotton	88	3	.268	.244	.524	.390
3.	*Euphorbia*	859	56	.095	.253	.650	.166
4.	*Merremia*	46	1	.000	.022	.956	.011
5.	*Rhyncosia*	45	0	.133	.288	.578	.277
6.	*Sida*	31	0	.355	.032	.613	.371
7.	*Wedelia*	69	1	.095	.221	.721	.170

Table 11: Frequencies of alleles at the main EST system, listed by host plant. Colombian data.

Plant	# indiv.	# inact.	Frequency of genotypes SS	FF	SF	VF	SVF	FVF	Allele freq. of S	F	VF
1. Beans	434	6	.280	.182	.362	.000	.007	.164	.464	.445	.086
2. Cotton	161	3	.177	.114	.127	.101	.095	.392	.288	.374	.344
3. Cowpea	58	5	.245	.038	.000	.000	.000	.736	.245	.406	.368
4. Cucurbits	112	1	.243	.018	.000	.000	.027	.712	.256	.374	.370
5. *Euphorbia*	1190	55	.440	.073	.078	.036	.047	.317	.502	.270	.218
6. *Merremia*	109	2	1.000	.000	.000	.000	.000	.000	1.000	.000	.000
7. *Rhyncosia*	55	0	.109	.382	.236	.000	.000	.236	.227	.618	.118
8. *Sida*	111	0	.892	.099	.009	.000	.000	.000	.896	.104	.000
9. Solanaceae	113	3	.255	.209	.118	.018	.000	.373	.314	.454	.204
10. *Wedelia*	30	0	.367	.133	.500	.000	.067	.000	.650	.383	.034
11. *Jatropha*	32	0	1.000	.000	.000	.000	.000	.000	1.000	.000	.000
12. *Croton*	40	0	1.000	.000	.000	.000	.000	.000	1.000	.000	.000
13. *Vigna*	32	0	.000	.000	.000	.000	.000	1.000	.000	.500	.500
14. *Macroptilium*	39	1	.342	.000	.000	.000	.658	.000	.671	.000	.329
15. *Desmodium*	32	2	.000	.000	.000	.000	.000	1.000	.000	.500	.500

can be expected (Table 12; the ff and wvf bands are not parts of this system). Table 12 lists the frequencies of the genotypes in samples from Colombian states, regardless of host plant.

Examination of Table 12 shows that the Colombian localities (including the two neighbouring countries) can be arranged in three groups (1) the "Valle" group (with the three standard genotypes FF, SS and FS). This group includes Valle, Santander, Meta and Cauca. (2) The Tolima group, with genotypes SS, VF and SVF - including Ecuador, Tolima and Huila. (3) The Guajira group, including Guajira, Bolivar, Cesar, and Venezuela. This group segregates for the genotypes FF, VF and FVF.

Not knowing whether the SS in group 2 is homologous with SS in group 1, or whether F in group 2 is homologous with F in group 3, we shall discuss the three groups separately.

(1) The Valle group

This group is the simplest to analyze, because there is no doubt as to the identity of the isozymes, and none of the other isozyme combinations were found there. The isozyme pattern of Valle is that illustrated in Fig. 13 and 14 (1-3). Within the group, there is a sharp genetic differentiation with Meta and Cauca fixed or almost fixed for the SS genotype (illustrated in Fig. 16), and Santander almost fixed for the FF genotype. Only Valle remains segregating for the two alleles, like the CIAT control population (which was originally established from a field sample from this area (Fig. 17).

(2) The Tolima group

The genotypic composition of the Tolima group, differs from the Valle by having the F and SF genotypes replaced by VF and the heterozygote SVF (actually a replacement of the fast allele by a faster one - assuming that S is the same). (Fig. 18).

There is not much differentiation within the Tolima group. Both Huila and

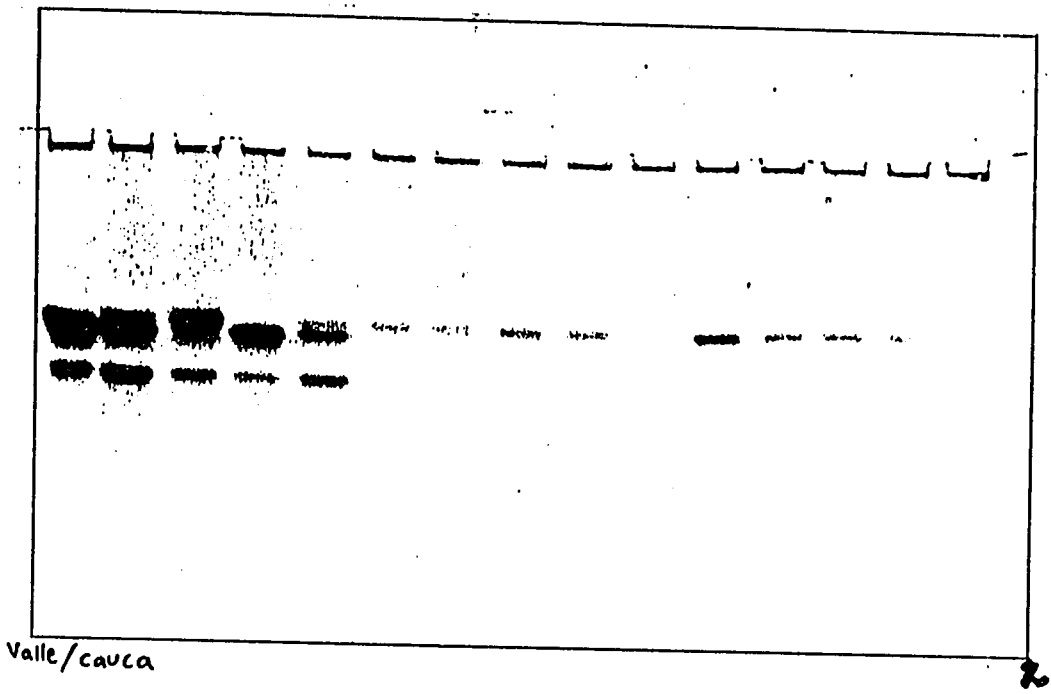


Fig. 16. EST pattern in *B. tabaci* samples from Quilcace, Cauca, Colombia, (cells 6-15, showing only the SS genotype). Cells 1-5 contain control individuals from CIAT (on beans). From left: F, FS, S, F, FS.

Table 12: Frequencies of EST genotypes in samples from Colombian states.

State	Total	Inactive	S	F	SF	VF	SVF	FVF	wvf	ff
Valle (not incl. CIAT)	212	0	23.1	27.4	36.8	12.3	0.9	0.9	1.4	98.1
Meta	231	43	98.4	1.1	0.5	0	0	0	76.6	100.0
Huila	200	0	82.0	3.5	1.5	0	12.5	0.5	0	64.5
Tolima	310	5	62.4	0.7	1.0	10.9	20.7	5.3	0.3	60.4
Sucre	16	0	0	0	0	0	0	100.0	0	81.2
Magdalena	16	9	0	0	0	42.9	0	57.1	0	100.0
Cordoba	51	1	0	0	0	0	0	100.0	0	92.2
Guajira	84	1	0	7.2	0	9.6	0	79.5	0	90.4
Santander	48	0	14.6	77.1	6.25	0	0	0	0	100.0
Bolivar	26	4	0	13.6	0	9.1	0	77.3	0	100.0
Atlantico - Caribe	12	0	0	0	0	0	0	100.0	0	100.0
Cesar	40	0	2.5	25.0	0	2.5	0	67.5	0	100.0
Cauca	261	2	100.0	0	0	0	0	0	0.8	98.1
Ecuador	108	4	57.7	3.8	1.0	10.6	16.3	0	0	94.2
Venezuela	528	9	0	0.6	0	0.2	0	99.8	98.8	0
CIAT	549	6	36.1	26.3	37.0	0	0	0	0	90.4

