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**Epidemiological Studies on Whitefly-
Transmitted Viruses in California and Israel**

S. Cohen, J.E. Duffus, M.J. Berlinger

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Names of Investigators:

Principal Investigator: S. CohenCooperating Investigator(s): J.E. Duffus, M.J. Berlinger

Name and Address of Affiliated Institutions:

Principal Institution: A.R.O., The Volcani Center, P.O.B. 6, Bet Dagan,
Israel

Cooperating Institution(s): U.S.D.A.-A.R.S. Sugarbeet ProductionResearch Unit, Salinas, California, U.S.A.A.R.O., Gilat Exp. Station, Negev Post 2, IsraelProject's starting date: 22/8/1984Type of Report: 1st Annual _____ 2nd Annual _____ Final X

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C. ABSTRACT

Bemisia tabaci-borne viruses cause severe damage in vegetables in Israel and California. The objectives of this project were to identify the viruses involved, to study their epidemiology, and suggest cultural control measures for the most important ones. Four viruses have been identified: two gemini-type persistent, TYLCV (tomato yellow leaf curl virus) and MLCV (melon leaf curl virus); a closterolike semipersistent, LIYV (lettuce infectious yellow virus); and a non-persistent nepo-like, TNDV (tomato necrotic dwarf virus). A simple system was developed to monitor the movement and infectivity of whiteflies. It is based on a light-weight rechargeable auto-type vacuum cleaner modified to collect the whiteflies directly into a mini inoculation chamber. Sticky yellow traps, bait plants and whiteflies marked with fluorescent dust were also used. By using biological and serological methods, the gemini SLCV (squash leaf curl virus) was found in the haemocoel of B. tabaci, which serves as a reservoir for the salivary gland system where virus-specific sites exist. The inability of the virus to pass through the salivary gland is probably the reason for the failure of a non-vector whitefly to transmit the virus. The location of the semi-persistent LIYV is apparently in the stylet or in the front part of the alimentary track. Based on the findings of the virus and the vector host range, the B. tabaci population dynamics and the virus-vector-host relationships, the apparent cycles of TYLCV (from Israel) and LIYV (from California) were described and cultural control measures were suggested.

D. Objectives of the Original Research Proposal

1. Purification of the viral agents and development of rapid diagnostic methods for detection of the viruses in plants and in their whitefly vectors.

2. The utilization of rapid diagnostic techniques for the detection of these viruses in wild and cultivated plants.
3. Development of trapping and biological methods and the use of serological techniques for monitoring the percent of viruliferous whiteflies in the population.
4. Studies of the dynamics of whitefly populations and virus spread.

E. BODY OF THE REPORT

Three main factors are involved in the spread of whitefly-borne (and other insect-borne) viruses: the vector, the virus and the host. The interactions among these three factors may determine the disease epidemic. Only a few epidemiological studies have been carried out on whitefly-borne viruses (Bock, 1983, Cohen et al., 1986ab; Fauquet and Fargette, 1986; Muniyappa, 1983; Shivanathan, 1983). However, more information is available on the above mentioned individual factors. The most important vector among the whiteflies is Bemisia tabaci Gennadius, commonly called the tobacco whitefly or the sweet potato whitefly. This insect has been reported to be responsible for the transmission of more than 70 diseases in the subtropical and tropical regions of the world (Duffus, 1987). In this report we related only to B. tabaci. Several factors have an effect on the population dynamics of B. tabaci, the most important among them being: the climatic condition (temperature and humidity), host plants and the biological equilibrium with the natural enemies. At temperature range of 27-30°C is required for the whiteflies to complete their development in a minimum of time (egg to adult) (Avidov, 1956; Buttler et al., 1983; Gerling et al., 1986), but this duration of time as well as the number of progenies are also host dependent (Gerling et al., 1986). However, no apparent

correlation was observed between these two last mentioned factors (Courdier, 1985, 1986).

Oviposition is impaired by rain, low humidity (under 60%) and low temperature (Avidov, 1956; Gerling et al., 1986; Ohnesorge et al., 1981). The host plants also play an important role in overwintering of the sweet potato whitefly. Thus, Ohnesorge et al., (1981) indicated that in host plants with persistent leaves, like cauliflower and lantana, larvae of B. tabaci can survive under winter conditions in the Jordan Valley. Similar results were obtained by Gerling (1983, 1984). Several commercial crops and weeds were found to serve as overwintering hosts for B. tabaci in the Imperial Valley of California (Courdier et al., 1985, 1986).

The spread of whitefly-borne viruses is by the winged adults. Therefore, factors which influence the survival and behavior of this form have an effect on virus spread. To the best of our knowledge, no information is available on the effect of the climatic conditions on the survival of migrating adults. However, adults died soon after being in contact with surfaces preheated to 35°C or more (Avidov, 1956; Cohen, 1982). There is indirect evidence of the way adult whiteflies move: a. short active movement (distance measured in meters) (Fauquet et al., 1986; Melamed-Melamed et al., 1979) b. long distance passive movement controlled mainly by the wind (Berlinger and Dahan, 1986; Fauquet et al., 1986; Gerling and Horowitz, 1983; Melamed-Madjar et al., 1982; Sharaf, 1982; Youngman et al., 1986).

The number of flight hours of B. tabaci during the day was tested in only a few cases. The main flight was found to take place during the morning, until noon (Cohen and Melamed-Madjar, 1978; Fauquet et al., 1986). There are only fragments of information on the take-off mechanism of the sweet potato whitefly. Besides being attracted to the yellow part of the spectrum they are also

attracted by ultraviolet light (Mound, 1962) and tend to leave old plants (Melamed-Madjar, 1979). The landing behavior of B. tabaci is probably mainly a passive process (Cohen and Berlinger, 1986). So far no olfactory reaction has been detected in B. tabaci (Mound, 1962); on the other hand, as already mentioned, they are sensitive to certain wave lengths. Thus, host finding is probably confined to sight senses. B. tabaci adults appear to be very short sighted (Cohen, 1982), and therefore they probably start looking for the host when they are close to the soil surface (Cohen and Berlinger, 1986). Very little work has been done on the effect of the host plants on the landing behavior and feeding preference. Al Musa (1982) showed in Jordan that when rows of cucumbers were planted between rows of tomatoes 30 days before tomato seedlings were transplanted, the spread of TYLCV (tomato yellow leaf curl virus) among the tomatoes was significantly delayed during the first 2 months. He suggested that whiteflies prefer cucumber to tomatoes, and tomatoes to corn and eggplants. In the above studies two main techniques were utilized for trapping the adult whiteflies; sticky (mainly yellow) traps and the sampler collector device (usually Di-Vac) (Ohnesorge and Rapp, 1986). The sampler collector, besides being heavy and consequently uncomfortable to use, suck the insects into a bag from which they must first be released into a transparent chamber before being classified. Moreover, the chances of the whiteflies surviving for the period of least 24 h which is required for virus-transmission tests is markedly reduced following the high sucking stress and the handling through the transferring process. Therefore, this method cannot be used for monitoring the percent of infected insects in the populations by having them feed on test plants.

So far, whitefly-borne viruses of six or seven morphological classes have been demonstrated: the gemini-viruses, carla-like, clostero-like, poty-like,

luteo-like, and nepo- or como-like viruses (Duffus, 1987). Only viruses from the gemini group are transmitted in a persistent-circulative way by the whiteflies i.e., they can be maintained and transmitted by the vectors for several days to life. So far there is no direct evidence for the multiplication of these viruses in the whiteflies. However, there are some indications that the pathway of these viruses in the vector body is not a passive one. Antiviral factors were found to be produced in the bodies of whiteflies carrying the tomato yellow leaf virus (TYLCV). The introduction of these materials into the whiteflies prior to or after virus acquisition resulted in reduced ability of the insects to acquire and transmit the virus (Cohen, 1967, 1969; Cohen and Marco, 1970; Marco et al., 1972, 1975). In the other whitefly-borne virus groups, only semipersistent or non-persistent relationships were described (Duffus, 1987), i.e., these viruses can be retained by the vectors for short periods lasting from hours in the cases of non-persistent viruses, up to a few days in the semi-persistent viruses (Duffus, 1987). The path of transmission is probably one of the main factors which controls the distance these viruses can spread. Longer acquisition and transmission feeding periods are required by the vector when persistent viruses are concerned in comparison to those needed for non-persistent viruses. Therefore, in several cases the spread of persistent viruses was successfully controlled using insecticides to kill the whitefly vectors (Bird and Maramorosch, 1978; Cohen, 1982; Cohen and Berlinger, 1986; Costa, 1976; Muniyappa, 1980, Nene, 1972). To the best of our knowledge, no conclusive work has been published on the pathway of these viruses in the vector bodies. The following B. tabaci-borne viruses have been used in the present project: Lettuce infectious yellows virus (LIYV), a semipersistent clostero-like RNA virus which causes heavy damage to lettuce, sugarbeet, carrot and melons in the southwestern United States (Duffus et al., 1986).

Squash leaf curl virus (SLCV) (Cohen et al., 1983) and Melon leaf curl virus (MLCV) (Duffus et al., 1985) are two closely related persistent gemini-type DNA viruses. These viruses are responsible for the leaf curl disease complex of cucurbits in the southwestern deserts of the U.S.A.

Tomato necrotic dwarf virus (TNDV) (Larsen et al., 1984) is a non-persistent nepo-like virus. It affects tomato, pepper and other solanaceous crops in the southwestern deserts of the U.S.A.

Tomato yellow leaf curl virus (TYLCV) (Cohen and Nitzany, 1966; Russo et al., 1980) is a persistent gemini-type DNA virus. It causes heavy damage to tomato crops in the Middle East, Africa, and probably India and Central America.

Very little information is available on the parameters involved in the virus-host-vector relationships, such as: virus titer in the host plants and in the different parts of individual hosts during the different seasons; availability of the virus to the insect vectors; and susceptibility of the different parts to infection. Thus, young leaves of Cassava plants contain the highest African cassava mosaic virus (ACMV) titer in comparison with the older leaves. The virus could not be detected in mature leaves positioned 7th or farther from the top. Also young leaves are the most susceptible to infection (Fargette et al., 1986). It is important to mention that B. tabaci adults prefer to feed on young leaves. A higher transmission rate was achieved by whiteflies previously fed on young leaves of Datura stramonium infected with TYLCV than in those fed on mature leaves located 3rd from the top (Cohen unpublished data).

The rate of ACMV-infected B. tabaci in the population was measured by biological means and found to be relatively low (0.18-0.67%) (Fauquet and Fargett, 1986).

In carrying out a systematic field survey on the occurrence and spread of viruses, a rapid and simple method for detection of the virus in plants and

insects should be used. ELISA (enzyme-linked Immuno sorbent assay) was used on a large scale to detect ACMV in cassava plants. However, this assay was not used in a host range survey of whitefly borne viruses. No work has been done on the detection of a whitefly-borne virus in the insects by ELISA, but SLCV DNA could be detected in B. tabaci using the spot hybridization technique with a cloned DNA probe made from SLCV (Al Musa et al., 1986). In the present work we tried to collect additional information on the different factors involved in the epidemiology of B. tabaci-borne viruses in Israel and USA. The research was concentrated on the following aspects: the identification of the virus involved; development of methods for field work with B. tabaci and detection of virus hosts; studies of factors influencing B. tabaci's distribution and behavior; and studies of the virus-vector-host relationships. All these have been done with the aim of determining out the epidemiological cycle of two of the most important whitefly-borne viruses, the tomato yellow leaf curl virus (tomato yellow leaf curl virus) in Israel (Cohen and Nitzany, 1966) and the lettuce infectious yellows virus (lettuce infectious yellow virus) (Duffus et al., 1986) in the Imperial Valley in California, U.S.A.

MATERIALS AND METHODS

Virus sources and whiteflies maintenance. TYLCV was maintained in D. stramonium L. or in tomato plants. Young leaves 3-5 weeks after inoculation were used as the virus source. D. stramonium plants at the first true leaf stage were used as test plants.

LIYV was maintained in lettuce, cv. Summer Bibb, or in Malva parviflora L. Plants at least 25 days after inoculation were used as virus sources in the different experiments. Lettuce plants (Summer Bibb) at the 3-4 leaf stage were used as test plants.

SLCV was maintained in squash (cv. Black Magic). Unless otherwise stated, the virus source in the various experiments was young leaves of squash plants which had been inoculated 2-3 months earlier. Squash plants ("Black Magic") with one or two leaves were used as test plants.

MLCV was maintained in watermelon. As a virus source young leaves of watermelon inoculated 15-20 days previously were used. Watermelon plants at the first- or second-leaf stage were used as test plants.

TNDV was maintained in tomato or Tomatillo plants.

Following inoculations with whiteflies, the plants were sprayed with resmethrin (USA) or with Smash or Simbush (Israel) before they were placed in the greenhouses. All plants were grown in screened greenhouses fumigated at weekly intervals with Vaporn and resmethrin (USA) or nicotine sulfate (Israel). B. tabaci colonies were kept on sweet potato (USA) or on cotton (Israel) grown in muslin-cover cages. The cages were kept in an insectary. Sweet potato and cotton are immune to the viruses under study. Trialeurodes abutilonea colonies (USA) were kept on Physalis alkekengi L. grown in muslin-covered cages. The cages were maintained in a growth room at temperatures of 21^o-27^oC.

THE VIRUSES

Purification and Identification

Tomato yellow leaf curl virus (Israel) - Purification procedures were carried out essentially according to Goodman et al. (1977) and Cohen et al. (1983), with some modifications. D. stramonium tomato plants 30-45 days after inoculation (1-2 weeks after symptoms appearance) served as a source for virus extraction.

