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Acquisition of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci*

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Tomato yellow leaf curl virus (TYLCV) genomic DNA can be detected by Southern blot analysis in nucleic acid extracted from a single whitefly. Acquisition of TYLCV by individual whiteflies in relation to the length of the access period, the virus concentration in, and the developmental stage of plant tissues was studied. The frequency of TYLCV detection increased with the length of the access period; DNA was detected in 15% of whiteflies tested after a period of access to infected tissue of 30 min, regardless of whether it had a high or a low virus content (5 ng or 0·05 ng TYLCV DNA/μg plant chromosomal DNA), and in all insects tested after an 8 h period of access to all the plants. Those insects which had access to the youngest leaves of source plants, which have a high virus content, acquired detectable TYLCV DNA within 2 h. Insects which had access to a tissue for the same period acquired variable amounts of TYLCV DNA; insects feeding on plants with a low virus concentration acquired amounts of viral DNA comparable to those acquired by insects feeding on plants containing a 100-fold greater concentration of virus. Viruliferous insects retained TYLCV DNA for at least 13 days when placed on uninfected tomato plants. In these tests, whitefly could not acquire more than 600 million virus genomes (1 ng viral DNA), suggesting the existence of factors controlling the number of virions present in an insect.

Introduction

Whiteflies are pests which affect ornamental and agricultural plants, both in greenhouses and outdoors, and are vectors of many viruses (Costa, 1976; Bird & Maramorosch, 1978; Gerling, 1990). The tobacco whitefly (*Bemisia tabaci* Genn.) is the insect vector of tomato yellow leaf curl virus (TYLCV), a geminivirus with a single genomic component (Cohen & Harpaz, 1964; Czosnek *et al.*, 1988*a*; Navot *et al.*, 1991). TYLCV affects tomato crops (*Lycopersicon esculentum* Mill.) in the Middle East and many other tropical and subtropical regions (Czosnek *et al.*, 1990). Understanding the epidemiology of TYLCV may help to establish efficient control measures and improve procedures for breeding virus-resistant culitvars.

We recently initiated a study of plant–virus–vector relationships using cloned TYLCV DNA probes. Analysing the infection process, we have found that young leaves and apices are the best target for whitefly-mediated infection (Ber *et al.*, 1990). In these tissues, the viral DNA replicates at the site of inoculation and is transported first to the roots then to the shoot apex, and finally to the neighbouring leaves and flowers. Inoculation of the oldest leaves and cotyledons is inefficient.

We are now examining the characteristics of the acquisition of TYLCV by its vector. For many years, bioassays have been the only tool available for the study of the acquisition of TYLCV by whiteflies. Using whitefly-mediated transmission assays, it has been shown that *B. tabaci* can acquire enough virus during a period of access to an infected plant of 30 min to transmit the disease to about 30% of test plants; access periods of 15 min were inefficient. Access periods of 4 h or greater increased the efficiency of disease transmission, 90% of test plants being affected (Cohen & Nitzany, 1966).

With the development of ELISA and molecular hybridization techniques, it is now possible to detect viral molecules in individual insects (Czosnek *et al.*, 1988*b*; Navot *et al.*, 1989; Polston *et al.*, 1990). The acquisition of the squash leaf curl geminivirus (SLCV) by its whitefly vector has been studied using DNA probes and antibodies (Polston *et al.*, 1990). It has been shown that the frequency of detection of SCLV DNA in single whiteflies increases with the length of the access period until saturation is reached at 96 h, when viral DNA is detectable in about 60% of the insects. The detection rate is dependent on the developmental stage of the insect, but not on its sex. The efficiency of detection of SLCV coat protein by ELISA is lower than that of the SLCV nucleic acid (Polston *et al.*, 1990).

We report the acquisition of TYLCV by individual whiteflies in relation to the length of the access period, and the virus concentration in and developmental stage of the source tissue.

Methods

Maintenance of virus cultures, whiteflies and plants. Whiteflies (*B. tabaci*) were reared on cotton plants (*Gossypium hirsutum* L. cv. Akala) grown in insect-proof wooden cages at 30 °C in a controlled temperature room provided with 16 h illumination per day. Virus cultures were maintained in tomato plants (*L. esculentum* Mill., cv. M82); uninfected tomato plants (*L. esculentum* cv. M82) were grown in an insect-proof growth chamber.