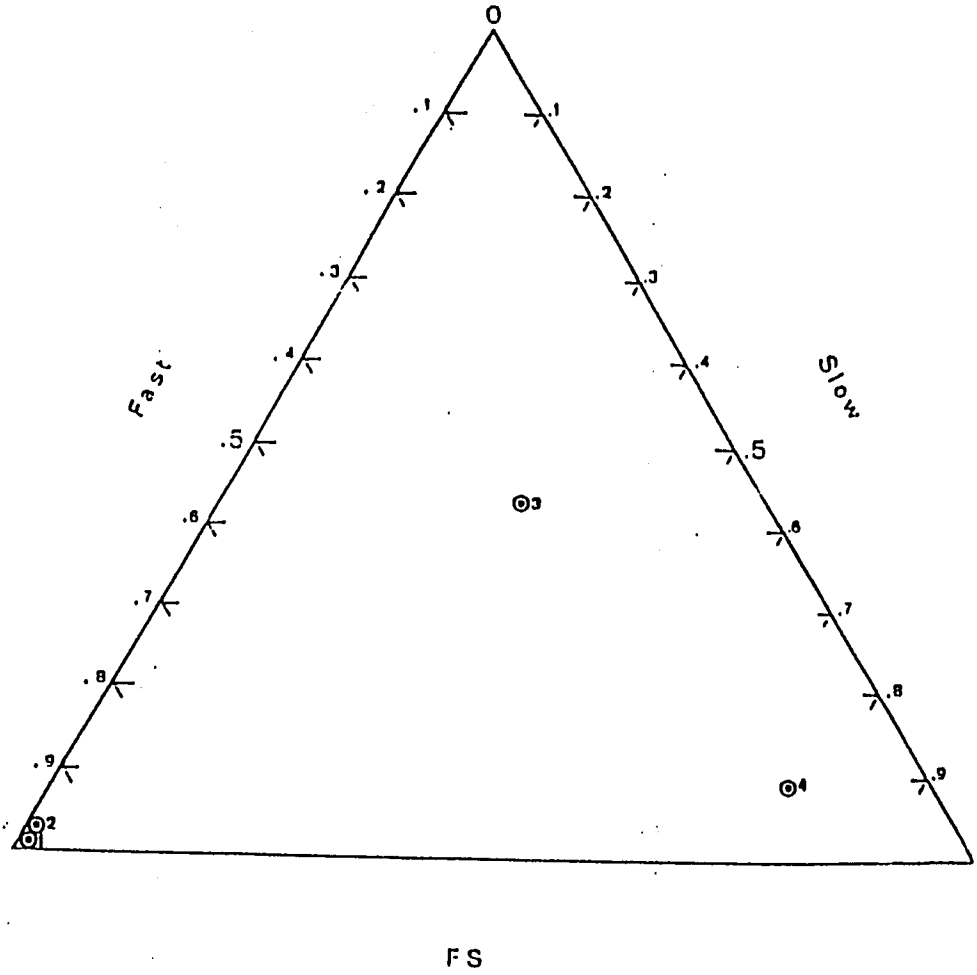


Fig. 17. Frequencies of EST genotypes at the major EST locus in samples from Colombian states Valle (3), Cauca (1), Meta (2) and Santander (4) (the "Valle group"). Data from Table 12, plotted in triangular coordinates as in Fig. 2.

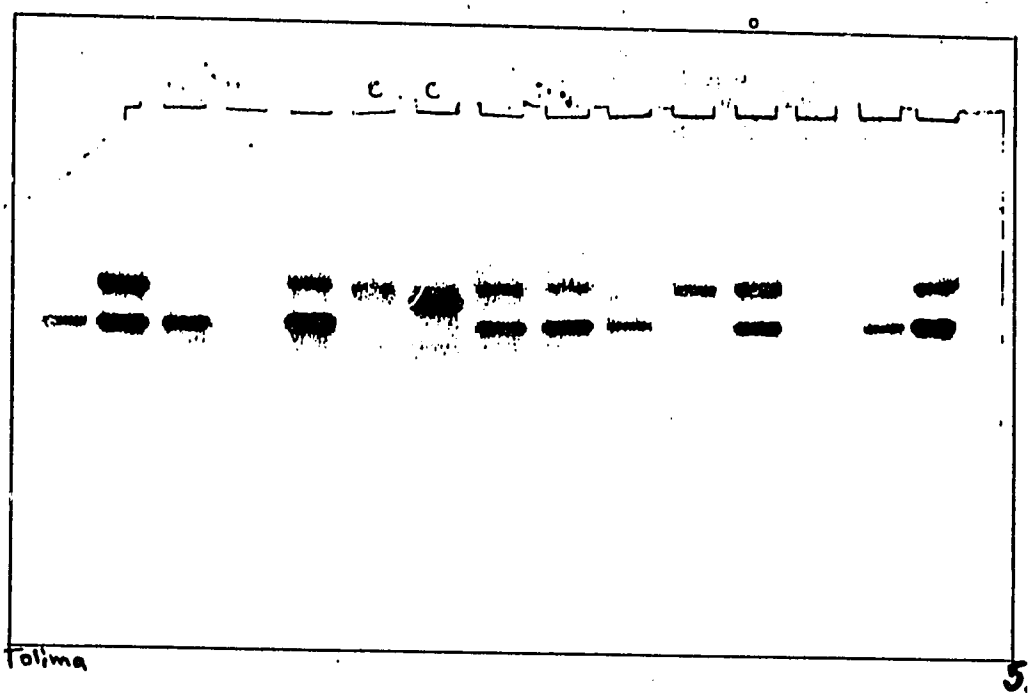


Fig. 18. EST pattern of *B. tabaci* individuals from Nataima, Tolima. Cells 6, 7 are control individuals from CIAT (both FS). Notice the S, VF pattern prevalent at Tolima.

Tolima contain a large majority of SS, a relatively small proportion of VF and about 25% of double-banded SVF individuals. It is strange that samples from Ecuador are very similar to Tolima although the distance between the two localities is very wide, with Cauca and Valle much closer to Ecuador than Tolima is (Fig. 19).

(3) The Guajira group

This group is distinct from the other two by the absence of the slow genotype, but retains the F, VF and their (tentative) heterozygote FVF. The genotype frequencies in these States are quite similar: most individuals are double-banded FVF. The samples from Cordoba and Venezuela are fixed for 100% FVF heterozygotes as illustrated by a sample from Caribe (Fig. 20). Guajira, Bolivar and Cesar are quite similar in proportions of the three genotypes (Fig. 21).

Summary

To summarize the complex geographical patterns of Colombian *B. tabaci*, we make use of the three-dimensional diagrams to plot the allele frequencies (rather than genotype frequencies) of F, S, and VF in Colombian states (artificially making $q_F + q_S + q_{VF} = 1.0$). Table 13 and Fig. 22 summarize the data in this way. The 3 dimensions of the equilateral triangle represent the allele frequencies of fast, slow, and very fast which should sum up to 1.0 (there are some discrepancies).

The plot of these data shows roughly two sets of localities (states): one group with high frequencies of S (Tolima, Ecuador, Huila, Santander, Cauca, and Meta; S is fixed in the latter two) and a second group almost fixed for F and having a high frequency of VF (Guajira, Cordoba, Bolivar, Cesar and Venezuela). Valle stands out as a separate group, with equal frequencies of F and S and (almost) no VF present.