Method I: Leaves frozen in liquid nitrogen were crushed to fine powder with a mortar and pestle and homogenized in a Waring Blendor after adding ice-cold

100 mM trisodium citrate - 0.32% ascorbic acid - 1% betamercaptoethanol (buffer A), using 2 ml/g tissue. The homogenate was treated with Triton X-100 (final concentration of 2.5%, v/v), stirred overnight at 4°C, squeezed through four layers of cheesecloth, and clarified by a 10 min centrifugation at 8,000 g. The supernatant was centrifuged for 3 h at 100,000 g in a SW2B Beckman rotor. Pellets were suspended in 20 mM trisodium citrate - 1 mM EDTA, pH 8.0 - 0.1% betamercaptoethanol (CEM buffer) and submitted to similar low speed followed by high speed centrifugation. Pellets were suspended in 10% sucrose in CEM (1 ml buffer per equivalent of 100 g leaves), homogenized with a Teflon-glass homogenizer, and clarified by a 10-min 4000 g centrifugation. The supernatant was applied to a 32 ml linear 10-50% sucrose gradient in CEM (extracts obtained from 100 g tissue were applied on each gradient). The gradients were fractionated (2 ml/fraction) after being subjected to a 14-h centrifugation at 100,000 g in a SW2B rotor.

Method II: Liquid nitrogen-treated leaf powder was stirred overnight at 4°C in three volumes of 0.1 M phosphate buffer pH 7.0 containing 2 mM EDTA, 10 mM sodium sulfate, 0.1% mercaptoethanol (v/v) and 1% (v/v) Triton X-100. The extract was filtered through muslin and the resulting sap was clarified by the addition of 10% (v/v) cold chloroform followed by a 10 min centrifugation at 8,000 g. The supernatants were loaded on 20% (w/w) sucrose cushions and centrifuged for 3.5 h at 25,000 rpm in a Sorvall AH627 rotor. Pellets were resuspended in 0.1 M phosphate buffer, pH 7.0, containing 2 mM EDTA (resuspension buffer), left overnight at 4°C, and subjected to low speed centrifugation (5 min at 4,000 g). Sucrose gradients (10-40%) were prepared in the resuspension buffer. The virus suspension (1 ml) was layered on the top of each gradient and centrifuged at 25,000 rpm for 2.5 h in a Sorvall AH627 rotor.

Gradients were fractionated by a syringe needle connected to a peristaltic pump (1 ml/fraction).

The presence of the virus in the sucrose gradient fractions was confirmed by electron microscopic observations and by infectivity tests. Virus concentrating was performed by a triple dilution of virus-containing fractions and high-speed centrifugation at 100,000 g for 3 h. Pellets were suspended in 10% sucrose-CEM (Method I) or in 0.1 mM phosphate buffer, pH 7.0 (Method II), and stored at -20°C.

Virus preparations were adjusted to 25% sucrose (w/v) and dyed yellow with liquid food coloring before being fed to whiteflies through membranes, as described before (Cohen, 1967). About 100 whiteflies per cage and five cages per fraction were used for the detection of virus infectivity in sucrose density gradient fractions and in concentrated preparations of virus.

LIYV (U.S.A.)

LIYV-infected Nicotiana clevelandii Gray plants were harvested in 200-g batches 2-3 wk after inoculation. Plants were homogenized in a meat grinder with cold 0.1 M tris-HCl plus 5 mM EDTA, 20 mM Na₂SO₄ and 10 mM diethyldithiocarbamate (DIECA). pH 7.6 (extraction buffer). The plant material was further homogenized in a ball mill for 2 h at 4°C. The homogenate was expressed through cotton muslin and Triton X-100 was added to a final concentration of 4%. The slurry was stirred overnight at 4°C, subjected to low-speed centrifugation for 3 min at 3,000 rpm (1,500 g) followed by centrifugation at 8,000 rpm (10,000 g) for 10 min in a Sorvall GSA rotor. The supernatant was ultracentrifuged for 3 h at 22,000 rpm (110,000 g) in a Beckman 35 rotor over a 5-ml cushion of 30% sucrose in extraction buffer plus 4% Triton X-100. The supernatant was discarded, and the pellets were rinsed carefully several times with distilled water to remove any remaining pigments and sucrose in the tubes.

Pellets were gently resuspended at 4°C with 0.1 M phosphate buffer ($\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$), and 5 mM EDTA, 20 mM Na_2SO_4 and 10 mM DIECA, pH 7.0 (resuspension buffer). Tube contents were pooled, Triton X-100 was again added to 4%, and the suspension was stirred overnight at 4°C. The suspension was centrifuged for 10 min at 8,000 rpm (7,800 g) in a Sorvall SS-34 rotor, and the supernatant was ultracentrifuged over a 5-ml cushion of 30% sucrose in resuspension buffer and 4% Triton X-100 for 2.5 h at 28,000 rpm (66,000 g) in a Beckman 30 rotor. Pellets were rinsed with distilled water; resuspended in 4 ml of 0.1 M phosphate buffer containing 2 mM EDTA, pH 7.0 (PE buffer); and stirred for 5 h at 4°C. The suspension was centrifuged for 5 min at 8,000 rpm (7,800 g) followed by 5 min at 10,000 rpm (12,000 g) in a Sorvall SS-34 rotor. The supernatant was brought to a final concentration of 25% Cs_2SO_4 (w/v) and layered over 1 ml of 40% Cs_2SO_4 (w/v) in PE buffer. The virus Cs_2SO_4 preparation was ultracentrifuged at 8°C for 15-18 h at 35,000 rpm (100,000 g) in a Beckman SW 39 rotor. Light-scattering bands were extracted with a bent needle and syringe, dialyzed exhaustively in PE buffer at 4°C, then stored at -20°C after the addition of 5% sucrose.

In several other experiments in which the same extraction method was used, viral preparations were layered on sucrose density gradients (10-40% in PE buffer) instead of Cs_2SO_4 gradients and centrifuged at 4°C for 2.5 h at 25,000 rpm (120,000 g) in a Beckman SW 27 rotor. Gradients were analyzed with an ISCO model 640 fractionator equipped with a model UA-5 analyzer.

Light-scattering bands from the Cs_2SO_4 preparations and samples from sucrose density gradient peaks were examined for virus particles by placing a drop of the suspension on carbon-coated Formvar grids, adding 2% uranyl acetate stain, and examining the grids in a Siemens Elmiskop 101. Healthy N. clevelandii

was also subjected to the same purification method described above, to serve as the control.

MLCV (U.S.A.)

MLCV was purified by a method developed for squash leaf curl (Cohen et al., 1983). Watermelon plants were harvested 4-7 days after the appearance of symptoms. Plants were coarsely ground in a Hobart food grinder with 0.1 M phosphate buffer ($\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$) at pH 7.2, containing 0.002 M disodium EDTA and 0.01 M Na_2SO_3 at the rate of 100 ml per 100 g of plant material. After thorough homogenization with a VirTis 60 homogenizer, the extract was filtered through muslin and stirred with 1% (v/v) Triton X-100 for either 2 h at room temperature or overnight at 4°C. Sap was clarified by adding 10% (v/v) cold chloroform, stirring for 10 min, and low-speed centrifugation (8,000 rpm) for 10 min in a Sorvall GSA rotor. Polyethylene glycol (mol wt 7,000-9,000), 12 g/100 ml, and 0.2 M NaCl were added to the aqueous phase. After being stirred for 1.5-2.0 h at 4°C, the precipitate was collected by centrifugation for 15 min at 8,000 rpm in a GSA rotor and resuspended in one-tenth the original volume of 0.1 M phosphate buffer containing 0.002 M EDTA (resuspension buffer) and 1% Triton X-100 (v/v). The virus suspension was stirred for a minimum of 1 h at 4°C, centrifuged for 10 min at 10,000 rpm in a Sorvall SS-34 rotor, and the supernatant was ultracentrifuged for either 3 h at 28,000 rpm in a Beckman 30 rotor or for 1 h at 55,000 rpm in a Beckman 70 rotor. Pellets were resuspended in 10 ml of resuspension buffer given a low-speed centrifugation at 10,000 rpm for 10 min in a Sorvall SS-34 rotor, and a final high-speed centrifugation in a Beckman 40 rotor at 35,000 rpm for 2 h. The pellet was resuspended in 4 ml of resuspension buffer, allowed to stir overnight at 4°C, and subjected to a low-speed centrifugation for 5 min at 6,000 rpm in an SS-34 rotor.

Linear sucrose gradients (10-35%) were prepared in 0.1 M phosphate buffer with 0.001 M EDTA, pH 7.2. The virus suspension (2 ml) was layered on the gradients and centrifuged at 25,000 rpm for 3.5 h in a Beckman SW 27 rotor. Gradients were examined for light scattering bands, followed by analysis with an ISCO model 640 fractionator equipped with a model UA-5 analyzer.

TNDV (U.S.A.)

TNDV was purified by an adaption of the method used for MLCV.

Electron microscopy - Virus preparations to be examined were stained with 2% phosphotungstic acid and 1.5% ammonium molybdate or with 2% aqueous uranyl acetate, mounted on carbon-stabilized Formvar-coated grids.

Antisera production - Antisera to LIYV, SLCV, MLCV, and TNDV were prepared by injecting purified viruses into a rabbit. The virus (32-75 ug/ml), in aliquot of 1 ml, was prepared for four to six injections following pelleting after density gradient centrifugation. Each aliquot was mixed with an equal volume of Freund's complete adjuvant just prior to the intramuscular injection. Injections were given every 7 days following a weekly bleeding beginning in the 5th-7th week.

THE VECTOR

A Collection and Marking System Suitable for Epidemiological Studies of Whitefly-borne Viruses

Sticky yellow traps: Yellow plastic boards 15x20 cm covered with a thin layer of "Rimifoot" glue (Jewnin-Joffe Chemicals Ltd., Tel Aviv), diluted 1:6 with petrol - were used for trapping the whiteflies. These traps were useful in population dynamic studies and in distribution tests using marked insects. The traps were positioned horizontally 50-70 cm above the soil surface. In some cases another type of sticky yellow trap was used, made of a disposable

transparent petri dish covered with glue on the inside. The dishes were placed on a piece of yellow plastic sheet inserted in a groove made in a wooden base.

Collection of adults: The technique of collecting whiteflies was developed gradually. When a Di Vac sampler collector was used, most of the whiteflies died in the first 24 h and therefore could not be used for virus transmission tests. As a second step a cordless rechargeable car vacuum cleaner was used. The vacuuming power of this device is sufficient to aspirate the whiteflies and most of them survived after 24 h. but a complicated procedure is involved in releasing the whiteflies from the sucking bag. This device was used for TYLCV. In the third stage we developed a device based on the above mentioned car vacuum cleaner which was modified to collect insects directly into clear cylindrical plastic sleeve cages (Fig. 1). During the collection process, the edge of the sleeve cage was brushed against the foliage suspected of having whiteflies. After insect collection, a test-plant plug was introduced directly into the cage with the vacuum on, and then the cage was capped.

Fig. 1. Whitefly collection system

- A. Cordless rechargeable auto vacuum cleaner
- B. Cylinder adaptor
- C. Clear plastic cylinder sleeve cage
- D. Lettuce test plant
- E. Field inoculation chamber (test plant sleeve cage).

The effect of the new device was tested on LIYV and on marked whiteflies.

Lettuce seedlings (cv. Summer Bibb) at the five- to six-leaf stage, grown by the plug method in Styrofoam trays, were used as test plants. The root plugs were wrapped with a thin layer of parafilm prior to inoculation in the cylinders (sleeve cages).

Survival of whiteflies during the collection process. The survival rate of the whiteflies was measured 48 h after the end of a 1-min vacuuming and compared with that of control insects which were not subjected to the treatment. After the treatment the whiteflies were kept in groups of ten in leaf cages positioned in pairs on the opposite half leaves of sweet potato.

Simulation of field studies. Whiteflies previously fed for 24 h on LIYV-infected cheeseweed were caged in groups of 15 in collection cylinders and exposed to 1 min of vacuuming. Lettuce seedlings previously kept for 3 days in a plastic bag in a dark room (simulating shipping conditions to the field) were introduced into the cylinders for a 24-h inoculation feeding access period at room temperature. The plants were then thoroughly washed with a stream of tap water to remove the whiteflies before being repacked in plastic bags and stored for 2 additional days in the dark room (simulating shipment back to the laboratory). After that period, the seedlings were transplanted into pots in a greenhouse. As a control, groups of 15 LIYV viruliferous whiteflies enclosed in leaf cages were placed for 24 h on the young leaves of the lettuce test plants. The whiteflies were then washed off with water and plants transferred to the greenhouse and placed next to the treated group.

Studies with fluorescent pigments. Daylight fluorescent pigment - "Fire Orange" (Day-Glo Color Corp., Cleveland, OH, U.S.A.) was used in experiments to track the flight of whiteflies. A small atomizer, "carborundum duster," was used

to apply the dust to whiteflies enclosed in leaf cages with screened covers and bottoms. Clean air was blown on whiteflies in control cages. The whiteflies were then released onto sweet potato or cotton plants in large muslin cages. The whiteflies were collected 24 h or more later and used for various tests. In the experiments where the effect of the vacuum collector was tested, the whiteflies were placed in the cylinder and vacuumed for 1 min before being used. The effect of "Fire Orange" on the life span of B. tabaci was tested as follows: Whitefly females collected from a single colony were divided into two groups. One group was treated as mentioned above with "Fire Orange" dust and the second group was used as a control and treated with clean air. Whiteflies were collected after 24 h in groups of ten per leaf cage and placed in pairs on the opposite half-leaves of cotton plants. The number of live and dead insects was recorded daily.

The persistence of the dust on the whiteflies under field conditions after they were trapped on sticky yellow traps was tested as follows: Whiteflies (in group of 20) were placed, at different intervals after marking, on the traps which were then put outdoors.

The chance of the newly hatching adults from a leaf previously covered with dust to become contaminated was tested as follows: A detached sweet potato leaf with larvae was dusted with "Fire Orange". The leaf was then placed in a tall glass cylinder with a vaseline-coated cover. This cover was placed under U.V. illumination to determine the number of adults contaminated with the dust.

The effect of the vacuum collector on the persistence of the dust on the whiteflies was determined as follows: Dusted whiteflies were collected into cylinders, which were allowed to cool. The number of glowing insects was counted through the cylinder walls. The whiteflies were then allowed to become active before being aspirated for 1 min. Then they were again cooled and counted. The

whiteflies were then blown out of the cyliner onto yellow sticky traps and the number of glowing insects was counted.

In all the above mentioned tests the detection of the dust was done in a dark room using a compact 4-watt U.VL-21 lamp (UVP Inc., San Gabriel, CA, U.S.A.). The results were analyzed by the "sign test" (Siegel, 1956).