Whitefly-mediated inoculation. Whiteflies which had had access to a TYLCV-infected tomato plant for 48 h were placed on uninfected tomato plants at the two-leaf stage for an additional 48 h (about 10 insects per plant). The plants were then sprayed with 0·3% Senprotathrin (Smash) and grown in insect-proof cages.

Acquisition of TYLCV by whiteflies. Adult female whiteflies were given access to TYLCV-infected plants at the four-leaf stage, each plant being in a separate insect-proof cage. After various acquisition access periods (as described in Results), the insects were collected individually by mouth aspiration using a plastic tip terminated with a cotton plug mounted on a rubber tube. The insects were stored at −20 °C until further processing.

Isolation of nucleic acids from whiteflies and plants. Each whitefly was placed in an Eppendorf tube containing 100 µl of 0·4% SDS, 100 µg/ml proteinase K and ground using a glass rod. Following a 1 h incubation at 55 °C the mixture was treated twice with phenol–chloroform–isoamyl alcohol (25 : 24 : 1) and the nucleic acid extract was immediately subjected to gel electrophoresis. Crude DNA extracts of tomato leaf (lysates) were prepared as described previously (Czosnek *et al.*, 1988*b*).

Detection of TYLCV DNA. A full-length TYLCV genomic DNA clone homologous to DNA A of other whitefly-transmitted geminiviruses (Lazarowitz, 1987) was obtained as previously described (Navot *et al.*, 1989, 1991) and used as a probe. Nucleic acids from individual whiteflies and leaf lysates (containing 0·5 µg tomato chromosomal DNA, estimated according to the method of Czosnek *et al.*, 1988*b*) were separated by gel electrophoresis in a 1% agarose gel containing 0·5 µg/ml ethidium bromide in TPE buffer (90 mM-Tris–phosphate, 2 mM-EDTA). After partial depurination in 0·25 M-HCl (15 min) and denaturation in 0·5 M-NaOH, 1·5 M-NaCl (20 min), the DNA samples were vacuum-blotted onto Hybond-N membranes (Amersham) and hybridized by the method of Carmon *et al.* (1982) to a probe radiolabelled with [α-^{32}P]dCTP (Amersham) (specific radioactivity about 10^8 c.p.m./µg DNA) by nick translation (Rigby *et al.*, 1977). Blots were washed twice at 65 °C for 30 min each in 150 mM-NaCl and 15 mM-trisodium citrate (1 × SSC), and exposed to pre-flashed Fuji films for 48 h at −80 °C using two intensifying screens. To quantify TYLCV DNA in insects, blots of whitefly DNA were hybridized together with blots of cloned TYLCV standards. Autoradiograms were scanned using a Computing Densitometer (Model 300 A; Molecular Dynamics).

Results

Detection of TYLCV DNA in individual whiteflies

When nucleic acids from a single viruliferous whitefly were subjected to electrophoresis, the insect chromosomal DNA migrated as a single ethidium bromide-stained band near the origin of the gel (Fig. 1, lane 1). After blotting and hybridization with the TYLCV-specific probe, a single band comigrating with the TYLCV genomic DNA present in infected plant tissue was detected by autoradiography (Fig. 1, lanes 2 and 3).

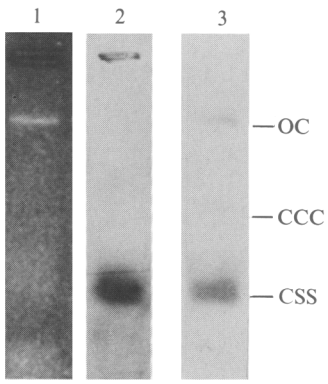

Fig. 1. Autoradiographic detection of TYLCV DNA in a whitefly following a 12 h period of access to a TYLCV-infected tomato plant. Lane 1, nucleic acid extract after electrophoresis in an ethidium bromide-containing agarose gel. Lane 2, Southern blot of DNA in lane 1 showing hybridization to the TYLCV probe. Lane 3, Southern blot of a TYLCV-infected tomato leaf DNA extract hybridized with the TYLCV probe. CSS, covalently closed circular single-stranded TYLCV genomic DNA; CCC and OC, covalently closed circular (supercoiled) and open circular double-stranded TYLCV-related DNA (intermediate replicative form).