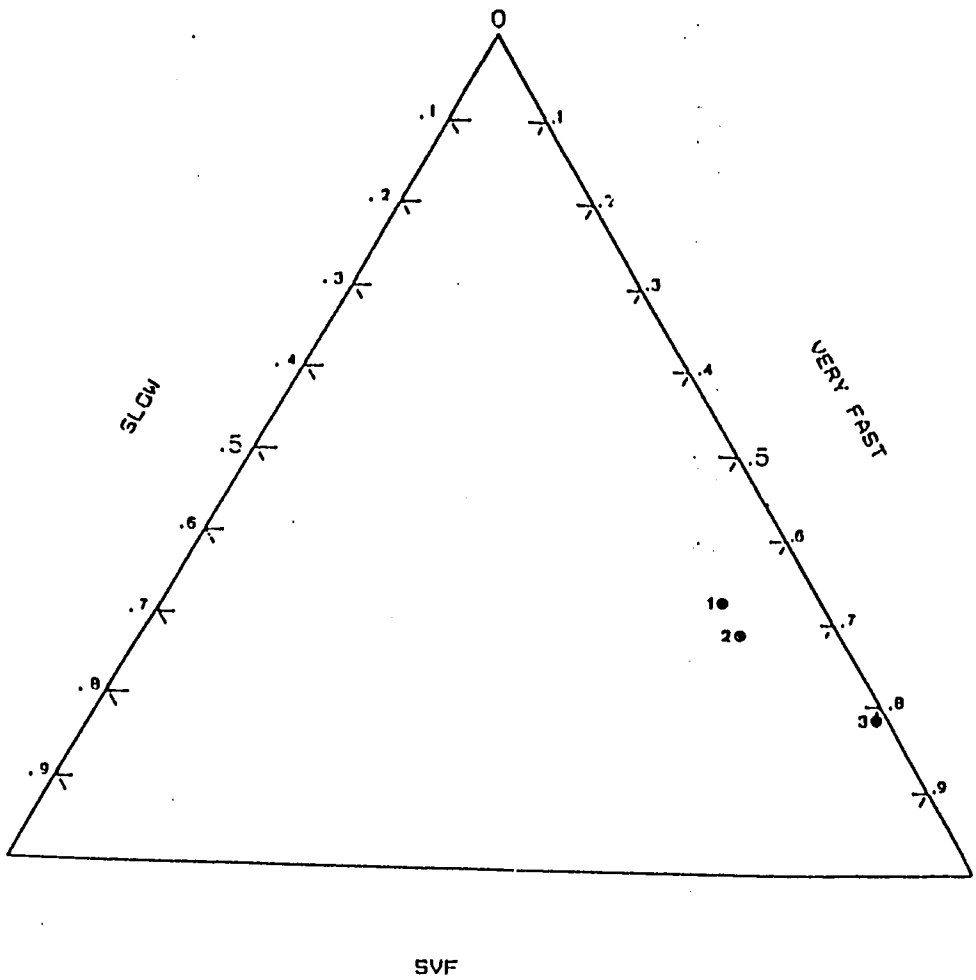


Fig. 19. Frequencies of EST genotypes S, VF and SVF in samples from two Colombian states [Tolima (2), Huila (3)] and from Ecuador (1). Data from Table 12, plotted in triangular coordinates as in Fig. 2.

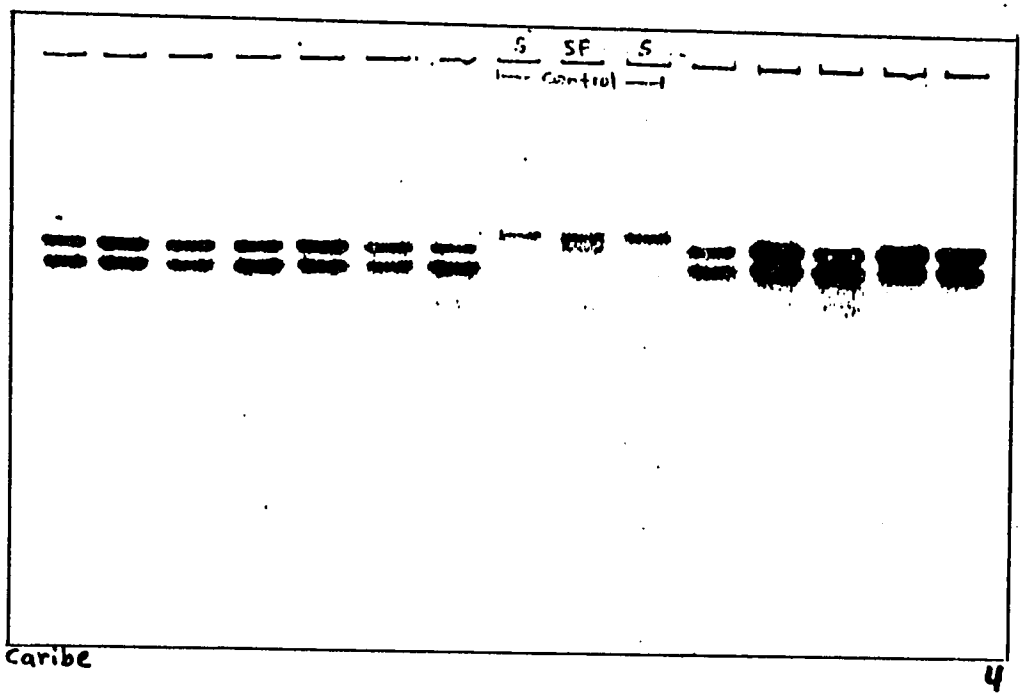


Fig. 20. EST pattern of *B. tabaci* in samples from Colombian state Caribe, illustrating a fixed pattern of F, VF isozymes (cells 8-10 are control individuals from CIAT: from left: S, FS, S).

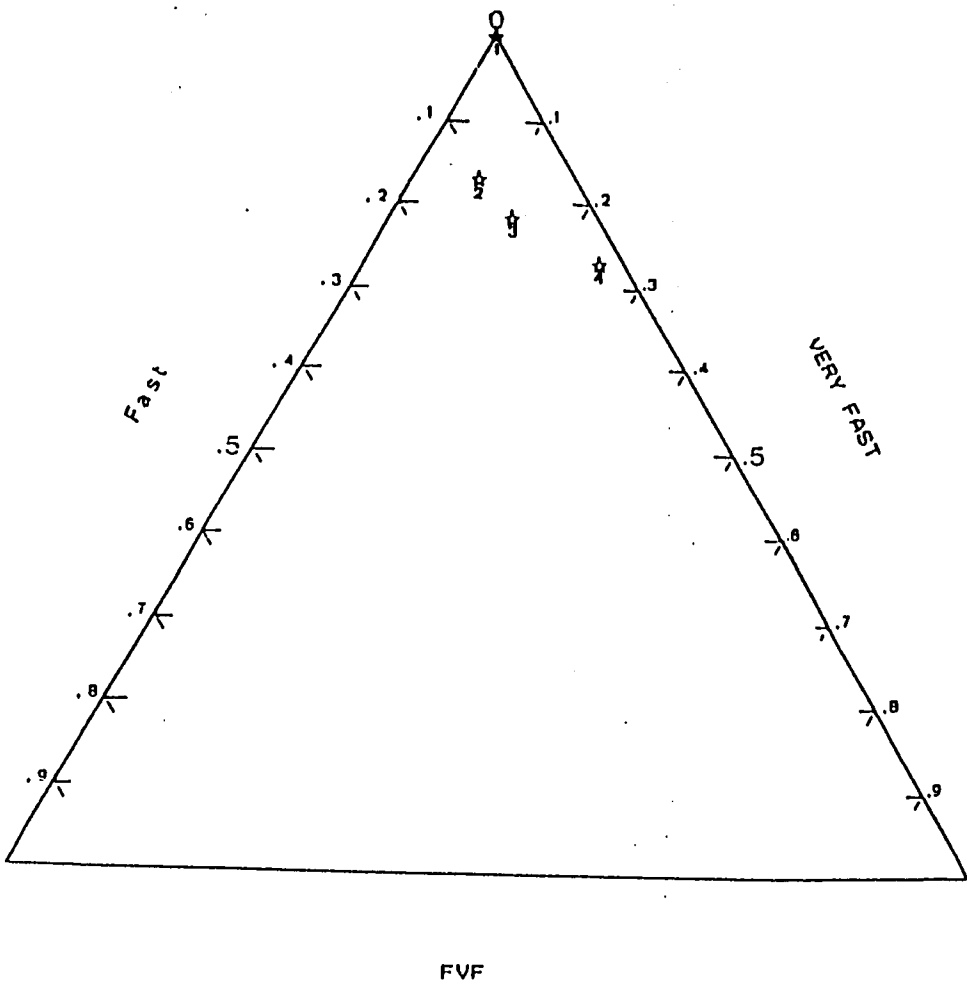


Fig. 21. Genotype frequencies in samples from Venezuela and Colombian states Guajira (2), Bolivar (3) and Cesar (4) (the "Guajira group"), segregating for genotypes F, VF and FVF. Data from Cordoba, Caribe and Venezuela are indistinghishable (1). Data from Table 12, plotted in triangular coordinates as in Fig. 2.

Table 13: Frequencies of three EST alleles in samples from Colombian states.