Studies of *Bemisia tabaci* Distribution Using Insects Marked with Fluorescent Dust

Two experiments were carried out at the Gilat Experiment Station. In those tests cotton plants previously heavily infested with *B. tabaci* in the laboratory were transferred to the field when most of the insect population had reached the late larval or pupal stage. The plants were then dusted with "Fire Orange" fluorescent dust using a mechanical hand duster. Sticky yellow traps were positioned 50 cm above the soil surface in circles located at different distances from the plants, and 24 h later the number of glowing insects adhered to the traps was counted under U.V. illumination. The total dusted adult population was estimated on the dusting day by counting the number of pupae on 20 plants and multiplying the number/plant by the total number of plants in the plot. An additional experiment was carried out in the Jordan Valley, where an area of about 100 m² covered with *Cynanchum acutum* (the natural host of TYLCV see hereunder) was dusted. The whiteflies' movement was tested as in the previous test. An additional test was carried out in the Imperial Valley, where cotton plants in a commercial field were dusted.

Effect of Physical Barriers on The Whitefly Population and on the Spread of TYLCV

Fourteen plots 121 m² each and located 30 m apart, were used. Five were surrounded by 1.5 m-tall black polyethylene partitions; four were surrounded similarly and tomato plants were planted in them (200/plot); and five plots were used as a control with tomato plants planted in them (200/plot) but they were not surrounded by the polyethylene. The plots for the different treatments were chosen at random. Nine sticky yellow traps were positioned at a height of 70 cm

above the soil surface in each of the plots. These traps were located in three circles, with a distance of 1.5 m between the barrier and the closest traps (the peripheral circle). The traps were replaced after 10 days by another batch which were left in the field for 10 days.

The results were analyzed by the Mann Whitney U test (Siegel, 1956).

Ability of *Bemisia tabaci* to Survive Migratory Conditions

The effect of the relative air humidity (RH) on the appearance of the whiteflies was observed in September 1986 by means of yellow sticky traps (the petri dish system) at Nevatim, in the northern Negev. The traps were changed in the evening of each day, after the daily flight activity had ceased. The number of trapped whiteflies was recorded. Data on the weather conditions were obtained from the meteorological station at Beer Sheva which is about 15 km distant.

In the tests carried out in the laboratory, adult whiteflies (males and females) of unknown age were collected by means of an aspirator and anesthetized slightly with CO₂.

The experiments which required constant temperature were conducted in an illuminated growth-chamber. The desired RH was achieved by using water solutions of various NaOH concentrations, adjusted for each temperature (Madge, 1961). Distilled water, creating an almost saturated (RH 100%) atmosphere, was used as a control treatment. Cobalt thiocyanate paper was used to check and verify the relative humidity (Solomon, 1957).

The experimental cage consisted of two compartments: a glass tube (25x100 mm) and a glass pipe (25x100 mm) joined together by a strip of parafilm. The bottom of the upper pipe was covered by a piece of Agryl (a porous unwoven polypropylene sheet, produced by Sodoca, France) which prevented the whiteflies from falling down but allowed air exchange between the two parts of the system.

The lower glass tube was filled to one-third of its capacity with the water/NaOH solution (Berlinger, 1973). The opening of the upper pipe was plugged by means of a syringe-piston which allowed us to change the test-tube volume. This procedure was necessary because the RH in the upper cage varied markedly with the distance from the lower test tube. During the experiment the piston was fixed close to its bottom lower position, leaving available a "test-cage" 10 mm in height. In the experiment in which the whiteflies were allowed to recover, the two compartments were disconnected and a cotton leaf was introduced into the upper glass tube, replacing the piston.

Whitefly survival was tested at 20, 50, 80 and close to 100% RH, at temperatures of 25, 30, 35 and 41 C and for 2, 4 and 6 h.

The possible recovery of the whiteflies from the RH/temperature/time treatments was examined with providing them by a fresh cotton leaf and transferring them to 28 C and 50-60% RH, for an additional period of 20 h, after which their survival was checked again.

In the outdoor experiment the whiteflies were introduced into a glass tube (25x100 mm), with both openings covered with Agryl. The test tubes were hung in a shaded place 1.5 m above ground. The effect of the outdoor conditions was checked after 1, 2 and 3 h. As a control treatment the test tube containing the whiteflies was kept at 28°C and RH 50-60% for 3 h. To study the effect of starvation time, (2, 4 and 6 h without food) on the whitefly survival, an experimental system similar to the outdoor one was used. In one treatment the whiteflies had no food, while in the control treatment they were provided with a cotton leaf as food resource. Survival was checked, using different series of cages, after 2, 4 and 6 h. The experiment was conducted at 28°C, RH 50-60%, in a lighted chamber. The results were analyzed by Duncan's multiple range test, for $P \leq 0.05$.

THE HOST

Host Range Studies

The host range was determined by allowing 30-100 viruliferous whiteflies previously fed on a virus source for at least 24 h, to feed for 48 h on at least five seedlings of each species tested. The presence of the virus in the inoculated plants was determined by recovery trials, in which at least 15 virus-free whiteflies were fed for at least 24 h on the inoculated plant before being transferred to a proper test plant (Summer Bibb lettuce in the case of LIYV, and D. stramonium in case the of TYLCV). These test plants were then kept for at least 25 days after the inoculation.

Field material was tested for the presence of viruses by recovery tests using insect vectors as mentioned previously and by ELISA. Basically, the double antibody sandwich described by Clark and Adams (1977) was used, but with, 1 ug/ml coating globulin and 1:400 dilution of the enzyme-conjugated globulin. A405 values were recorded 30 min after the substrate was added. During the 30-min period the plate was left in an incubator at 37°C. Plant tissue samples were prepared from five leaf discs of 5-mm diam each, ground in a Vortex in 1 ml of buffer. Four to six samples collected from healthy plants grown in the greenhouse using the same species and cultivar to be tested, were used as controls in each plate.

VIRUS-VECTOR-HOST RELATIONSHIPS

Host Selection

The experiments were carried out in an aphid flight chamber as described by Cohen and Melamed (1974). Host selection through landing was examined as follows: The leaves to be tested were attached to cardboard plates (6x6) cm in such a way that the whole surface was covered. The leaves were then covered with

vaseline prior to introduction of groups of 200-400 whiteflies into the chamber for 2 h. The number of whiteflies which adhered to the leaves was counted.

Host preference through feeding was determined with young plants (three to four leaves) grown in 10 cm diam pots. The total leaf surface area of the various tested plants was calculated to be approximately the same size. The whiteflies were introduced into the chamber in groups of 200-400 for 24 h, after which time the number observed on the leaves was counted. The results were analyzed by the sign test (Siegel, 1956).

Association Between Bemisia tabaci Population Dynamics and the Spread of TYLCV and LIYV

The TYLCV population rate was monitored using sticky yellow traps located near tomato bait plants which were replaced weekly. TYLCV infectivity rate was examined as follows: Groups of 100 tomato seedlings (bait plants) previously grown in an insect-protected nursery were transplanted weekly in the field. A week after the transplanting the plants were covered for the next 3 weeks with Agryl, or treated daily with synthetic pyrethroids to control the whiteflies. Then the percent of TYLCV-infected plants was calculated. This procedure provides information on the infectivity rate during the first week of exposure to the vector. The rate of virus-carrying whiteflies in the population was examined by collecting whiteflies from different hosts using the collector (previously mentioned). The insects were then placed in groups of 20 on tomato test plant for 48 hr of inoculation feeding. The results were analyzed by the Spearman rank correlation coefficient (Siegel, 1986).

For studies of the LIYV, the whiteflies were obtained using the collector. Calculation of the infectivity rate was based on the transmission established by the number of whiteflies collected per unit of time. This number was determined

by counting at each collecting spot the total number of insects collected, in at least two replicates. The inoculation took place using lettuce seedlings grown by the plug method, as described above.

Acquisition, Interferences and Retention of Cucurbit Leaf Curl Viruses in Whiteflies

Unless otherwise stated, young leaves about 3-4 cm in length from squash plants that had been inoculated 2-3 months earlier were used as the SLCV source. Squash plants in the first true leaf stage of growth were used as test plants. Young leaves of watermelon plants 15-20 days after inoculation were used as the MLCV source. Watermelon plants in the first true leaf stage of growth were used as test plants.

Virus transmission tests. Transmission tests were done by using a leaf-cage method described previously (Cohen and Harpaz, 1964). Virus retention tests were performed using females collected from the same colony. The whiteflies were allowed different time periods for acquisition feedings and then transferred individually at 2-day intervals onto healthy squash plants. Percent transmission or transmission rate was calculated by using the formula:

$$\frac{\text{No. of infected plants} \times 100}{\text{Total no. of plants inoculated}}$$

during a certain period of time. The results were analyzed by the Mann-Whitney U-test (Siegel, 1956), at $P \leq 0.05$.

ELISA tests. Leaf extracts were prepared by grinding a 5-mm-diam disc in a micromortar in 0.2 ml of phosphate-buffered saline with 0.5 ml/l Tween-20, 20 g/l polyvinylpyrrolidone (MW 44000) and 2 g/l egg albumin. Whitefly homogenates were prepared by grinding them in a micromortar in a volume of 0.2 ml of the buffer mentioned above. The antiserum to SLCV was prepared in the Salinas laboratory (Cohen et al., 1983).

The double antibody sandwich method described by Clark and Adams (1977) was used as described previously. In each of the microtiter plates, six to nine samples of 20 whiteflies previously fed on sweet potato, two samples from SLCV-infected squash, and two from healthy squash were routinely used. In cases where homogenates from more than 20 whiteflies were used, an additional six to nine control samples were taken with the corresponding numbers of virus-free whiteflies. In the case of T. abutilonea, additional controls were prepared from whiteflies fed on Physalis alkekengi. With this system the mean values of the appropriate control in each plate were taken as 100%. The results were analyzed by the Mann Whitney U test (Siegel, 1956) at $P \leq 0.05$.

Interference between SLCV and MLCV in Bemisia tabaci B. tabaci females collected from young colonies were divided into two or three groups. One group was given an acquisition access of 48 h on SLCV-infected squash followed by a 24-h acquisition feeding on MLCV-infected watermelon leaves. The second group was fed on healthy squash for 48 h. followed by a 24 h acquisition access simultaneously with the first group, and on the same MLCV-infected watermelon leaves. The third group was used as a control to examine the rate of acquisition of SLCV. In this case the whiteflies were first fed for 48 h on SLCV-infected squash, followed by a 24-h feeding on healthy watermelon. The whiteflies of the first and second groups were then placed individually for a 48 h inoculation feeding on watermelon seedlings. Those of the third group were placed for 48 h on healthy squash plants. It is important to mention that watermelon is immune to SLCV (Duffus et al., 1985): therefore, only the transmission of MLCV could be observed. Results of the experiments were analyzed by the sign test (Siegel, 1956) at $P \leq 0.05$.

Use of antisera to examine the location of LIYV and SLCV in the vector Bemisia tabaci. The whiteflies were fed in groups of 50-100 (in leaf cages) for

24 h on young leaves of squash plants which had been inoculated 3-4 months earlier with SLCV or on middle leaves of Malva inoculated one month earlier with LIYV. The cages were located in pairs or in groups of three on the same leaf. The cage cover was then replaced by a parafilm membrane and the whiteflies were allowed to feed for 5-6 h on SLCV or LIYV antiserum, or normal serum. In some cases a third group was fed on buffer. The whiteflies were then placed individually, in the case of SLCV, on young leaves of young squash plants for an inoculation feeding of 3 days. When LIYV was tested, the whiteflies were placed in groups of 15 on young lettuce (Summer Bibb) for an inoculation feeding of 48 h. The results were analyzed by the sign test, at $P \leq 0.05$ (Siegel, 1956).

RESULTS AND DISCUSSION

The Viruses

Purification and Identification

Tomato yellow leaf curl virus (TYLCV). *Datura* plants were found to be superior to tomato plants as a source for virus purification. Characteristic gemini particles, about 20-30 nm in size, were detected at approximately the middle of the gradients. Gemini structures were never found in mock preparations obtained from non infected control plants. Spherical particles (15 nm in diameter) similar to phytoferritin (Goodman et al., 1977) could be observed in the upper fractions of sucrose gradients loaded with either control or infected plant extracts.

Fractions from sucrose gradients were fed to whiteflies which were used to inoculate Datura test plants. High infectivity rates, leading to typical disease symptoms, were associated always with the presence of a high concentration of gemini particles. Preparations obtained after pooling and concentration of

virus-containing fractions were also infective. Fractions from mock virus preparations were always non infective (Czosmek et al., in press).

Our results indicate that the TYLCV is a twinned particle of 20x30 nm, which is characteristic of the gemini group of plant viruses (Harrison, 1985, Stanley 1985).

Lettuce infectious yellows virus (LIYV). A new yellowing disease of lettuce, sugarbeet, carrot, and other crop and weed hosts was found in the desert areas of the southwestern United States. The inciting virus LIYV, was transmitted by the sweet potato whitefly (Bemisia tabaci) in a semipersistent manner, but it was not mechanically transmissible. The virus was retained by viruliferous whiteflies for 3 days in serial transfers on susceptible hosts. LIYV had a wide host range (45 species in 15 plant families) and caused economically significant losses in a number of important crop plants. Purified preparations had an A260/280 nm ratio of 1.28 and contained long flexuous particles 13-14 nm wide and 1,800-2,000 nm long. An antiserum with a homologous titer of 1/1.024 showed no relationship to beet pseudo-yellows virus and could be used to detect greenhouse and field-infected plants by the ELISA method. The host range, particle size, insect transmission and serology clearly distinguished LIYV from previously described viruses (Duffus et al., 1986).

Tomato necrotic dwarf virus (TNDV). A new whitefly-transmitted virus causing leaf necrosis and severe stunting of tomato has been isolated from plants in the Imperial Valley, California. The infectious agent, TNDV, which affects tomato, pepper, tomatillo, eggplant and Physalis wrightii, is transmitted in a non-persistent manner by B. tabaci as well as mechanically. Purified virus has been shown to contain three distinct isometric components ~ 30 nm in diameter. Preliminary investigations of the virions by electron microscopy and spectrophotometric analysis suggest that only the middle and bottom components

contain nucleic acid. The A260/280 ratio, uncorrected for light scattering, was typically 1.0, 1.78 and 1.91 for the top, middle and bottom components, respectively. Sedimentation coefficients (S₂₀, w) of the virions are 57S (T), 117S (M) and 138S (B). Tests by mechanical inoculation indicate that both middle and bottom components are required for infection (Larsen et al., 1984; Duffus, 1987).