Allele	S	F	VF
Valle	42.0	46.2	13.2
Meta	98.4	1.1	0
Huila	89.0	4.5	6.5
Tolima	73.3	3.8	24.0
Sucre	0	50.0	50.0
Magdalena	0	28.6	71.4
Cordoba	0	50.0	50.0
Guajira	0	47.0	49.4
Santander	17.7	80.2	0
Bolivar	0	52.2	47.8
Cesar	2.5	58.8	36.2
Cauca	100.0	0	0
Ecuador	66.4	4.3	18.8
Venezuela,Caribe	0	50.4	50.0
CIAT	54.6	44.8	0

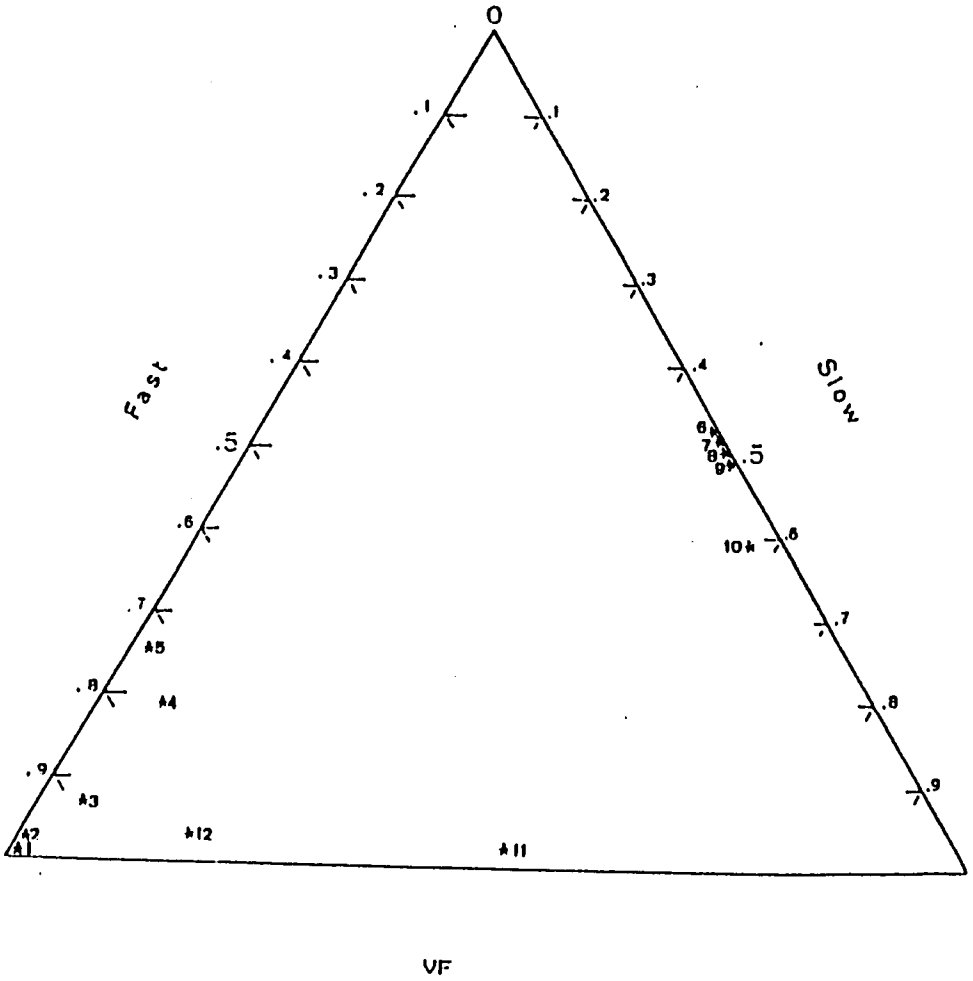


Fig. 22. Frequencies of EST alleles in different Colombian states, assuming a locus with 3 alleles - F, S and VF. Data of Table 13, plotted in triangular coordinates as in Fig. 2. (1) Meta, (2) Cauca, (3) Huila, (4) Ecuador, (5) Tolima, (6) Bolivar, (7) Guajira, (8) Cordoba, (9) Caribe, (10) Cesar, (11) Valle, (12) Santander.

DISCUSSION

The genetic interpretation of the Colombian patterns of electrophoretic isozymes is more complex than that of the Israeli samples, but whatever the genetic basis, it is clear that Colombian *Bemisia tabaci* whiteflies are differentiated geographically and may be considered "geographical races", where certain alleles are fixed in samples from large areas (such as Cauca, Caribe or Maracaibo, Venezuela).

The genetic problem associated with the identity of genotypes in Cordoba, Caribe and Venezuela (the apparent fixation of FVF in the heterozygote stage) may be solved if these whiteflies reproduce parthenogenetically. If reproduction at these sites is parthenogenetic (some whiteflies do reproduce parthenogenetically) it will be major evidence in support of genetic race formation in Colombian *B. tabaci*. However, we have no data on this point and our genetic interpretation of the isozymes may be wrong

The difference between Venezuelan samples from around Maracaibo and the Colombian samples may be explained by a geographical barrier to gene flow. On the borderline between the two countries in that area there is a high mountain range, the Serrania de Perija (L.M. Constantino, pers. comm.) which may prevent intermixing of the two "races".

Colombia is approximately 200 times larger than Israel, and is dissected (in a south to north direction) by three Andean mountain ranges which reach altitudes of 5,000 m and more in some places, and thus form presumably impassable barriers to the movement of *B. tabaci* (most of the agricultural areas with suitable host plants occur in the valleys). These conditions are of course favourable for the formation of geographical races, unlike the situation in Israel.

With the exception of cotton, most of the host plants from which we collected *B. tabaci* in Colombia were different from the Israeli host plants.

Cotton was not one of the preferred hosts of the whitefly in Colombia, and when we tried to collect them from cotton fields we often found the adults concentrated on weeds inside or in the periphery of the field and not on the cotton plants themselves.

The striking difference between Colombian and Israeli collection data is illustrated by the list of host plants (Table 11). In Israel, the majority of the whiteflies were collected from commercial crops (cotton and cucurbits in particular). In Colombia, commercial crops provided only a minority of the samples (excluding the control population on beans) - almost all the host plants were weeds growing along roadsides or bordering the cotton fields. Many of these host plants were species of *Euphorbia*; (only one (ornamental) plant belonging to this family was found as a host of *B. tabaci* in our study in Israel). The same plant species were collected from in most localities. We may, therefore, assume that the differentiation among populations in Colombia is probably not due to host-race formation.

Of interest is the "Valle" population in Colombia, in particular the CIAT control (on beans). It is so very similar in pattern to the Israeli samples that identity of origin was first suspected, but our work shows that, physiologically, the S isozyme in the two countries may not be the same, and thus the genetic basis may be different (the specific substrate preference of S to the α- configuration was not observed in Colombian whiteflies). In addition, the high level of heterozygosity in CIAT control samples is unparalleled in Israel, suggesting that despite similar migration patterns of isozymes on the gels, the genes coding for them are not identical.

<u>*B. tabaci* from other countries</u>. Data on *B. tabaci* EST patterns in different countries are rare. The publications known to us are our own (Wool *et al.* 1989; Wool 1990); Prabhaker, Coudriet & Meyerdirk 1987 and Burban, Fishpool &

Abisgold 1989), which were mentioned in the Introduction. During this study we obtained small samples from Europe (Denmark, France), Africa (Kenya) and the U.S. (California, Arizona). The samples were too small to permit quantitative analysis, but the impressions were that EST patterns in these samples - especially the one from Kenya - were quite different from the Israeli or Colombian whiteflies.

All these data point to the conclusion that the worldwide *B. tabaci* is not a single genetic entity, but rather a complex of local, regional or geographical races. This has major implications for whitefly control and quarantine.

PART III. DISCRIMINATION AMONG DIFFERENT SPECIES OF WHITEFLIES BY ELECTROPHORESIS

This part of the report was published (Wool *et al.* 1989).

Introduction

The taxonomy of whiteflies is based on morphological characters of the pupal case (Martin 1987). These characters may be affected by environmental factors (Mound 1983; Mohanty & Basu 1986). Pupal morphology in polyphagous whitefly species may vary depending on the host plant (Lopez-Avila 1986). Adult whiteflies of some species are difficult to tell apart. But for practical reasons, identification of adults is of primary importance, because the adults are vectors of plant viral diseases (see review by Duffus 1987). We have attempted to find out if we could add discriminating power to the taxonomy of whiteflies by using electrophoresis.

Electrophoretic techniques became quite widespread in biological research, particularly in population genetics, in the last 20 years. In the present context, the use of electrophoresis in insect taxonomy is relevant (Berlocher 1979). In several cases "cryptic" strains and species were detected (examples: Snyder 1977, 1982; Hsiao and Stutz 1985; Steiner *et al.* 1985; Tomiuk, Woehrmann and Eggers-Schumacher 1979). Prabhaker, Coudriet & Meyerdirk (1987) and Wool *et al.* (1989) specifically addressed the issue of species identification in whiteflies. Prabhaker, Coudriet & Meyerdirk (1987), showed that *B. tabaci* and *Trialeurodes vaporariorum* have different EST patterns (actually, no activity could be detected in the latter species) and *T. abutilonea* was different from *B. tabaci*.

We set out to test for differences among species of whiteflies in Israel and Colombia.

Materials and methods

The following species of whiteflies were included in this part of the study (Table 14): in Israel - *Bemisia tabaci*, *B. afer* (a small sample),

Table 14: List of whitefly species included in this study.

Species	Host plants	Country
Bemisia tabaci	see tables A1, A2	Israel & Colombia
Bemisia tuberculata	*Manihot esculenta* (Euphorbiaceae)	Colombia
Bemisia afer	*Smilax* sp. (Liliaceae)	Israel
Dialeurodes kirkoldyi	*Jasminum* (Oleaceae)	Israel
Dialeurodes citri	*Melia azedarach* (Meliaceae)	Israel
	Citrus (Rutaceae)	Israel & Colombia
Trialeurodes vaporariorum	*Lantana* (Verbenaceae)	Israel
	Tomato (Solanaceae)	Israel
	Beans (Leguminosae)	Colombia

Dialeurodes kirkoldyi, *D. Citri*, and *Trialeurodes vaporariorum*. In Colombia: *Bemisia tabaci*, *B. tuberculata*, *Dialeurodes citri*, and *Trialeurodes vaporariorum*.

Individual adults were homogenized and run on acrylamide gels as described above. Four enzyme systems were analyzed: EST, -GPDH, MDH and ME.

Results

The results show that the electrophoretic patterns of different whitefly species are often very distinct. Fig. 1 in Wool *et al.* (1989) illustrates the EST patterns of two congeneric species - *B. tabaci* and *B. tuberculata*. The same comparison can be made by inspecting Fig. 13 in the present report. The *B. tabaci* pattern (in a sample from CIAT) shows a "major" system similar to that described from Israel - either an S band or an F band in homozygotes, and a double band in heterozygous individuals. A weaker, much faster isozyme (ff) occurs in all individual *B. tabaci* adults in Colombia. The same isozyme is rare in Israel and is visualized on good gels only. *B. tuberculata*, which infests cassava, has a very different isozyme pattern.

Wool *et al.* (1989) (see Fig. 23c) illustrates diagrammatically the EST patterns of three more species - *Dialeurodes kirkoldyi* which infests *Jasminum* (Oleaceae), *D. citri* (on citrus and *Melia*) and *Trialeurodes vaporariorum*, a pest of many greenhouse plants. Their EST patterns when compared with *B. tabaci*, are quite different. We did find EST activity in *T. vaporariorum*, although Prabhaker *et al.* (1987) did not detect such activity. The single isozyme is very weak and appears only upon prolonged staining.

Another system which can be used to discriminate *B. tabaci* from the morphologically similar *T. vaporariorum* adults, is αGPDH (Fig. 23b). This system was found monomorphic within species, with a single isozyme, but the isozyme migrates to a different distance in each species (note the single

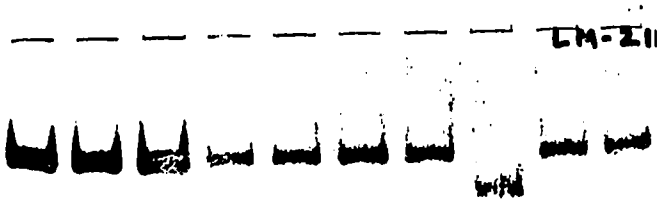


Fig. 23. (a) Pattern of α-GPDH in *B. tabaci* and one *Trialeurodes vaporariorum* individual in Colombia. (b) Comparison of α-GPDH patterns in several whitefly species. (c) Diagrammatic comparisons of EST patterns in several whitefly species (from Wool *et al.* 1989).

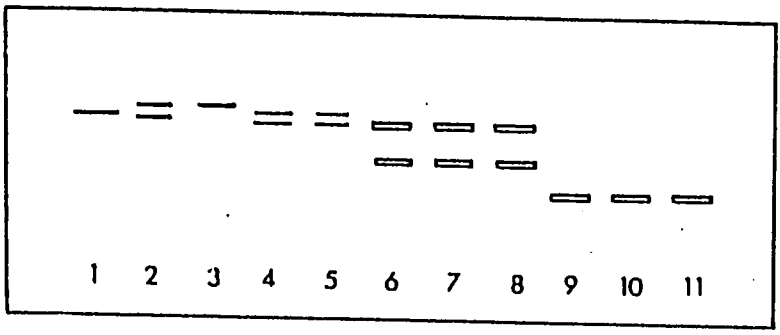


*Fig.*23bDiagrammatic representation of EST patterns of whitefly species from Israel. From left: (1–3) *B. tabaci* f, fs, s; (4–5) *Dialeurodes kirkoldyi*; (6–8) *Dialeurodes citri*; (9–11) *Trialeurodes vaporariorum*. Thin bands – weak staining. Quadrangles indicate very weak bands, often detectable only after long staining

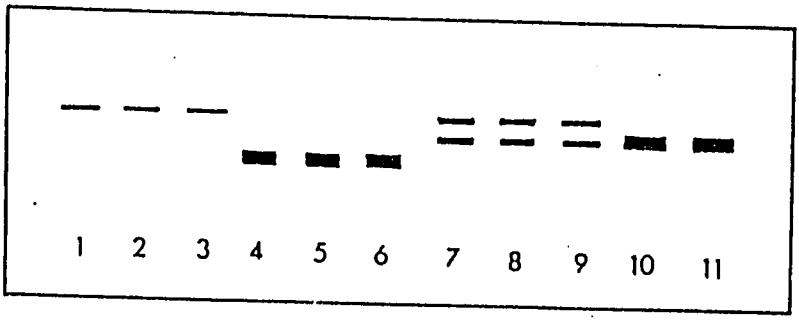


Fig. 23c Patterns of α-GPDH in whiteflies from Israel. From left: 1–3 *B. tabaci*; 4–6 *Trialeurodes vaporariorum*; 7–9 *Dialeurodes citri*; 10–11 *D. kirkoldyi*

aberrant individual in Fig. 23a - which was a *T. vaporariorum* adult morphologically misclassified as *B. tabaci*). The other species of whiteflies also had α-GPDH patterns different from *B. tabaci* and from each other (Fig. 4 in Wool *et al.* 1989, and see Fig. 23b)

The *Dialeurodes* and *Trialeurodes* species can be readily distinguished from *Bemisia* in having far less active esterases (using our procedures at least), and often seem not to stain at all with this system. Upon prolonged staining, weak bands are detectable in these species (illustrated in diagrammatic form in Fig. 23c). *Dialeurodes kirkoldyi* has two weak bands, *D. Citri* also two but with a wider difference in mobility between them. *Trialeurodes vaporariorum* has a single, very weak, fast migrating EST. α-GPDH was monomorphic within species in all individuals of *B. tabaci* examined in Israel, *T. vaporariorum* also had a single band but it migrated to a different distance in different species. *Dialeurodes citri* had a very strong single band but the difference in migration distance from the α-GPDH of *B. tabaci* is small. *D. kirkoldyi* has two, closely-migrating α-GPDH bands (illustrated diagrammatically in Fig. 23b).

MDH and ME were tried on many samples of Israeli whiteflies but their value as diagnostic species markers is uncertain. It seems that MDH loses activity when stored at -20°C for extended periods. There was a sex-related difference in migration distance of MDH in *B. tabaci*.

Conclusion:

Whitefly adults which are sometimes very similar morphologically, can be told apart with confidence by electrophoresis. The minute whitefly adults may easily be carried by wind over great distances and may land on crops to which they transmit viruses but also on crops to on which they are harmless. Since whitefly adults can be captured by various inexpensive means on crops,

electrophoresis can be used to identify them and decide whether or not their presence threatens the crops and require treatment, without having to wait until infestation has occurred to send pupae for identification.

PART IV. WHITEFLY IDENTIFICATION AND MORPHOLOGICAL CHARACTERIZATION USING SCANNING ELECTRON MICROSCOPY (SEM) AT CIAT

In the latter part of the project, CIAT acquired a scanning electron microscope, and Dr. Lee Calvert (who joined CIAT at about that time) decided to apply SEM technology in the identification of whiteflies, in particular the adults. It will be recalled that the taxonomy of whiteflies is based on pupal morphological characters (Gill 1989), and no taxonomic keys have been published for adults (see Introduction, p. 3). (A provisional key for adult whiteflies of California was kindly provided us by its author, Dr. R.J. Gill).