Melon Leaf Curl Virus (MLCV). A new whitefly-transmitted gemini virus causing leaf curl symptoms on melons has been isolated from plants in the Imperial Valley, California. The infectious agent, MLCV, which affects melon, watermelon, cucumber, cataloupe, pumpkin, squash and bean is transmitted by B. tabaci as well as mechanically. MLCV virions appeared identical to SLCV on the basis of particle morphology and ELISA tests. However, SLCV does not affect melon, watermelon or cucumber; also, SLCV antiserum did not react with MLCV in agar double diffusion tests (Duffus et al., 1985).

The Vector

A Collection and Marking System Suitable for Epidemiological Studies of Whitefly-borne Viruses

Effect of the vacuuming on the survival of the whiteflies. A significant reduction of about 23% was noted in the survival of whiteflies 48 h after the 1 min treatment with the collector (Table 1). A 48 h inoculation feeding access period is however, long enough for successful transmission of all of the known whitefly-borne viruses (Duffus, 1987).

Effect of the collector and the shipping condition simulation on the transmission of LIYV by the whiteflies. A total of 95% transmission was achieved by the groups of whiteflies in the simulation of collection, and the shipping tests (Table 2). This figure does not differ significantly from that established

by the control groups (88%). Therefore, the combined effect of the collecting process and shipping conditions on the transmission rate is minor.

Effect of Fire Orange dust on the life span of B. tabaci. The results (Table 3) indicate no effect of the Fire Orange pigments on the life span of B. tabaci over 7 days. Thus the pigments would seemingly not interfere during the period of LIYV retention (3 days) (Duffus et al., 1986).

Persistence of Fire Orange dust on the insects and sticky yellow traps at different days after dusting. As previously mentioned yellow sticky traps are one of the tools used in epidemiological studies of whitefly-borne viruses. It was important to test the persistence of the pigments on the trapped whiteflies in the field.

The results show that whiteflies leaving the site (source) as long as 5 days after dusting, still retained detectable pigments for at least 6 additional days (Table 4).

Treatment of whitefly larvae with Fire Orange. All of the adults (17 out of 17) which emerged 2 days after dusting, and 86% (30 out of 35) of those which emerged 3 days after dusting were contaminated. The results indicate that heavy dusting with Fire Orange can effectively coat newly emerging adults.

Effect of the "whitefly field collector" on the persistence of the dust on the whiteflies. The possibility that the vacuuming process of the collector may clean the dust from the whiteflies was tested. Also, it was important to compare the detection rate of the living whiteflies enclosed in the collection cylinder with that on the sticky traps. The results in Table 5 show that the fluorescent pigment was still detectable on the whiteflies after a period of 7 days and that no reduction in the detection of marked insects is caused by the vacuuming and the cylinder walls.

A simple system designed to monitor the movement and infectivity of whiteflies in epidemiological studies of whitefly-borne viruses is suggested. The collection device and shipping procedures are currently being used in studies of the epidemiology of LIYV in the southwest desert of the U.S.A. The collection device captures living insects in small cages that can be used directly as inoculation chambers, as a measure of population infectivity rate and densities, and as a means of monitoring movement (with the fluorescent dyes). Under field conditions whiteflies could be collected at very low population levels that were virtually impossible to detect by visual methods.

The method offers perhaps a better alternative to placing bait plants in the field for monitoring insect infectivity levels and would be applicable to epidemiological studies with other types of insect-borne viruses.

Table 1. Effect of vacuuming on the survival of whiteflies

Cage No.	Number of living insects following the treatments ^a	
	1-minute vacuuming	/ without vacuuming
1	7	10
2	10	10
3	7	10
4	3	9
5	6	10
6	7	8
7	8	10
8	7	9
9	10	10
10	10	10
11	5	10
12	10	10
Total	90/120	116/120
%	75	97

^aTen whiteflies per leaf cage (collected from the same colony) placed after the treatments in pairs for 48 h on the opposite half leaves of sweet potatoes.

Table 2. Effect of the whitefly collector and the shipping condition simulation on the transmission of lettuce infectious yellows virus (LIYV) by the whiteflies

Test No.	Percent transmission	
	Collection and simulation ^a	Control ^b
1	100 (10/10) ^c	75 (6/8)
2	40 (10/10)	100 (8/8)
3	80 (4/5)	75 (6/8)
4	80 (4/5)	100 (9/9)
5	100 (10/10)	88 (7/8)
6		88 (7/8)
7		75 (6/8)
8		100 (9/9)
Total	(38/40) 95	(58/66) 88

^a The inoculative whiteflies were vacuumed for 1 min. The lettuce seedlings were maintained for a total period of 4 days in the dark. Inoculation took place in the collector cylinder, with 15 insects/plant.

^b Inoculation was carried out using 15 whiteflies/leaf cage. The leaf cages were placed on young leaves of lettuce seedlings.

^c In parentheses, number of plants infected divided by total number of plants inoculated.

Table 3. Effect of Fire Orange dust on the life span of whiteflies^a (The data are number of live whiteflies divided by total number)

Days after treatment	Cage Number										Total
	1	2	3	4	5	6	7	8	9	10	
1 treatment	10/10	10/10	8/10	10/11	11/11	11/11	11/11	11/11	10/10	9/10	101/105
control	11/11	11/11	10/10	8/10	10/10	10/10	10/10	8/10	5/10	8/10	91/102
2 treatment	9/10	10/10	8/9	10/10	11/11	11/11	11/11	11/11	10/10	9/10	100/103
control	11/11	11/11	10/10	8/10	10/10	9/9	9/10	8/10	5/10	8/10	89/101
3 treatment	8/10	10/10	5/8	10/10	11/11	11/11	11/11	11/11	9/10	9/10	95/102
control	11/11	11/11	10/10	8/10	10/10	9/9	9/10	8/10	5/10	8/10	89/101
4 treatment	8/10	10/10	5/8	10/10	11/11	11/11	11/11	11/11	9/10	9/10	95/102
control	11/11	11/11	8/10	7/10	9/10	9/9	9/10	8/10	5/10	8/10	84/101
5 treatment	8/10	10/10	5/8	10/10	11/11	11/11	11/11	11/11	9/10	9/10	95/102
control	11/11	11/11	8/10	7/10	9/10	8/9	8/10	8/10	5/10	8/10	82/101
6 treatment	8/10	10/10	5/8	10/10	11/11	9/11	11/11	11/11	9/10	9/10	94/102
control	10/11	11/11	8/10	7/10	9/10	8/9	7/10	8/10	5/10	8/10	81/101
7 treatment	8/10	10/10	4/8	escape	11/11	8/11	11/11	10/11	8/10	8/10	78/ 92
control	10/11	11/11	8/10	7/10	7/10	8/9	6/10	8/10	5/10	8/10	78/101

^aWhitefly females collected from the same colony were divided into two groups and placed in leaf cages. Fire Orange powder (Day-Glo Color Corp.) was forced into one cage using a "Carborundum duster" atomizer. Clean air was blown into the second cage which was used as the control. The insects were then released onto cotton plants located in large muslin cages. 24 hours later the whiteflies were collected in groups of ten per leaf cage and these were placed on new cotton plants. The number of live and dead insects in each case was recorded daily.

Table 4. Persistence of the Fire Orange fluourscent dust on whiteflies on vaseline-coated traps at different days after dusting.

Days after Dusting	Days on the traps													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1	^a +	+	+	+	+	+	+	+	+	+	+	+		+
2	+	+	+	+	+	+	+	+		+				
3	+	+	+	+	+	+	+		+					
4	+	+	+	+	+	+		+						
5	+	+	+	+	+		+							

^a Dusted whiteflies collected at different intervals after the marking date were stuck to traps outdoors. The traps were examined daily under U.V. light. The plus sign indicates that the whiteflies were still glowing.

Table 5. Effect of the whitefly collector on the persistence of Fire Orange fluorescent dust on the whiteflies (The figures in parentheses are the number of glowing insects divided by the total number of dusted insects).

Treatments	% of Glowing insects						
	Days after dusting						Total
	1	2	3	4	5	6	
Before vacuuming ^a	82 (18/22)	75 (18/24)	100 (20/20)	100 (26/26)	100 (11/11)	100 (25/25)	92 (118/128)
After 1 min of vacuuming	82 (18/22)	75 (18/24)	100 (20/20)	100 (26/26)	100 (11/11)	100 (25/25)	92 (118/128)
On sticky yellow ^b trap	100 (22/22)	100 (24/24)	100 (20/20)	100 (26/26)	100 (11/11)	100 (25/25)	100 (128/128)

^a The whiteflies were enclosed in the collector cylinder.

^b The whiteflies were released from the cylinder onto sticky yellow traps.

Distribution of the Whiteflies as Determined Using Fluorescent Dust

The chance that airborne whitefly corpses will reach the small sticky yellow trap is very low. Therefore the results summarized in Tables 6 and 7 indicate that the whiteflies can survive and travel a distance of 7 km (Table 7). The decrease in the mean number of glowing whiteflies per trap with the increased distances from the dusted center (Table 6) may be misleading because of the dilution effect. However, in the experiment summarized in Table 6, the spaces between the traps in each of the circles was approximately the same. Therefore, to avoid the "dilution effect," the total number of glowing insects trapped should be taken into account. In this way the conclusion can be reached that along the first 100 m the "losses" among the moving population are slight and that most of the whiteflies can reach the distance tested. Similar calculations cannot be made for the experiment carried out in the Jordan Valley (Table 7). There, the traps in the circles were positioned in groups facing downwind.

Table 6. Distribution of marked whiteflies at Gilat

Distance from the dusted cotton plants ^a (m)	<u>Test No. 1</u>			<u>Test No. 2</u>		
	Number	<u>Mean number/trap</u>		Number	<u>Mean number/trap</u>	
	of	total	glowing	of	total	glowing
	traps	white- flies	white- flies	traps	white- flies	white- flies
10	6	17	(56) 9.3	6	1006	(96) 16
50	31	10.1	(61) 1.9	25	1002	(34) 1.4
100				60	1004	(39) 0.65

^a In both tests the estimated dusted adult population was approx. 100,000

() In parentheses, the total number of glowing whiteflies

Table 7. Distribution of marked whiteflies in the Jordan Valley

Distance from the dusted (<i>Cynachum acutum</i>) plants (m)	Number of traps	Mean number of whiteflies/trap	Total number of glowing whiteflies	Mean number of glowing whiteflies/trap
10	20	1153	169	8.5
100	18	1686	67	3.7
200	18	1464	30	1.7
300	16	3258	21	1.2
1000	3	2512	22	7.3
7000	2	1696	2	1.0

In the Imperial Valley marked whiteflies were located 300 m from the dusted field.

Influence of Physical Barriers on the Whitefly and TYLCV Spread

The results in Table 8 show that significantly more whiteflies landed in the surrounded plots than in the non-surrounded ones. Concomitantly, the percentage of TYLCV infected plants was significantly increased in the surrounded plots. Thus, one month after transplanting 70% of the plants in the surrounded plots were already infected as compared with 40% in the control plots. The number of whiteflies caught in the second batch of traps (which replaced the first group 10 days after transplanting) was similar to that trapped in the first set (Table 8). Therefore there is no indication of an accumulation of whiteflies in the surrounded plots and the influence of the

barrier is apparently solely on landing behavior. The barrier is probably responsible for an air turbulence which forces the whiteflies into the plot. A similar effect has been described in the case of wind-breaks and their influence on aphid movement (Marrou et al., 1979).

Table 8. Effect of physical barriers on the population of Bemisia tabaci

Location of the traps	Mean number of whiteflies/trap		
	Barrier + tomato	Barrier only	Tomato only
Catch 1 (1-10.X)			
External circle	195 ^{a*}	225 ^a	54 ^c
Second circle	94 ^b	105 ^b	50 ^c
Center	72 ^b	92 ^b	40 ^c
Catch 2 (10-20.X)			
External circle	218 ^x	242 ^x	73 ^z
Second circle	110 ^y	115 ^y	54 ^z
Center	91 ^{yz}	113 ^y	53 ^z

*Within a catch figures followed by a common letter do not differ significantly at $P \leq 0.05$.

Ability of B. tabaci to Survive Migratory Conditions

The general pattern of whitefly catches during September at Nevatim (Fig. 2) was an increase in the number of trapped whiteflies from the beginning of the month until day 18, and followed by a general decrease.

Daily catches of whiteflies in the traps showed that at temperatures of 32-33°C the whitefly catches increased, e.g. on days 1-3, 6, and 12-13, as long

as the RH was at least ~ 50%. When the temperature was as high as 39.5°C (as on day 16), although the RH was above 50% there was a decrease in the number of trapped whiteflies to a level of 100/trap/day. When the RH dropped below 50%, as on days 3, 9 and 19, it resulted in a decrease in the number of whiteflies trapped. However, the decrease in whitefly catches was the strongest, to less than 50/trap/day, when the temperature exceeded 35°C and the RH at the same time was far below 50%, as on days 21-27 of the month (Fig. 2).

Fig. 2 No. of Bemisia tabaci/Trap/Day (At Nevatim) Vs. Temperature and RH% (Beer Sheva Meteor. Sta.)

Whitefly survival was examined at various combinations of temperature and RH during 2, 4 and 6 h without food, to mimic migration conditions. The final rate of survivors was strongly influenced by the tested climatic conditions (Table 9). Increasing the duration of the treatment from 2 to 6 h resulted in a marked decrease in survival rate, although the influence of a 2-h treatment was detected only when the temperature reached 35°C.

Increasing temperatures (from 25 to 35°C) caused a gradual decrease in survival rate. This decrease was moderate, and ranged from 88-90% to 60% at RH 100% and from 83-85% to 33% at RH=80%. The decrease in survival was accelerated when RH dropped to 50-20%: then survival rates dropped quickly to very low values, of 10% and 2%.