Materials and Methods

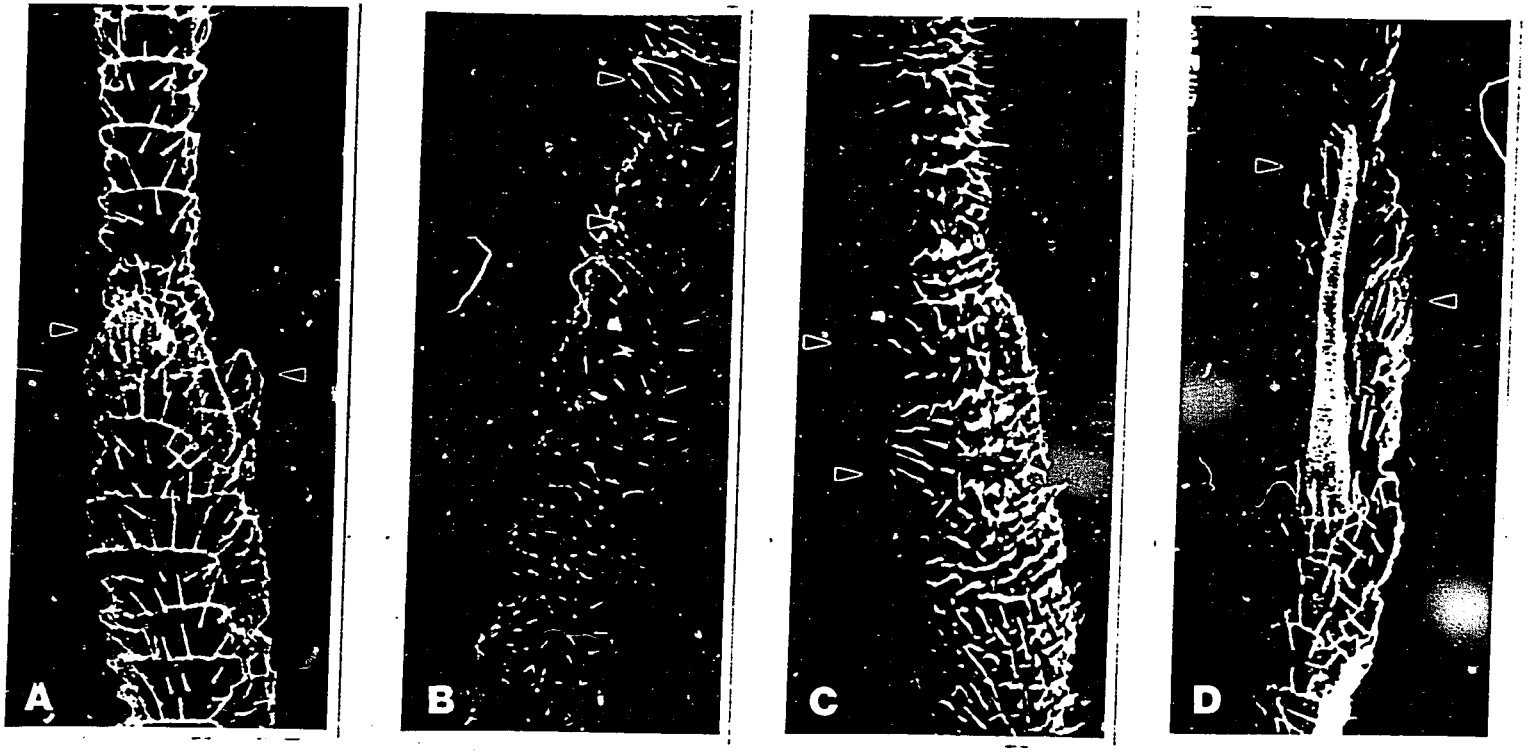
Preliminary investigation of adult whitefly morphology was carried out on field samples (stored in 70% ethanol) of five whitefly species. Adult, as well as pupal characters, were observed.

Results

Differences between species of whiteflies were detected in the numbers of ommatidia and the shapes of the compound eyes (see photographs in Fig. 24), and in the shape and number of sensorial cones on the third antennal segment (Fig. 25). Differences were also observed in structural details of legs and genitalia.

Discussion

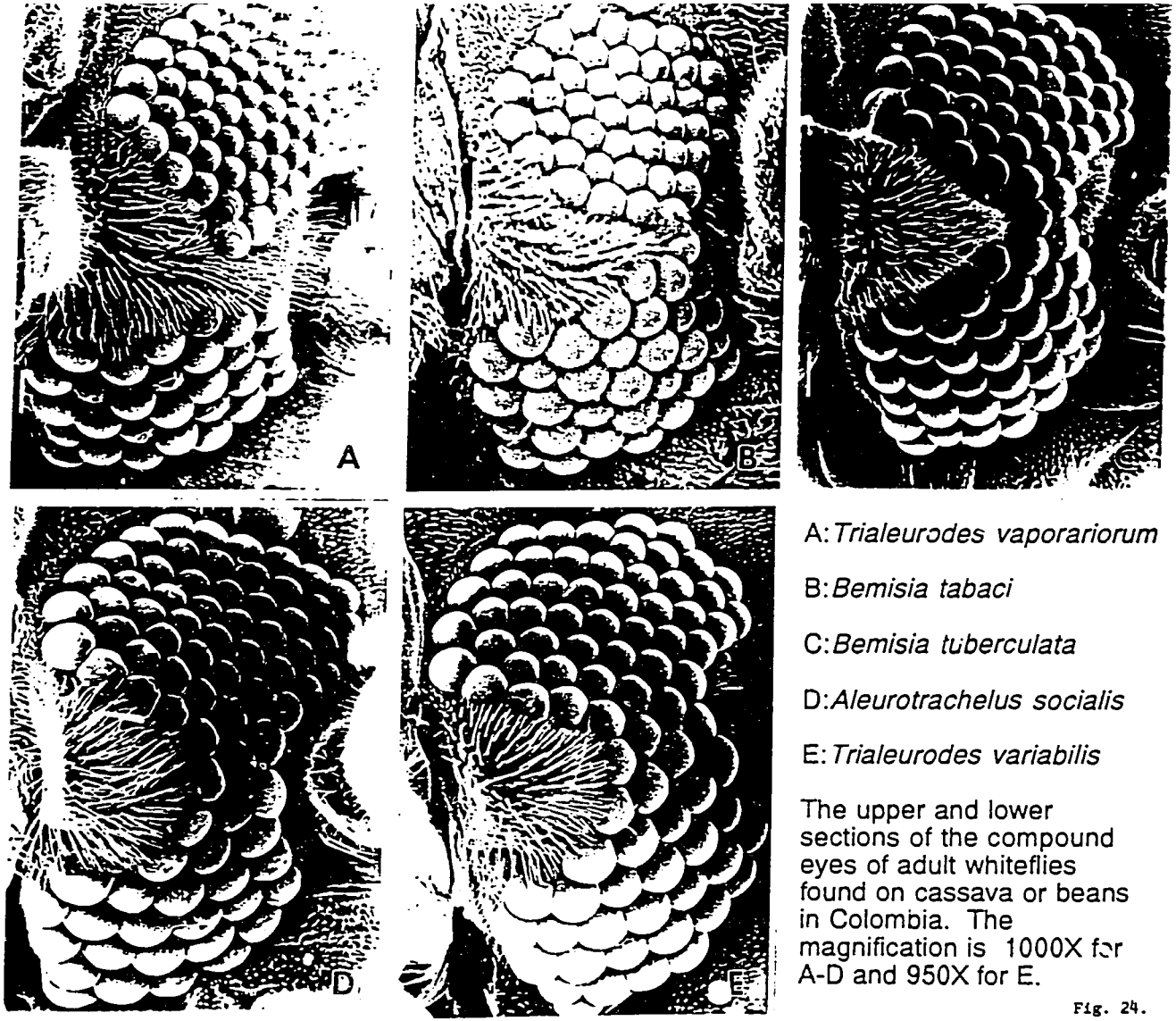
The use of SEM can certainly help whitefly taxonomists in doubtful cases concerning species identification. A major advantage of SEM, compared with electrophoresis, is that samples can be collected and stored in alcohol in the field rather than kept frozen for enzyme analysis. The disadvantage, of course, is that SEM is available only in well-equipped laboratories and the procedure is expensive.