When the temperature reached a high level, such as 41°C, the influence of air humidity was minimized and even at RH=100% no more than 11% of the whiteflies survived. Nevertheless, even at as high a temperature as 41°C, and at low air humidity such as RH 20%, not all the whiteflies died after a 2-h treatment.

Table 9. The combined influence of temperature and relative humidity for 2, 4 and 6-h periods on the percent survival of Bemisia tabaci

Duration (h)	Temp. (°C)	% RH			
		100	80	50	20
2	25	87.7b	83.4ab	79.5ab	77.3a
	30	91.9b	82.2ab	76.9a	71.9a
	35	87.6b	81.3b	69.8a	66.4a
	41	11.2b	6.8ab	2.1a	1.1a
4	25	89.7b	84.8ab	81.3ab	77.7a
	30	84.3c	73.8b	72.5b	61.4a
	35	66.6c	58.3c	28.0b	11.4a
6	25	85.5b	84.4b	71.9a	66.3a
	30	80.1c	67.3c	45.2b	20.2a
	35	61.7c	32.2b	9.6a	1.9a

The experiment was performed in ten replicates of ten adult whiteflies each. The statistical analysis was performed separately for each line. figures followed by a common letter do not differ significantly at $P \leq 0.05$.

Within rows, the survival of whiteflies provided with food varied from 78% to 88.4% (Table 10). Lack of food, during this period caused additional mortality, and the survival rates varied between 61.7% and 71.4%. The calculated net-mortality due to the lack of food increased with time, and was 17.1%, 20.9% and 27.3% for 2, 4 and 6 h, respectively.

Table 10. Influence of food on the percent survival of Bemisia tabaci adults held at 25-28°C and RH=50-60% 2, 4 and 6 h.

Exp. no.	Duration (hr)	% survival		% Mortality* due to lack of food
		with food	without food	
1	2	86.1a	71.4b	17.1
2	4	78.0a	61.7b	20.9
3	6	88.4a	64.3b	27.3

* Mortality was calculated as follows $100 - (100 \times 71.4 / 86.1) = 17.1\%$
The experiment was performed in ten replicates of ten adult whiteflies each. Within rows, figures followed by the same letter do not differ significantly $P \leq 0.05$.

A series of combined temperature/RH/time-treatments was followed by a period of 20 h at 28°C, RH=50-60% and food, in order to give the whiteflies a chance to recover from the previous treatments (Table 11). The survival of the whiteflies decreased from 66.6% to 21.4% after 4 h when RH decreased from 100% to 20%, and after 6 h of treatments the survival rate decreased from 59.7% to 1.9%. The whiteflies scored as "dead" immediately after the treatment did not recover after their transfer to improved conditions of temperature and RH and provision of food. In all cases the rate of survival continued to decrease after the treatment had ceased. From these results it was concluded that survival rate as scored immediately after the treatments, represents the results adequately and thus the testing method can be simplified and the recovery period can be omitted in future experiments.

Table 11. Recovery of Bemisia tabaci after their exposure to various relative humidities at 35°C and without food for 4 and 6 h

Treatment	Survival (%)			
	% RH			
	20	50	80	100
<hr/>				
	after 4 h			
After treatment	21.4a	28.0b	58.2c	66.6c
After "recovery"	6.5a	19.3b	45.1c	64.3d
Difference	-14.9	-8.7	-13.1	-2.3
<hr/>				
	after 6 h			
After treatment	1.9a	9.5a	35.1b	59.7c
After "recovery"	0.0a	5.0a	23.3b	45.0c
Difference	-1.9	-4.5	-11.8	-14.7

Each experiment was performed in 14 replicates of ten adult whiteflies each. Within rows, figures followed by the same letter do not differ significantly at $P \leq 0.05$.

In the outdoor experiment the prevailing temperature increased within 3 h from 36° to 42°C (Table 12). At the same time air humidity was ~ 20 - 25% and did not change significantly. Whitefly survival decreased rapidly and significantly from 63.8% after 1 h to 3.3% after 2 h, and to zero after 3 h, whereas in the control group 71.7% survived.

Table 12. Percent survival of Bemisia tabaci adults outdoors, on a hot and dry day, after 1, 2 or 3 h of exposure, as compared with a control group held for 3 h at 28°C and RH=50%

Exp no.	Hours of day	Duration (h)	Temp. (°C)	RH (%)	Survival (%)
1	9.00-10.00	1	36	20	63.8a
2	9.00-11.00	2	40	25	3.3b
3	9.00-12.00	3	42	24	0.0b
4	Control (9.00-12.00)	3	28	50	71.2a

Figures followed by the same letter do not differ significantly at $P \leq 0.05$

Healthy whiteflies survived the experimental conditions somewhat better than the TYLCV-viruliferous-whiteflies; both groups of whiteflies were sensitive to RH and survived better at the higher RHs (Table 13).

Table 13. Percent survival of TYLCV-viruliferous (+V) and healthy (-V) whiteflies at 30°C and RH=50% or 100%, for 4 h

Exp. No.	RH 50%		RH 100%	
	+V	-V	+V	-V
1	38.0	56.4	65.7	74.6
2	25.7	45.7	56.2	70.4
3	66.2	78.6	83.4	75.2
4	55.8	84.4	73.6	93.0
Mean	46.4	66.3	69.7	78.3

Each experiment was performed in five replicates of 11-12 adult whiteflies each.

The number of trapped whiteflies represents only that fraction of the population which left its host plants and flew away in the search for other host plants. Since the number of trapped whiteflies was found high correlation with the number of TYLCV-infected plants, it is this fraction of the population which is most important in whitefly/TYLCV epidemiological studies.

The experimental conditions were aimed at duplicating temperature and RH, components of weather, which migratory whiteflies may encounter on their flight. Therefore, the effect of various temperature/RH combinations, during 2-6 h, on un-fed adults, was studied under controlled as well as under outdoor conditions.

Daily trapping of whiteflies showed that on warm ($>35^{\circ}\text{C}$) and dry ($\text{RH}<50\%$) days, the number of trapped whiteflies was lower than on cooler and more humid days (Fig. 2). The comparison of the whitefly catches with the daily maximum temperatures and RH at 08.00 am revealed that the whiteflies are probably affected by both high temperatures ($>35^{\circ}$) and low RH ($<50\%$). This assumption was confirmed by a series of experiments which were performed under controlled conditions (Table 9). The lack of food (Table 10) for 2-6 h, although the climatic conditions were favorable, led to a decrease in the survival-rate of the whitefly population; about 15-27% died due to lack of energy, i.e., starvation, or to lack of water i.e., desiccation. Both energy and water are usually provided when the aphid feed on plants. The harmful effect of starvation means that only a part of the population which has left its host plants will arrive at its new destination, even when temperature and RH values are fairly reasonable.

Whiteflies which at the end of the experiments were scored as dead, did not "recover" even when transferred to favorable conditions ($25-28^{\circ}\text{C}$, $\text{RH}=60\%$) and provided with food (Table 11). This means that after they have been affected by

weather conditions during their flight, they will not recover after landing in a cultivated field even when the conditions become more favorable, e.g. in the evening.

The high sensitivity of unfed whiteflies to climatic conditions was confirmed in an outdoor experiment on a very warm and dry day, on which the temperature rose within 3 h from 36°C (at 09.00 am) to 42°C (at 12.00 noon) and the RH was 20-25%. The fact that a few whiteflies survived all tests conditions (Tables 9-13) supports the hypothesis that B. tabaci is able to migrate over distances of several kilometers. The whiteflies may migrate only on a daily basis, since they fly only during the day time hours and at night must feed, or they will starve to death (M. Berlinger, unpublished).

The results that were obtained in the above experiments may explain the whitefly's appearance at some isolated sites, such as on the top of a building about 40 m high (Berlinger, unpublished) and in the southern desert of Israel, far from any suitable host-plant.

The Hosts

Survey of the Occurrence of TYLCV and LIYV in Common Plants Cultivated in the Jordan Valley (Israel) and the Imperial Valley (U.S.A.)

TYLCV

Host range of tomato yellow leaf curl virus on common weeds and ornamentals in Israel

Amaranthaceae:	<u>Amaranthus retroflexus</u> L.	- Immune
Apocynaceae:	<u>Nerium oleander</u> L.	- Immune
Asclepiadaceae:	<u>Calotropis procera</u> (Willd.) R.Br.	- Immune
	<u>Cynanchum acutum</u> L.	- Symptomless carrier
Capparidaceae:	<u>Capparis aegyptia</u> Lam.	- Immune
Caprifoliaceae:	<u>Lonicera</u> sp.	- Immune
Compositae:	<u>Sonchus oleraceus</u> L.	- Symptomless carrier
Compositae:	<u>Xanthium strumarium</u> L.	- Immune

Cucurbitaceae:	<u>Momordica balsamina</u> L.	- Immune
Euphorbiaceae:	<u>Ricinus communis</u> L.	- Immune
Leguminosae:	<u>Prosopis farcata</u> (Banks et Sol.) Eig	- Immune
Malvaceae:	<u>Althaea</u> sp.	- Immune
	<u>Hibiscus rosa-sinensis</u> L.	- Immune
	<u>Lavatera cretica</u> L.	- Immune
	<u>Malva micaensis</u> All.	- Symptomless carrier
	<u>Malva parviflora</u> L.	- Symptomless carrier
Plumbaginaceae:	<u>Plumbago capensis</u> Thunb.	- Immune
Polygonaceae:	<u>Polygonum equisetiforme</u> S. et S.	- Immune
Portulacaceae:	<u>Portulaca oleracea</u> L.	- Immune
Resedaceae:	<u>Ochradenus baccatus</u> Del.	- Immune
Solanaceae:	<u>Hyoscyamus desertorum</u> (Asch.) Eig	- Susceptible (symptoms similar to tomato)
	<u>Lycium</u> sp.	- Immune
	<u>Solanum incanum</u> L.	- Immune
	<u>Solanum villosum</u> (L.) Lam.	- Immune
	<u>Withania somnifera</u> (L.) Dun.	- Immune
Tamaricaceae:	<u>Tamarix</u> sp.	- Immune
Zygophyllaceae:	<u>Tribulus</u> sp.	- Immune

Among the plants found susceptible in the laboratory only Cynanchum acutum and Malva parviflora were found to be also a natural host of the virus. Thus, more than 50% of the 65 samples of C. acutum collected along the Jordan River during July-September were infected and 13 out of 19 plants of M. parviflora collected in December in the Jordan Valley were infected.

LIYV - In addition to lettuce, the following plants were found to be naturally infected in the Imperial Valley:

Chenopodiaceae: Beta vulgaris L., Chenopodium murale L. Compositae: Lactuca

seriola L., Helianthus annuus L. Cucurbitaceae: Cucumis melo L. Malvaceae: Malva parviflora L. Polygonaceae: Rumex crispus L. Solanaceae: Physalis wrightii Gray Umbelliferae: Daucus carota L.

It is important to mention that from mid-July until September, only three wild hosts are widespread in the field (mainly along the borders of alfalfa fields): Lactuca serriola, Helianthus annuus and Physalis wrightii. The infectivity rates on these weeds in samples collected from the end of June to September were 21/124 (17%), 20/104 (16%), and 11/68 (16%) in L. serriola, H. annuus and P. wrightii, respectively.

At the beginning of September when the earliest autumn melon plantings were about one month old, 0.6% of 176 samples (only the older leaves were used for ELISA tests) collected from five fields were infected. The infectivity rate in those fields increased to 8-70% by the end of September (50 samples of older leaves were tested from each of the fields). The rate of viruliferous whiteflies (obtained with the collector described previously) in the population which developed on those melons could be roughly calculated from Table 14, which shows that 90, 60, 90, 55 and 50% of the samples from five fields were infected. Since 22.2, 66.6, 77.5, 92.1 and 97.4% transmission rates were established when 1, 5, 10, 20 and 40 viruliferous whiteflies were placed on a plant (Duffus et al., 1986), then roughly 20, 5, 20, 4 and 4 viruliferous whiteflies are present on the different field samples, respectively. The calculated percent of infected insects in the population is therefore 8.6 (20/232), 1.0, 4.0, 2.6 and 3.0, the respectively.

Table 14. Infectivity rate of the whitefly population in melon in the Imperial Valley (U.S.A.) in September

Field	Mean number of whiteflies collected/cage	% Transmission (out of 9 or 10 replicates)
Imperial Res. Station	232 (15 sec)	90
South of Brawley	520 (15 sec)	60
Valin and Vail Rd	426 (15 sec)	90
Boone Rd	151 (15 sec)	55
S 30 and Lyons Rd.	131 (1 min)	50

Virus-Vector-Host Relationships

Host Selection

Cynachum acutum is a perennial natural host of TYLCV, and, as such, it was important to learn its relationships with the vector. The results on Table 15 show that C. acutum is less attractive to B. tabaci than squash. No differences were observed between the attraction of the whiteflies to tomato, squash or cucumber. However, after landing, (Table 16), the whiteflies prefer squash and cucumber to tomato: 2.75 and 1.9 times more whiteflies were found on the same leaf area of squash and cucumber as on tomato after 2 h. However, the plant to which they are attracted least is C. acutum (8.6 and 7.5 times less in comparison with squash and cucumber). Indeed, in eight samples (15 leaves in each) where the 6-7 leaves from the top of C. acutum were collected in the field at 2-week intervals between 20.VIII and 30.X, the total number of larvae 15 leaves did not exceed 23 (mean of 1.5/leaf). These findings show that C. acutum is a poor host for B. tabaci. However, this plant is a very good source for the spread of the virus, since B. tabaci stays on the plants long enough to acquire the virus and leaves them soon after that.

Table 15. Host selection as expressed by landing

Host pairs ^a	Number of whiteflies in each replicate												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
<u>C. acutum</u>	8	10	6	5	3	11	18	18	29	64	5	16	193*
and squash	37	57	25	32	60	77	85	67	80	85	28	20	653
Tomato and	17	33	16	23	26	8	17	26	29	30	17	11	253
squash	41	61	36	37	26	22	15	24	13	11	8	15	309
Tomato and	25	27	20	13	13	33	41	30	16				218
cucumber	23	18	22	11	25	29	37	20	30				215

^aDetached leaves of similar surface area covered with vaseline. The whiteflies were counted 2 following their introduction into the chamber.

*Significant difference at $P \leq 0.05$ in pair.

Table 16. Host preference after feeding, as expressed by landing

Host pairs ^a	<u>Number of whiteflies in each replicate</u>									Total
	1	2	3	4	5	6	7	8	9	
<u>C. acutum</u>	26	9	17	4	5					61*
and squash	133	148	99	35	110					525
<u>C. acutum</u>	5	3	14	1	5					28*
cucumber	19	49	39	67	37					211
Tomato and	20	29	7	3	20	31	27	4	19	160*
squash	44	65	31	41	35	47	77	40	60	440
Tomato and	15	16	16	28	33	36	25	21		190*
cucumber	37	30	35	41	56	40	48	65		352

^aIntact pot-grown plants with similar leaf area. Live whiteflies were counted 24 h after their introduction into the chamber.

*Significant difference at $P \leq 0.05$ in pair.

Association between LIYV Titer and Acquisition of the Virus by *Bemisia tabaci* from Different Hosts

Virus titer was tested by ELISA (Table 17). In the recovery tests (Table 18) the whiteflies were placed in leaf cages (100-200/cage) for 24 h on the young, intermediate and old leaves of the tested hosts 30-60 days after the inoculation. The whiteflies were then divided into groups of 15 insects each, which were given a 48-72-h feeding period on healthy Summer Bibb lettuce test plants (3-4 true leaves old). Table 17 shows that in most of the cases, higher virus titers were observed in the older leaves. However, virus recovery efficiency by the whiteflies from the young leaves was relatively high (Table 18). It is important to mention the two extreme cases, of *C. murale* and melons. A very high antigen titer was found in all the leaves of *C. murale*, but the whiteflies could pick up virus only from the young leaves. No virus was detected in the young leaves of melons, but positive transmissions were achieved by whiteflies fed on those leaves.

The relatively high efficiency of the whiteflies in acquiring virus from young growth may be explained by their better feeding behavior on such leaves. Also, the possibility exists that in some hosts some of the virus particles in the old parts of the plants are non-infective.

The above results emphasize the importance of preliminary work on the potential hosts of viruses before starting field studies. Thus this work can supply information on the proper part of plant to be collected in surveys and the importance of the host as the virus source.

Table 17. LIYV titer in different hosts as examined by ELISA

Host	A405 from leaves at the following positions on the stem ^a			
	Top (Young)	Middle	Bottom (old)	Control
<u>Chenopodium murale</u>	1.120	1.087	1.166	0.009 ^b
Lettuce	0.076	0.229	0.322	0.016
<u>Lactuca serriola</u>	0.168	0.730	0.601	0.023
<u>Malva parviflora</u>	0.115	0.121	0.489	0.014
Melon	0.027	0.069	0.230	0.016
<u>Physalis wrightii</u>	0.115	0.372	0.745	0.033
<u>Sonchus oleraceus</u>	0.327	0.148	1.483	0.013
Sugarbeet	0.178	1.262	1.961	0.026
Sunflower (wild)	0.125	0.298	0.492	0.017

^aMeans of at least five replicates. Five discs each 5 cm in diam were used in each replicate. These were crushed by a Vortex in 1 ml of buffer solution.

^bAt least two replicates of five discs each, collected at random from different places on the plants.

Table 18. LIYV recovery by Bemisia tabaci from different hosts

Host	% Transmission from leaves at the following positions on the stem ^a		
	Top	Middle	Bottom
<u>Chenopodium murale</u>	5(2/39)	0(0/15)	0(0/38)
Lettuce	42(19/45)	39(18/46)	11(5/46)
<u>Malva parviflora</u>	88(29/33)	88(29/33)	91(30/33)
Melon	38(23/60)	4(2/46)	47(27/58)
<u>Sonchus oleraceus</u>	85(34/40)	88(35/40)	95(38/40)
Sugarbeet	64(28/44)	50(17/34)	51(21/41)
Sunflower (wild)	53(21/40)	58(23/40)	43(17/40)
Wild lettuce	61(19/31)	84(26/31)	30(9/30)
<u>Physalis wrightii</u>	31(16/51)	38(17/45)	40(19/47)

^aMeans of at least five replicates

^bIn parentheses, number of plants infected divided by total number of plants inoculated.

Association between Bemisia tabaci Population Dynamics and the Spread of TYLCV and LIYV

TYLCV - The results of the observations carried out in the Jordan Valley during the last 3 years show a positive and significant correlation between population size and the spread of TYLCV. Thus, r_s values of 0.65 ($P=0.05$), 0.72 ($P=0.05>0.01$) and 0.78 ($P<0.01$) were measured in the years 1984, 1985, 1986, respectively. It is important to mention that the maximum number of whiteflies trapped per week in each of the above mentioned years was: 800 in the first week

of September 1984, 9500 in the second week of October 1985 and 1000 in the fourth week of September 1986.

The results for 1985 are depicted graphically in Figure 3.

The results summarized in Table 19 show that toward the end of the season when the population size of B. tabaci decreases, there is a rise in the number of infected whiteflies in the population, probably because of an increase in the number of susceptible hosts. However, the infectivity rate was found to be correlated with the population size (Fig. 3). As mentioned in the Materials and Methods section, the infectivity rate of the whiteflies in the field was examined by arranging the collected whiteflies in groups of 20 before they were placed on test plants. The probability of a single, 3, 5, 10 and 15 whiteflies transmitting TYLCV is 32, 83, 84, 86 and 100% (Cohen and Nitzany 1966). Since the rate of transmission in the sample reached a maximum of 55%, it may be assumed that only a single infected whitefly in a sample is responsible for the transmission. Therefore, the actual number of infected insects in the population increased to a maximum of approximately 2.7% in 1986 (Table 19).

Fig. 3

The connection between Bemisia tabaci population dynamics and tobacco yellow leaf curl virus infectivity rate in the Jordan Valley, Israel.

Table 19. TYLCV - Infectivity rate of the whitefly population in the Jordan Valley in 1986

Date of collection	% Infectivity in field samples collected from		% Infected insects in the population (calculated)	
	<u>C. acutum</u>	tomato	<u>C. acutum</u>	tomato
24.IX	12(3/25) ^a	10(2/20)	0.6(3/500) ^b	0.5(2/400)
1.X	0(0/15)	5(1/20)	0(0/300)	0.25(1/400)
6.X	0(0/30)	0(0/20)	0(0/600)	0(0/400)
18.X	0(0/20)	8.7(4/46)	0(0/400)	0.43(4/920)
22.X	55(0/11)	43.5(20/46)	2.7(6/220)	2.17(20/920)
28.X	0(0/15)	32(13/41)	0(0/300)	1.6(13/820)
5.XI	12.5(1/8)	0(0/12)	0.6(1/160)	0 (0/240)

^aIn parentheses, number of plants infected divided by total number of plants inoculated, 20 whiteflies were placed on each plant.

^bNumber of plants infected divided by the total number of whiteflies placed on the plants.

LIYV - The observations on the connection between the population size and infectivity rate were started in 1987. In this case, as with TYLCV, there is an indication of a correlation between these two parameters. Thus, during July only two Summer Bibb lettuce test plants became infected out of 204 (1%) inoculated with whiteflies collected from weeds and cultivated crops in the Imperial

Valley. During August the number was 4 out of 316 (1.3%), and in the first week of September - 16 out of 226 (7%). At the end of August a mean of 100 whiteflies were collected during 1 min of operation of the collector in the Brawley region. The number rose in that region to 2100 insects/min at the end of September.

Acquisition, Interference and Retention of Gemini Cucurbit Leaf Curl Viruses in Whiteflies

Effect of source plant on the uptake of SLCV by *B. tabaci*. The relative virus titer was determined by ELISA in young and old leaves, collected from old SLCV-infected squash plants (2 to 3 months after inoculation) and from young plants (15 to 20 days after inoculation). The results indicate a significantly higher virus titer in young leaves in comparison with older leaves of the same plant, and a significantly higher virus content in the young leaves of older plants than in the young leaves of young ones (Table 20). Similarly, a higher virus content was found in whiteflies fed on young leaves of old plants, than among those fed on young leaves of young plants (Fig. 5).

In previous work, a high percentage of the whiteflies fed on young leaves of young plants became infective (Cohen et al., 1983). Based on the results of the ELISA tests (Table 20), it was important to compare virus acquisition from young and old plants. In this experiment, whitefly females were given a 48 h. acquisition access on five young leaves each from young and old sources. At least ten insects were selected from each leaf and placed individually on healthy squash for a 48-h inoculation feeding. Sixty percent (30 out of 50) transmission was achieved by the whiteflies fed on young plants in comparison with 60.3% (41 out of 68) by those fed on old plants. These results indicate no apparent difference in efficiency of transmission between whiteflies fed on a relatively high or low titer source under the conditions of this experiment.

Detection of SLCV in *B. tabaci* by ELISA. Significant differences were found between A405 values of homogenates prepared from ten or more whiteflies previously fed on a SLCV source in comparison with the appropriate control. The differences were significant in all tests made with 10 or 20 whiteflies. No significant differences were observed between whiteflies fed on two different control plants, viz., healthy squash and sweet potato (Fig. 4).

SLCV could also be detected in homogenates when groups of 20 inoculative whiteflies were mixed with 80 virus-free ones. In three experiments with a total of 21 replications, ELISA readings averaged 32%, 23% and 48% above the controls and were significantly different from them.

Effect of acquisition access period on SLCV in *B. tabaci*. A significant increase in virus content in the insect's body as measured by ELISA, was indicated when the acquisition access period was increased from 1 to 6 days (Fig. 5).

Retention of SLCV by *B. tabaci*. In these experiments the whiteflies were given an acquisition access period of 48 h on young leaves from old or young plants and then transferred to sweet potato. After removal from the virus source, virus content decreased with time (Fig. 6). Virus could not be detected by ELISA in insects fed on a relatively poor virus source within as soon as 2 days (Fig. 6B) after leaving the source. When the whiteflies were fed on a higher virus content source (Fig. 6A), the virus was still detectable 6 days after removal from the source, but the virus titer was one third of its initial level.

A significantly higher titer was observed in homogenates of whiteflies one day after being removed from high virus content sources (Fig. 6A), in comparison with the titer found in those tested immediately after the end of acquisition

access. This was thought to a result of weaker insects dying in the transfer process from the acquisition source to the sweet potato holding plants.

Retention of SLCV by *T. abutilonea*. No transmission of SLCV was achieved by *T. abutilonea* in tests where several thousands of these insects were used. The experiments on the retention of SLCV in these insects were conducted employing the same techniques as used for the *Bemisia* experiments, except that 15 *T. abutilonea* were crushed in each sample. This number of *Trialeurodes* whiteflies weighs approximately the same as 20 *B. tabaci* whiteflies. The results summarized in Figures 6 and 7 show that the initial amount of SLCV acquired by *T. abutilonea* during an 18-h acquisition access is roughly six to nine times more than that acquired by *B. tabaci*.

The results in Figure 7 are quite variable and probably reflect virus concentration in individual plants. Possibly the deviations observed with *Trialeurodes* are larger because of the greater potential of *T. abutilonea* to acquire virus. Nevertheless, *T. abutilonea* retained SLCV for at least the same period and probably longer than *B. tabaci*.

Persistence of SLCV in *B. tabaci*. The results of serial transfers show that 86% transmission was achieved by the whiteflies during the first 10 days following a 48-h acquisition feeding (group A) in comparison with 13% transmission during the 11th through 34th days (Fig. 8), the difference is significant ($P < 0.01$).

Following a 5-day acquisition feeding (group B), 80% transmission was recorded during the first 12 days in comparison with 59% from the 13th to 34th days. The difference is significant ($P < 0.025$). During the first 10 days, significant differences were not found in the transmission rate between the two groups 86% and 79% in group A and B respectively. However, they differed significantly in the period from the 11th to 34th days of the serial transfers.

Transmission rates of 43% and 64% were established by the whiteflies of groups A and B, respectively.

Interference between SLCV and MLCV in *B. tabaci*. As mentioned previously, MLCV infects all the hosts which are susceptible to SLCV but not vice versa. Therefore, in the interference tests SLCV was used as the protective virus and MLCV as the challenger. In all five experiments (Table 21), a reduction in the transmission rate of MLCV was established by whiteflies previously fed on SLCV in comparison with those fed on MLCV only. The reduced rates were between 36% and 87% in the different tests; the difference is significant. It should be noted that SLCV was acquired and transmitted by the whiteflies much more efficiently than was MLCV.

The detection of SLCV in the whiteflies *B. tabaci* and *T. abutilonea* one day or more after removal from the virus source apparently indicates the passage of the virus into the haemolymph system. There is no evidence of multiplication of SLCV in the insect vector since the detected virus content decreased shortly after the whitefly left the virus source (Fig. 6). However, the method used here is not sufficiently sensitive to detect minor changes in virus titer, and the possibility of the occurrence of limited multiplication cannot be excluded. The virus content in *B. tabaci* rose with an increase in the acquisition feeding period (Fig. 5). An increase in transmission rate accompanied the increase in the acquisition access period (Cohen et al., 1983) (Fig. 8). However, during the first 10 days, no differences in the transmission rate were observed between whiteflies previously fed for 48 h or 5 days on SLCV (Fig. 8). This phenomenon is apparently a result of the occurrence of virus in the haemocoel. Only after the virus detected by ELISA is exhausted, do significant differences in the transmission between the two groups become apparent.

In all cases, B. tabaci females retained their inoculativity many days after the virus could no longer be detected in their bodies by ELISA. The decrease in the virus titer in time may be due to the production of antiviral factors in the whiteflies, as reported previously for TYLCV (Cohen, 1969; Marco et al., 1972, 1975) or to the natural loss in the feeding process. The apparent discrepancy between the rapid decrease in detectable virus in the insects and the prolonged transmission rate could be due to the possibility that the transmissible form is uncoated virus which cannot be detected by ELISA. However, no conclusive evidence was obtained of a correlation between transmission level and the rate of SLCV nucleic acid in B. tabaci (Al Musa et al., 1986).