A: *Trialeurodes vaporariorum*
B: *Bemisia tabaci*
C: *Bemisia tuberculata*
D: *Aleurotrachelus socialis*

The antennal segment III of adult whiteflies that are found on cassava or beans in Colombia. The arrows point to the primary sensoria. The magnification is 2300X for A-D.

Fig. 25.



A: *Trialeurodes vaporariorum*

B: *Bemisia tabaci*

C: *Bemisia tuberculata*

D: *Aleurotrachelus socialis*

E: *Trialeurodes variabilis*

The upper and lower sections of the compound eyes of adult whiteflies found on cassava or beans in Colombia. The magnification is 1000X for A-D and 950X for E.

Fig. 24.

One particular aspect of whitefly identification which could be carried out with SEM is the comparison of samples of a widespread species such as *Bemisia tabaci* from different parts of the world (Different countries in Central and South America, the U.S.A., Africa, Israel and India). As mentioned earlier, we have some electrophoretic evidence that these populations are not the same genetically. It would be interesting to know if any morphological differences can be detected by SEM.

PART V. CONCLUDING REMARKS

The project summarized in this report was a model of fruitful collaborative scientific research between Israel and a developing country. Collaboration between TAU and CIAT was very close throughout the project. D. Wool paid two visits to CIAT and stayed there long enough to transfer electrophoretic methods from TAU, to coordinate research effort, and to collect samples of whiteflies from different Colombia states together with CIAT personnel. Data sheets from CIAT were routinely sent to Israel, and data from both countries were stored and processed almost as soon as they were recorded. This enabled real-time evaluation of the progress of the project.

The project has yielded important evidence in support of our hypothesis that geographical populations of the world-wide pest, *Bemisia tabaci*, are genetically heterogeneous. From the point of view of a population geneticist, who is familiar with studies on other insects distributed over large geographical areas, this result is hardly surprising and was expected. But for whitefly control and quarantine, these results have important consequences, in view of the inability of current taxonomic procedures to differentiate between the variants or races which we detected electrophoretically. It is possible that these variants differ in economically relevant properties such as insecticide resistance, host preference, or virus vector potential. That this may be so is indicated by our discovery of an electrophoretic difference between *B. tabaci* samples from agricultural fields and unsprayed control populations.

The markers we developed can be used to discriminate between and identify different species of whiteflies. We have also shown that the scanning electron microscope can be used to this end. The CIAT laboratory is now capable of using three techniques efficiently.

At the time of writing, two publications came out of this project (Wool

et al. 1989; Wool 1990, now in press). Much remains to be published.

Finally, the project opened the way to future research by leaving the most important questions unanswered: are the genetic races of *B. tabaci* in fact different in biologically and economically important characteristics such as mentioned above? We applied recently to CDR-AID for support of the continuation of this research. We firmly believe that both Colombia and Israel, as well as world agriculture in general, will benefit from the continued collaborative research between our laboratories.

Literature cited

Ahmed, A.H.M., Elhaj, E.A., Bashir, N.H.M. 1987. Insecticide resistance in the cotton whitefly (*Bemisia tabaci* Genn.) in the Sudan Gezira. Trop. pest Management 33:62-72.

Bellows, T.S., Perring, T.M., Arakawa, K., Farrar, C.A. 1988. Patterns in diel flight activity of *Bemisia tabaci* (Homoptera: Aleyrodidae) in cropping systems in southern California. Environ. Entomol. 17:225-228.

Berlocher, S.H. 1979. Biochemical approaches to strain, race and species discrimination. pp. 137-144 in "Genetics in relation to insect management", M.A. Hoy and J.J. McKelvey (eds.) Rockefeller Foundation.

Bird, J. 1982. Relationship between whiteflies and whitefly-borne viruses. Workshop on Plant Virus Detection, Univ. of Puerto Rico. exp. Sta., pp.1-9.

Brittnacher, J.G., Sims, S.R., Ayala, F.J. 1978. Genetic differentiation between species of the genus Speyeria (Lepidoptera, Nymphalidae). Evolution 32:199-210.

Burban, C., Fishpool, L.D.C., Abisgold, J.D. 1989. Characterisation des populations de *Bemisia tabaci* en fonction des plantes hotes: Recherche de marqueurs electrophoretique et transferts d'hotes. Proceedings 4th International Plant Virus Epidemiology Workshop.

Byrne, D.N., Bellows, T.S., Parella, M.P. 1990. Whiteflies in agricultural systems. pp. 227-261 in "Whiteflies: their bionomics, pest status, and management", D. Gerling (ed.). Intercept.

Cock, M.J.W. (ed.) 1986. *Bemisia tabaci* - a literature survey. FAO & C.A.B Publications.

Costa, A.S., Russell, L.M. 1975. Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera, Aleyrodidae). Ciencia e Cultura 27:388-390.

Dittrich, V., Hassan, S.O., Ernst, G.H. 1986. Development of a new primary pest of cotton in the Sudan: *Bemisia tabaci*, the whitefly. Agriculture, Ecosystems and Environment 17:137-142.

Dittrich, V., Uk, O., Ernst, G.H. 1990. Chemical control and insecticide resistance of whiteflies. pp. in "Whiteflies: their bionomics, pest status, and management", D. Gerling (ed.), Intercept.

Dittrich, V., Hassan, S.O., Ernst, G.H. 1985. Sudanese cotton and the whitefly: a case study of the emergence of a new primary pest. Crop protection 4:161-176.

Dittrich, V. 1987. Resistance and hormoligosis as driving forces behind pest outbreaks. pp. 169-181 in "Rational pesticide use", K.Y. Brent & R.K. Alein, (eds.), Cambridge Univ. Press.

Duffus, J.E., 1987. Whitefly transmission of plant viruses. pp. 73-91 in "Current topics in vector research", K.R. Harris (ed.), Springer.

Geiger, H.J., Scholl, A. 1985. Systematics and evolution of holarctic Pierinae (Lepidoptera): an enzyme electrophoretic approach. Experientia 41:24-29.

Gerling, D. 1990 (ed.). Whiteflies: their bionomics, pest status, and management. Intercept.

Gerling, D., Motro, U., Horowitz, R. 1980. Dynamics of *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae) attacking cotton in the coastal plain of Israel. Bull. Ent. Res. 70:213-219.

Gerling, D., Horowitz, A.R. 1984. Yellow traps for evaluating the population levels and dispersal patterns of *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae). Ann. ent. Soc. Amer. 77:753-759.

Gerling, D. 1984. The overwintering mode of *Bemisia tabaci* and its parasitoids in Israel. Phytoparasitica 12:109-118.

Gerling, D., Horowitz, A.R., Baumgartner, J. 1986. Autecology of *Bemisia tabaci*. Agriculture, Ecosystems & Environment 17:5-19.

Gill, R.J. 1989. The morphology of whiteflies. pp. 11-44 in "Whiteflies: their bionomics, pest status, and management", D. Gerling (ed.), Intercept.

Horowitz, A.R., Toscano, N.C., Youngman, R.R., Georghiou, G.P. 1988. Synergism of insecticides with DEF in sweetpotato whitefly (Homoptera, Aleyrodidae). J. Econ. Entomol. 81:110-114.

Hsiao, T.H., Stutz, J.M. 1985. Discrimination of alfalfa weevil strains by allozyme analysis. Entomol. exp. & Appl. 37:113-121.

Jayaraj, S., Rangarajan, A.V., Murugesan, S., Santharam, G., Vijayaraghavan, S., Thangaraj, D. 1986. Studies on the outbreak of whitefly *Bemisia tabaci* (Gennadius) on cotton in Tamil Nadu. pp. 103-115 in "Resurgence of sucking pests", S. Jayaraj (ed.), Tamil Nadu Agric. Univ., Coimbatore, India.

Lopez-Avila, A. 1986. Taxonomy and Biology. pp. 1-12 in "*Bemisia tabaci* - a literature survey", M.J.W. Cock (ed.), F.A.O. & C.A.B. Publication.

Loxdale, H.D., Castanera, P., Brookes, C.P. 1983. Electrophoretic study of enzymes from cereal aphid populations. I. Electrophoretic techniques and staining systems for characterizing isoenzymes from six species of cereal aphids (Hemiptera, Aphididae). Bull. Ent. Res. 73:645-657.

Martin, J.H. 1987. An identificaton guide to common whitefly pest species of the world (Homoptera: Aleyrodidae). Trop. Pest Manage. 33:298-322.