The evidence on the interference between the SLCV and MLCV in B. tabaci (Table 21) led to the conclusion that the mechanism involved in the transmission of SLCV is similar to that described for luteoviruses in aphids (Gildow, 1982, 1985; Gildow and Rochow, 1980, 1980a; Tamada and Harrison, 1981) and for the gemini beet curly top virus in its leaf hopper (Bennet and Wallace, 1938). Our results may support the concept suggested by Rochow (1969) and for Gildow and Rochow (1980a,b,c) on the active role of the accessory salivary gland in determining vectoring and regulating virus transmission, apparently by virus coat protein-specific receptors located on its membrane surface.

This concept may explain the failure to detect the virus by ELISA after a relatively short period (Fig. 6). Thus, while attaching to the membrane of the accessory salivary glands, at least part of the capsid surface is blocked - which may lower the probability to react with the antiserum. However, Al Musa et al. (1986) failed to detect viral nucleic acid in insect extracts in as short a period as 72 h after acquisition. Thus, the possibility exists that the amount of virus required to achieve transmission is much lower than the amount that can be detected by ELISA or spot hybridization.

The suggested mechanism by which SLCV is transmitted by B. tabaci is as follows: The acquired virus passes intact through the gut wall into the haemolymph, which serves as a virus reservoir. SLCV then enters into the salivary gland system via specific sites and from there into plants. As long as there is virus in the haemolymph, the salivary gland system is saturated and no decrease in the transmission rate occurs. As the haemolymph reservoir is depleted, the rate of transmission slowly decreases. The ability to detect SLCV in B. tabaci using ELISA depends on the virus source, the acquisition access period, and especially on the length of time after B. tabaci leaves the source plant. The ELISA technique cannot be used as the only tool in epidemiological studies to evaluate the rate of inoculative vectors in the population. However, T. abutilonea, which is not a vector but is common in the area of SLCV (Duffus et al., 1986), acquired markedly more virus than B. tabaci and therefore may be used in epidemiological studies as a marker for the presence of SLCV in the region.

Table 20. The content of squash leaf curl virus in squash A405 values of samples from SLCV-infected squash; means of 10-20 samples)

Exp. no.	2-3 months after inoculation			10-20 days after inoculation		Control (healthy squash)
	1st	3rd	Leaf from the top 1st	3rd		
1	0.612 ^a (0.301-0.968)	0.434 ^b [*] (0.316-0.593)	0.363 ^c (0.165-0.518)	0.248 ^d (0.172-0.354)	0.0	
2	0.627 ^w (0.345-0.972)	0.249 ^y (0.168-0.327)	0.382 ^x (0.183-0.816)	0.174 ^z (0.108-0.297)	0.0	

Within each experiment, figures followed by a common letter do not differ significantly at $P \leq 0.05$.

*In parentheses, minimum and maximum values.

Table 21. Interference between SLCV and MLCV in Bemisia tabaci (Results are expressed as percent transmission).

Test no.	Feeding order					
	1) 48 h SLCV-squash	1) 48 h Healthy squash	1) 48 h SLCV-squash	2) 24 h Healthy watermelon	3) 48 h Healthy watermelon	3) 48 h Healthy squash
1	14.2(2/14)*	22.2(4/18)	89.4(17/19)			
2	27.7(5/18)	50 (15/30)	--			
3	27.7(5/18)	77.7(16/18)	94.4(17/18)			
4	5.5(1/18)	37.5(6/16)	--			
5	7.6(1/13)	57.1(8/14)	--			
Total	17.3(14/81)	48.9(47/96)	91.8(34/37)			

* In parentheses, number of plants infected divided by number of plants inoculated.

Fig. 4

Detection of squash leaf curl virus in samples containing different numbers of *Bemisia tabaci*

A₄₀₅ values with different letters differ significantly from each other; those marked with an asterisk differ significantly from the control. Numbers in parenthesis denote the number of samples tested. A was analyzed statistically independently of B and C.

A. *B. tabaci* fed for 18 h on young leaves of 2-3 month-old SLCV-infected squash plants

B. *B. tabaci* fed on sweet potato

C. *B. tabaci* fed for 48 h on healthy squash

Fig. 5

Effect of acquisition access period on the squash leaf curl virus content in *Bemisia tabaci*

Percentages refer to homogenates of 20 insects/sample. Figures with different letters differ significantly from each other; those marked with an asterisk differ significantly from the control. Numbers in parenthesis denote the number of samples tested. A and B were analyzed statistically independently of each other.

A. Virus source from young leaves of 2-3-month-old SLCV-infected squash.

B. Virus source from young leaves of young SLCV-infected plants 15-20 days after inoculation.

Fig. 6

Retention of squash leaf curl virus by *Bemisia tabaci*.

Percentages refer to homogenates of 15 insects/sample. Figures with different letters differ significantly from each other; those marked with an asterisk differ significantly from the control. Numbers in parenthesis denote the number of samples tested. A and B were analyzed statistically independently of each other.

- A. Virus source from young leaves of 2-3-month-old SLCV-infected squash.
- B. Virus source from young leaves of young SLCV-infected plants 15-20 days after inoculation.

Fig. 7

Retention of squash leaf curl virus by *Trialeurodes abutilonea*

Percentages refer to homogenates of 15 insects/sample. Figures with different letters differ significantly from each other; those marked with an asterisk differ significantly from the control. Numbers in parenthesis denote the number of samples tested. A and B were analyzed statistically independently of each other.

A. Virus source from young leaves of 2-3-month-old SLCV-infected squash.

B. Virus source from young leaves of young SLCV-infected plants 15-20 days after inoculation.

Fig. 8

Persistence of squash leaf curl virus in Bemisia tabaci females following a 5-day acquisition feeding (A) and a 2-day acquisition feeding (B).

Use of Antisera to Examine the General Location of LIYV and SLCV in the Vector,

B. tabaci

The results summarized in Table 22 show a significant reduction of 72% in the transmission of LIYV by the whiteflies fed on LIYV-antiserum after virus acquisition. However, no significant differences were observed in the transmission of SLCV when SLCV-antiserum was given to the whiteflies after or before virus acquisition (Tables 23 and 24). The above mentioned results provide an indication of the location of the viruses in the insect body. Thus LIYV, a semipersistent virus, is probably located at the beginning of the gut, where the antiserum could be effective without being destroyed by the enzymatic activity which occurs in the rest of the gut. SLCV, on the other hand, is a persistent virus which is probably concentrated in the haemolymph (see previous experiment), and therefore the antiserum is destroyed in the gut before coming in contact with the virus.

Table 22. Effect of LIYV-antiserum on the transmission of LIYV

Test no.	% Transmission after the end of a 24 h feeding on LIYV followed by a 5-6 h feeding through membrane on:		
	LIYV antiserum (1:10)	Normal serum (1:10)	Buffer
1	8(1/12)	50(6/12)	-
2	25(3/12)	92(11/12)	-
3	13(1/8)	100(8/8)	100(8/8)
4	42(5/12)	91(10/11)	-
5	21(3/14)	64(7/11)	-
Total	22(13/58) ^a	78(42/54)	100(8/8)

^aP=0.031 as analyzed by the sign test (Siegel, 1956).

^bIn parentheses, number of plants infected divided by total number of plants inoculated.

Table 23. Effect of SLCV antiserum on the acquisition of SLCV

Test no.	% Transmission after the end of a 24-h feeding on SLCV preceded by a 5-6-h feeding through membrane on:	
	SLCV antiserum (1:10)	Normal serum (1:10)
1	100(12/12)	91(10/11)
2	100(9/9)	50(5/10)
3	73(8/11)	89(8/9)
Total	91(29/32)	77(23/30)

*In parentheses, number of plants infected divided by total number of plants inoculated.

Table 24. Effect of SLCV-antiserum on the transmission of SLCV

Test no.	% Transmission after the end of a 24 h feeding on SLCV followed by a 5-6-h feeding through membrane on:		
	SLCV antiserum (1:10)	Normal serum (1:10)	Buffer
1	100(6/6)*	100(7/7)	80(8/10)
2	50(6/12)	50(6/12)	-
3	100(10/10)	100(10/10)	-
4	89(8/9)	100(11/11)	-
5	100(15/15)	77(10/13)	93(14/15)
Total	87(45/52)	83(44/53)	96(22/25)

*In parentheses, number of plants infected divided by the total number of plants inoculated.

GENERAL DISCUSSION

The information collected in the course of the present research in respect to the viruses and the methods of their detection in plants and vector, the data on the population dynamics and behavior of B. tabaci, and the findings on the host-virus-vector relationships enable us to propose the epidemiological cycles of the two main viruses: the TYLCV in the Jordan Valley (Fig. 9) and the LIYV in the Imperial Valley (Fig. 10). So far, C. acutum was found to be the only perennial natural host of TYLCV. This weed is concentrated along the bank of the Jordan River, a few kilometers east of the main tomato production area in the Jordan Valley. During the cold winter months (December-February) only the subterranean parts of the plants survive. The plants start to grow again in the spring, reaching full vegetation in August-September, when flowering starts. From that time on, the vegetation starts to decline. B. tabaci overwinters and multiplies mainly on plants immune to TYLCV. It is important to note that the information on the hosts of B. tabaci during the spring and early summer is very limited. However, during that period the migrating population is very small. Only in June were infected whiteflies first observed and their number increased sharply with the approach of August-September, concomitantly with the increase in the total number of migrating whiteflies. During September the tomato crops are transplanted. At that time whiteflies which had been marked with fluorescent dust while staying on the C. acutum, reached the tomato area. C. acutum is not a preferred host for the vector. The whiteflies stay on the plants for a short period - about 3 h - before they take off. Therefore, this weed serves as a good distributor of the virus. Once TYLCV has been introduced into the tomato fields the plants and the infected M. parviflora serve as a huge TYLCV reservoir. Tomato plots surrounded by a wind break are in greater jeopardy than those sown in an open field. Attacking the TYLCV cycle (Fig. 9) at its weak point, i.e.,

eradication of C. acutum in June-July before the beginning of the B. tabaci migration season, may control the spread of TYLCV.

In the case of LIYV (Fig. 10), natural and cultivated hosts of the virus are widespread from September (the beginning of the lettuce planting season) to June. However, from April to August the number is still very low. During the second half of July the B. tabaci migrating population and the infectivity rate among the whiteflies is still very low. The virus has been found in only three weeds, Lactuca serriola, Helianthus annuus and Physalis wrightii; the two latter weeds are also good hosts of B. tabaci. At the beginning of August, melons are planted. This crop is one of the best hosts for B. tabaci and is susceptible to LIYV. Therefore, at the beginning of the lettuce season (September) the infectivity rate among the melon plants and of the population developing on these plants is very high which explains the 100% infection which occurs in the lettuce fields. There are very few data on the multiplication site of B. tabaci during the spring and early summer, before the time when melons and cotton are planted. During our survey we observed an increasing population of B. tabaci from April on, almost limited to on Convolvulus sp. plants. During that period the migrating population was very small. Eradication in the early spring of the bind weed (Convolvulus) and the eradication during July of the three virus hosts mentioned previously, may reduce the B. tabaci population and the virus source and consequently also the spread of LIYV into lettuce fields. Based on the findings amassed on the virus-vector relationships, further studies may uncover the way viruses are attached to the sites on the membranes of the salivary glands or in the stylet. This in turn may lead to the development of control measures based on blockage of these sites by synthetic nonviral materials.

Fig. 9

Schematic diagram of the epidemiology of tobacco yellow leaf curl virus.

A. Migration rate of the *B. tabaci* population

B. TYLCV hosts and rate of infectivity; C = *Cynanchum acutum*, T = tomato, M = *Malva parviflora*

C. Inoculativity rate of the *B. tabaci* population.

Fig. 10

Schematic diagram of the epidemiology of lettuce infectious yellows virus.

A. Migration rate of the *B. tabaci* population.

B. LIYV hosts and rate of infectivity; W = weeds, C = cultivated crops

C. Inoculativity rate of the *B. tabaci* population.

Literature Cited

Al-Musa, A. (1982) Incidence, economic importance and control of tomato yellow leaf curl in Jordan. *Plant Dis.* 66:561-563.

Al-Musa, A., Polston, J.E., Perring, T.M., and Dodds, J.A. (1986). The use of spot hybridization with a cloned probe for squash leaf curl virus to investigate vector relationships. *Proc. of the Workshop on Epidemiology of Plant Virus Diseases* (Orlando, Florida) pp. X-14-16.

Avidov, Z. (1956) Bionomics of the tobacco whitefly (Bemisia tabaci Gennad.) in Israel. *Ktavim* 7:25-41.

Bennett, C.W. and Wallace, H.E. (1938). Relation of the curly top virus to the vector, Eutettix tenellus. *J. Agric. Res.* 56:31-52.

Berlinger, M.J. (1973). Effect of relative humidity and temperature on the survival of Clausenia josefi (Hymenoptera: Encyrtidae). *Entomologia exp. appl.* 16:373-379.

Berlinger, M.J. and R. Dahan (1986). Flight patterns of Bemisia tabaci a vector of plant viruses. *Abstr., International Conference on Tropical Entomology*, p.23 Nairobi, Kenya.

Bird, J. and Maramorosch, K. (1978). Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22:55-109.

Bock, K.R. (1983). Epidemiology of Cassava mosaic virus in Kenya. *in*: [Eds.] *Plant Virus Epidemiology*. Plumb, R.T., and Thresh, J.M., Blackwell Scientific Publications, Oxford. pp. 337-347

Buttler, G.D. Jr., Henneberry, T.J. and Clayton, T.E. (1983). Bemisia tabaci (Homoptera:Aleyrodidae): development, oviposition and longevity in relation to temperature. *Ann. ent. Soc. Am.* 76:310-313.

- Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. gen. Virol. 34:475-483.
- Cohen, S. (1967). The occurrence in the body of Bemisia tabaci of a factor apparently related to the phenomenon of "periodic acquisition" of tomato yellow leaf curl virus. Virology 31:180-183.
- Cohen, S. (1969). In vivo effects in whiteflies of a possible antiviral factor. Virology 37:448-454.
- Cohen, S. (1982). Control of whitefly vectors of viruses by color mulches. in: Harris, K.F. and Maramorosch, K. [Eds.] Pathogens, Vectors and Plant Diseases, Approaches to Control. Academic Press, New York, NY. pp. 45-56.
- Cohen, S. and Berlinger, M.J. (1986). Transmission and cultural control of whitefly-borne viruses. Agric. Ecosyst. Envir. 17:89-97.
- Cohen, S., Berlinger, M., Lehman-Sigura, N., Kern, J., Harpaz, I. and Ben Joseph, R. (1986a). The epidemiology of a whitefly-borne virus in Israel. Proc. of the Workshop on Epidemiology of Plant Virus Diseases (Orlando Florida), pp. VII-35-36.
- Cohen, S., Duffus, J.E., Larsen, R.C., Liu, H.Y. and Flock, R.A. (1983). Purification, serology and vector relationships of squash leaf curl virus, a whitefly-transmitted geminivirus. Phytopathology 73:1669-1673.
- Cohen, S. and Harpaz, I. (1964). Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (Bemisia tabaci Genn.). Entomologia exp. appl. 7:155-166.
- Cohen, S., Keren, J., Harpaz, I. and Ben-Joseph, R. (1986b). Studies of the epidemiology of a whitefly-borne virus, tomato yellow leaf curl virus, in the Jordan Valley. Phytoparasitica 14:158 (abstr.).

Cohen, S. and Marco, S. (1970). Periodic occurrence of an anti-TMV factor in the body of whiteflies carrying the tomato yellow leaf curl virus (TYLCV). *Virology* 40:363-368.

Cohen, S. and Melamed-Madjar, V. (1978). Prevention by soil mulching of the spread of tomato yellow leaf curl virus transmitted by Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) in Israel. *Bull. ent. Res.* 68:465-470.

Cohen, S., Melamed-Madjar, V. and Hameiri, J. (1974). Prevention of the spread of tomato yellow leaf curl virus transmitted by Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) in Israel. *Bull. ent. Res.* 64:193-197.

Cohen, S. and Nitzany, F.E. (1966). Transmission and host range of the tomato yellow leaf curl virus. *Phytopathology* 56:1127-1131.

Costa, A.S. (1976). Whitefly-transmitted plant diseases. *A. Rev. Phytopath.* 14:429-449.

Coudriet, D.L., Meyerdirk, D.E., Prabhaker, N. and Kishaba, A.N. (1986). Bionomics of the sweetpotato whitefly (Homoptera: Aleyrodidae) on weed hosts in the Imperial Valley, California. *Envir. Ent.* 15:1179-1183.

Coudriet, D.L., Prabhaker, N., Kishaba, A.N. and Meyerdirk, D.E. (1985). Variation in developmental rate of different hosts and overwintering of the sweetpotato whitefly, Bemisia tabaci (Homoptera: Aleyrodidae). *Envir. Ent.* 14:516-519.

Duffus, J.E. (1987). Whitefly transmission of plant viruses. in: Harris, K.F. [Ed.] *Current Topics in Vector Research*. Springer-Verlag, New York, NY. pp. 73-91

Duffus, J.E., Larsen, R.C. and Liu, H.Y. (1986). Lettuce infectious yellows virus - a new type of whitefly-transmitted virus. *Phytopathology* 76:97-100.

Duffus, J.E., Liu, H.Y. and Johns, M.R. (1985). Melon leaf curl virus a new geminivirus with host and serological variations from squash leaf curl virus. *Phytopathology* 75:1312.

Fauquet, C. and Fargette, D. (1986). A summary of the epidemiology of african cassava mosaic virus. Proc. of the Workshop on Epidemiology of Plant Virus Diseases (Orlando, Florida), pp VII-1-6.

Fargette, D., Fauquet, C. and Thouvenel, J.C. (1986). African cassava mosaic virus: the virus, the vector, the plant and the reservoirs. Proc. of the Workshop on Epidemiology of Plant Virus Diseases (Orlando, Florida), pp VII-7-9.

Fauquet, C., Fargette, D., Van Helden, M., Vanhalder, I. and Thouvenel, J.C. (1986). Field dispersal of Bemisia tabaci vector of african cassava mosaic virus. Proc. of the Workshop on Epidemiology of Plant Virus Diseases. (Orlando, Florida), pp VII-10-12.

Gerling, D. (1983). Ecology of Bemisia tabaci in Israel. Lecture at the First Congress of the Entomological Society, Israel.

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

Gerling, D. and Horowitz, R. (1983). Flight of adult Bemisia tabaci as determined in yellow trap catches. *Phytoparasitica* 11:64 (abstr.).

Gerling, D., Horowitz, A.R. and Baumgaertner, J. (1986) Autecology of Bemisia tabaci. *Agric. Ecosystems & Envir.* 17:5-19.

Gildow, F.E. (1982). Coated-vesicle transport of luteoviruses through salivary glands of Myzus persicae. *Phytopathology* 72:1289-1296.

Gildow, F.E. (1985). Transcellular transport of barley yellow dwarf virus into the hemocoel of the aphid vector. *Phytopathology* 75:292-297.

Gildow, F.E. and Rochow, W.F. (1980a). Role of accessory salivary glands in aphid transmission of barley yellow dwarf virus. *Virology* 104:97-108.

Gildow, F.E. and Rochow, W.F. (1980b). Transmission interference between two isolates of barley yellow dwarf virus in Macrosiphum avenae. *Phytopathology* 70:122-126.

Gildow, F.E. and Rochow, W.F. (1980c) Importance of capsid integrity for interference between two isolates of barley yellow dwarf virus in an aphid. *Phytopathology* 70:1013-1015.

Goodman, R.M., Bird, J. and Thongmeearkom, P. (1977). An unusual virus-like particle associated with golden yellow mosaic of beans. *Phytopathology* 67:37-42.

Harrison, B.D. (1985) Advances in geminivirus research. *A. Rev. Phytopath.* 23:55-82.

Larsen, R.C., Duffus, J.E. and Liu, H.Y. (1984). Tomato necrotic dwarf, a new type of whitefly transmitted virus. *Phytopathology* 74:795.

Madge, S.D. (1961). The control of relative humidity with aqueous solutions of sodium hydroxide. *Entomologia exp. appl.* 4:143-147.

Marco, S., Cohen, S., Harpaz, I. and Birk, Y. (1972). In vivo suppression of plant virus transmissibility by an anti-TMV factor occurring in an inoculative vector's body. *Virology* 17:761-766.

Marco, S., Cohen, S., Harpaz, I. and Birk, Y. (1975). An anti-TMV factor in the tobacco whitefly after acquisition of tomato yellow leaf curl virus. *J. Insect Physiol.* 21:1821-1826.

- Marrou, J., Quiot, J.B., Duteil, M., Labonne, G., Leclant, F. and Reinoust, M. (1979). Ecologie et epidemiologie du virus de la mosaïque du concombre dans le Sud-Est de la France. VIII: Influence des bris-vents et des cultures environnants sur la dissemination du virus de la mosaïque du concombre. Ann. Phytopath. 11:375-392.
- Melamed-Madjar, V., Cohen, S., Chen, M., Tam, S. and Rosilio, D. (1979). Observations on populations of Bemisia tabaci Gennadius (Homoptera: Aleyrodidae) on cotton adjacent to sunflower and potato in Israel. Israel J. Ent. 13:71-78.
- Melamed-Madjar, V., Cohen, S., Chen, M., Tam, S. and Rosilio, D. (1982). A method for monitoring Bemisia tabaci and timing spray applications against the pest in cotton fields in Israel. Phytoparasitica 10:85-91.
- Mound, L.A. (1962). Studies on the olfaction and colour sensitivity of Bemisia tabaci (Genn.) (Homoptera: Aleyrodidae). Entomologia Exp. appl. 5:99-104.
- Muniyappa, V. (1980). Whiteflies. in: Harris, K.F. and Maramorosch, K. [Eds.] Vectors of Plant Pathogens. Academic Press, New York, NY. pp. 39-85.
- Muniyappa, V. (1983). Epidemiology of yellow mosaic disease of horsegram (Macrotyloma uniflorum) in south India. in: Plumb, R.T. and Thresh, J.M. [Eds.] Plant Virus Epidemiology. Blackwell Scientific Publications, Oxford. pp. 331-335
- Nene, Y.L. (1972). A survey of the viral diseases of pulse crops in Uttar Pradesh. Res. Bull. G.B. Pant Univ. Agric. Technol., Pantnagar no. 4.
- Ohnesorge, B. and Rapp, G. (1986) Monitoring Bemisia tabaci. Agric. Ecosystem Envir. 17:21-27.

Ohnesorge, B., Sharaf, N. and Allawi, T. (1981). Population studies on the tobacco whitefly Bemisia tabaci Genn. (Homoptera: Aleyrodidae) during the winter season. Z. angew. Ent. 92:127-136.

Rochow, W.F. (1969). Specificity in aphid transmission of a circulative plant virus. in: Maramorosch, K. [Ed.] Viruses, Vectors and Vegetation. Interscience Publ. New York, NY. pp. 175-198.

Russo, M., Cohen, S. and Martelli, G.P. (1980). Virus-like particles in tomato plants affected by the yellow leaf curl disease. J. gen. Virol. 49:209-213.

Sharaf, S.N. (1982). Factors limiting the abundance of the tobacco whitefly (Bemisia tabaci) Gennad. (Homoptera: Aleyrodidae) on tomatoes during the spring season in the Jordan Valley. Duraset 9:97-103.

Shivanathan, P. (1983). The epidemiology of three diseases caused by whitefly-borne pathogens. in: Plumb, R.T. and Thresh, J.M., [Eds.] Plant Epidemiology. Blackwell Scientific Publications, Oxford. pp. 323-330

Siegel, S. (1956). Nonparametric Statistics for the Behavioural Sciences. McGraw-Hill, London.

Solomon, M.E. (1957). Estimation of humidity with cobalt thiocyanate papers and permanent colour standards. Bull. ent. Res. 48:489-506.

Stanley, J. (1985). The molecular biology of geminiviruses. Adv. Virus Res. 30:139-177.

Tamada, T. and Harrison, B.D. (1981). Quantitative studies on the uptake and retention of potato leafroll virus by aphids in laboratory and field conditions. Ann. appl. Biol. 98:261-276.

Youngman, R.R., Toscano, N.C., Jones, V.P., Kido, K. and Natwick, E.T. (1986). Correlations of seasonal trap counts of Bemisia tabaci (Homoptera: Aleyrodidae) in southeastern California. J. econ. Ent. 79:67-70.

F. DESCRIPTION OF COOPERATION

The cooperation among the investigators throughout the project was very close. Most of the efforts in Israel were invested in the biological aspects of the project, while the biochemical and serological aspects of the research were investigated mainly in the U.S.A. The results obtained in one country were immediately adopted and are being used by all the partners involved. Cooperative work was done on the virus-vector-host relationships, the development of the whitefly-collector device, and on the epidemiology of LIYV when S. Cohen spent 15 months of sabbatical leave in J.E. Duffus's laboratory in Salinas.

G. EVALUATION OF THE RESEARCH ACHIEVEMENTS

The original aim was to purify the viral agents and to develop rapid diagnostic methods for detection of the viruses in plants and in their whitefly vectors. Four viruses have been purified during the course of the project: TYLCV, LIYV, MLCV and TNDV. Antisera have been prepared for three of them. Using the serological ELISA technique it was possible to detect the viruses in plant tissues and to detect gemini viruses in B. tabaci.

The original plan was to utilize the rapid diagnostic techniques for the detection of these viruses in wild and cultivated plants. The ELISA method was found to be effective in field surveys only when the plants were first inoculated in the laboratory and attempts were made to detect the virus in different plant parts. Thus, LIYV could not be detected in young leaves of infected melons but could be detected easily in the older leaves. Therefore, in a field survey only the collection of old leaves would give the proper results.

Also, different background effects are established by different plants and by different concentrations of the same source. Therefore, healthy control plants of the same species to be tested should be grown in the laboratory and in each sample the same leaf area weight or unit of buffer volume should be used.

The aim was to develop trapping and biological methods and to use serological techniques for monitoring the rate of viruliferous whiteflies in the population. The ability to detect the gemini virus SLCV in B. tabaci using ELISA depends on the virus source, the acquisition period, and the length of time after the whitefly leaves the source plants. Therefore, this technique cannot be used as the only tool in epidemiological studies to evaluate the rate of inoculative vectors in the population. However the ELISA technique was used to study the virus-vector relationships. The semipersistent LIYV could not be detected in the vector. However, a simple system designed to monitor the movement and the infectivity of whiteflies by biological means was developed. Using this system the epidemiology of the most important whitefly-borne diseases in the U.S.A. (LIYV) and in Israel (TYLCV) was studied and cultural control measures were suggested.

H. LIST OF PUBLICATIONS

Cohen, S. and Ben-Joseph, R. (1986) Preliminary studies of the distribution of whiteflies (Bemisia tabaci), using fluorescent dust to mark the insects. *Phytoparasitica* 14:152-153 (Abstr.).

Cohen, S., Keren, J., Harpaz, I. and Ben-Joseph, R. (1986) Studies of the epidemiology of a whitefly-borne virus, tomato yellow leaf curl virus, in the Jordan Valley. *Phytoparasitica* 14:158 (abstr.).

Duffus, J.E., Larsen, R.C. and Liu, H.Y. (1986) Lettuce infectious yellows virus - a new type of whitefly-transmitted virus. *Phytopathology* 76:97-100.

Duffus, J.E., Liu, H.Y. and Johns, M.R. (1985) Melon leaf curl virus - A new gemini virus with host and serological variations from squash leaf curl virus. *Phytopathology* 75:1312.

Larsen, R.C., Duffus, J.E. and Liu, H.Y. (1984) Tomato necrotic dwarf - a new type of whitefly-transmitted virus. *Phytopathology* 74:795.

Submitted for publication

Cohen, S., Duffus, J.E. and Liu, H.Y. Acquisition, interference and retention of cucurbit leaf curl viruses in whiteflies. Submitted to Phytopathology in 1987.

Cohen, S., Duffus, J.E., Perry, R. and Dawson, R. A collection and marking system suitable for epidemiological studies of whitefly-borne viruses. Submitted to Plant Disease in 1987.

Czosnek, H., Ber, R., Antignus, I., Cohen, S., Navot, N. and Zamir, D. (1987) Isolation of the tomato yellow leaf curl virus, a geminivirus. *Phytopathology* (accepted).