Mohanty, A.K., Basu, A.N. 1986. Effect of host plant and seasonal factors on intraspecific variation in pupal morphology of the whitefly vector, *Bemisia tabaci* Genn. (Homoptera, Aleyrodidae). J. Ent. Res., 10:19-26.

Mound, A.K. 1983. Biology and identity of whitefly vectors of plant pathogens. pp. 305-313 in "Plant-virus epidemiology", R.T. Plumb & J.M. Thersh (eds.), Blackwell.

Nevo, E. 1978. Genetic variation in natural populations - pattern and theory. Theoret. pop. Biol. 13:121-177.

Nevo, E., 1983. Adaptive significance of protein variation. pp. 239-282 in "Protein polymorphism: adaptive and taxonomic significance", G.S. Oxford & D. Rollinson (eds.), Academic Press.

Nevo, E., Beiles, A., Ben Shlomo, R. 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates: Evolutionary dynamics of genetic diversity, Lecture notes in Biomathematics, 53:13-213.

Onillon, J.C. 1990. The use of natural enemies for the biological control of whiteflies. pp. 287-313 in "Whiteflies: their bionomics, pest status, and management", D. Gerling (ed.), Intercept.

Prabhaker, N., Coudriet, D.L., Meyerdirk, D.E.,1985. Insecticide resistance in the sweetpotato whitefly, *Bemisia tabaci* (Homoptera, Aleyrodidae). J. Econ. Entomol. 78:748-752.

Prabhaker, N., Coudriet, D.L., Meyerdirk, D.E. 1987. Discrimination of three whitefly species (Homoptera: Aleyrodidae) by electrophoresis of non-specific esterases. J. Appl. Ent. 103:447-451.

Prabhaker, N., Toscano, N.C., Coudriet, D.L. 1989. Susceptibility of the immature and adult stages of the sweetpotato whitefly (Homoptera, Aleyrodidae) to selected insecticides. J. Econ. Entomol. 82:983-988.

Robertson, I.A.D. 1987. The whitefly, *Bemisia tabaci* (Gennadius), as a vector of African cassava mosaic virus at the Kenya coast, and ways in which the yield losses in cassava, *Manihot esculenta* Crantz, caused by the virus can be reduced. Insect Sci. Appl. 8:797-801.

Snyder, T.P. 1977. A new electrophoretic approach to biochemical systematics of bees. Biochem. Syst. Ecol. 5:133-150.

Snyder, T.P. 1982. Electrophoretic characterization of blackflies in the *Simulium venustum* and *verecundum* species complexes (Diptera, Simulidae). Can. Ent. 114:503-507.

Sokal, R.R., Rohlf, F.J. 1981. Biometry. Freeman.

Steiner, W.W.M., Voegtlin, D.J., Irwin, M.E., Kampmeyer, G. 1985. Electrophoretic comparison of aphid species detecting differences based on taxonomic status and host plant. Comp. Biochem. Physiol. 81B:295-299.

Tomiuk, J., Woehrmann, K., Eggers-Schumacher, H.A. 1979. Enzyme patterns as a characteristic for the identification of aphids. Z. Ang. Ent. 88:440-446.

Wool, D., Gerling, D., Cohen, I. 1984. Electrophoretic detection of two endoparasite species, *Encarsia lutea* and *Eretmocerus mundus*, in the whitefly *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae). Z. Ang. Ent. 98:276-279.

Wool, D., Gerling, D., Nolt, B., Constantino, L.M., Bellotti, A.C., Morales, F.J. 1989. The use of electrophoresis for identification of adult whiteflies (Homoptera:Aleyrodidae) in Israel and Colombia. J. Appl. Ent. 107:344-350.

Wool, D. 1990. Spatial and temporal genetic variation in populations of the whitefly *Bemisia tabaci* (Genn.) in Israel and Colombia: an interim report. Insect Sci. Appl. (in press).

Wool, D., Greenberg, S. 1990. Esterase activity in whiteflies (*Bemisia tabaci*) in relation to insecticide resistance. Ent. exp. & Appl. (in press).

Table A1: Collection Localities and host plants - Israel
Wild plants are identified by the genus name. Commercial plants are listed by common names. Localities can be found in the map (Fig. 4).

I. Galilee (Upper)	Host plants
Hagash (Kiriat Shmona)	Cotton; Melon
II. Beit Shean Valley	
Tirat Zvi	*Lantana*; Cotton
Sde Eliyahu	*Lantana*; Cotton
Eden	Cotton; Melon
Mevo Hama	Cotton
Reshafim	Cotton
III. Western Galilee	
Beit Haemeq	Cotton
Kfar Masarik	Cotton
Carmel	*Inula*
Hanita	Cotton
Shavei Zion	Cotton
IV. Sharon	
Kfar Sava	*Lantana*
Gan HaShomron	*Amaranthus*
Tel Aviv (TAU)	Melon
Herzlia	*Lantana*
Petah Tiqva	*Lantana*
Beit Halevi	Eggplant
Givat Haim	Cotton
Miqve Israel	Cotton

V. Southern coastal plain	Host plants
Masmia	Cotton
Kiriat Gat	Cotton; Sunflower
Givat Brenner	*Lantana*
Keremya	Cotton
Berechia	Cotton
Kfar Aza	Cotton
Hahal Oz	Cotton
Sde Yoav	Sunflower
Gvar Am	Cotton; Sunflower
Beit Hanan	*Lantana*
Beit Oved	*Lantana*
VI. Ayalon valley	
Nachshon	Cotton; Sunflower
Ayalon - Latrun	Cotton; Sunflower
Gezer	Cotton
Kfar Uriah	Squash
Yad Rambam	Eggplant; Tomato
Shaalvim	Cotton; Sunflower
Azaria	*Amaranthus*
Ramla	Cotton
VII. Beit Guvrin	Squash; *Inula*; *Amaranthus*; *Abutilon*; Sunflower
VIII. Beer Sheva - Negev	
Beer Sheva	*Lantana*; *Inula*; Cotton
Nevatim	Potato; Pepper

IX. Arava valley & Dead Sea	Host plants
Ein Yahav	*Ipomoea*; Melon; Eggplant; Pepper; Tomato
Eilat	*Malva*; Melon
Iddan	Tomato; *Mentha; Lantana*
Paran	Melon; Tomato
Samar	Melon
Almog	Squash
X. Ramat Hasharon	*Lantana; Ipomoea; Malva; Amaranthus; Tespesia; Poinsettia; Datura*
XI. Tzora	Cotton; *Lantana*

Table A2:

Collection sites in Colombia and neighbouring countries, host plants and sample sizes. Wild plants are listed by scientific names; commercial crops by common names. Some Colombian states are marked on the map (Fig. 12)

State	# of sites	Principal host plants	Individuals analyzed
Valle	(1) CIAT (control)	beans (*Phaseolus vulgaris*)	158
Valle	6	cotton (*Gossypium hirsutum*)	51
		Euphorbia pruniflora	108
		E. hirta	53
		E. hypercifolia	8
		E. heterophylla	13
		Other hosts	243
Cauca	3	*Merremia* sp.	44
		Other hosts	13
Santander (incl. Norte Santander)	5	*Euphorbia hypercifolia*	30
		E. hirta	20
		Other hosts	25
Cesar	2	*Euphorbia hypercifolia*	30
Guajira	3	*Euphorbia hypercifolia*	30
		E. hirta	60
		Tomato	15
Magdalena	3	*E. hypercifolia*	30
Bolivar	3	*Euphorbia hypercifolia*	10
		E. hirta	10
		Rhyncosia minima	10
Sucre	2	*Euphorbia hirta*	10
		E. hypercifolia	10

State	# of sites	Principal host plants	Individuals Analyzed EST only
Atlantico	2	*Euphorbia prunifolia*	10
		E. hypercifolia	6
Cordoba	3	*Euphorbia hirta*	30
		E. hypercifolia	40
Meta	4	*Euphorbia hirta*	55
		Other hosts	10
Tolima	7	*Euphorbia hypercifolia*	140
		E. hirta	30
		E. heterophylla	70
		Cotton	92
		Other hosts	26
Huila	5	*Euphorbia hypercifolia*	100
		E. hirta	40
		Beans	30
		Squashes	40
		Other hosts	30
Ecuador (Manabi)	5	*Euphorbia hypercifolia*	10
		E. hirta	50
Venezuela	12	*Euphorbia hypercifolia*	70
		E. hirta	150
		Beans	90
		Cotton	80
		Cucurbits	40
		Other hosts	230