Effect of the length of the access period and plant tissue used on the efficiency of TYLCV detection

Two plants with clear symptoms (A and B) and two plants with very mild symptoms (C and D) were chosen from a batch of tomato plants infected at the same time as the virus source. About 350 insects were placed on each plant, ensuring that the insect population was evenly distributed among the four leaves and five whiteflies from each leaf were removed 0·5, 1, 1·5, 2, 3, 4, 6, 8, 10 and 12 h later, being careful not to disturb the other insects. The 880 insects collected were assayed individually for detectable TYLCV DNA as described in Fig. 1. The amount of TYLCV DNA in each insect was estimated using cloned TYLCV DNA as a standard (Fig. 2).

Immediately after the last group of whiteflies had been collected, the amount of TYLCV DNA in each plant leaf was estimated by hybridization (Fig. 3). The leaves of plants A and B contained about 100-fold more TYLCV genomic DNA than those of plants C and D (about 5 ng compared to 0·05 ng/µg plant chromosomal DNA). In plants C and D the amount of viral DNA in the youngest leaf (L1) was about fivefold that in the older leaves (L2 to L4). The ratio of TYLCV genomic DNA (CSS) to its

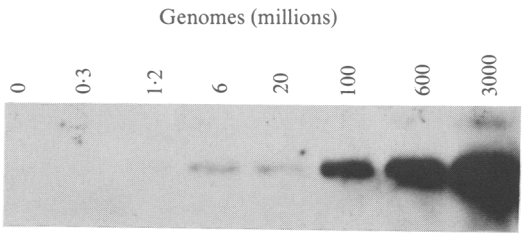

Fig. 2. Cloned TYLCV DNA standards. Full-length TYLCV DNA was excised from its cloning vector and amounts of DNA corresponding to the numbers of TYLCV genomes indicated (1 pg DNA is equivalent to 600000 genomes) were analysed by Southern blotting and hybridized with the TYLCV DNA probe.

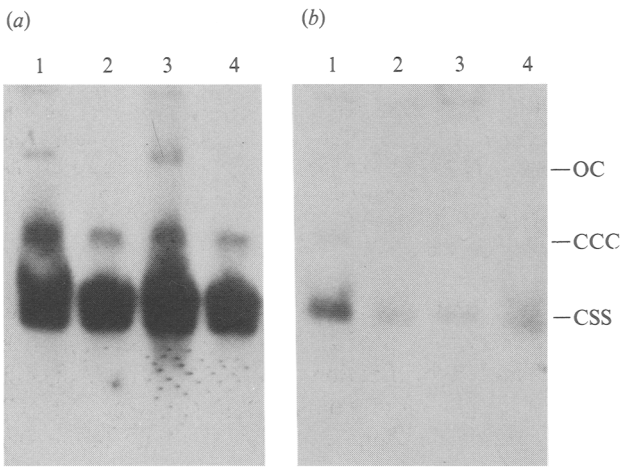

Fig. 3. TYLCV DNA in the tomato plants used as the virus source for whitefly acquisition feeding. DNA extracts from each leaf were analysed by Southern blotting and hybridized with the TYLCV DNA probe. Plant A (*a*) showed severe symptoms and plant C (*b*) showed mild symptoms. Each lane contains a different leaf extract, L1 to L4 (lanes 1 to 4); leaves were numbered from the apex downwards (the sample from the youngest leaf included the shoot apex).

replicative forms (CCC and OC) was about 50:1 in plants A and B, but only 2:1 to 5:1 in plants C and D, confirming that the disease was in a more advanced stage in plants A and B than in plants C and D.

The insects collected after the various access periods were scored for the presence of detectable viral DNA. Table 1 shows the effect of the length of access period and feeding tissue on the rate of TYLCV DNA detection (number of insects with a detectable amount of TYLCV DNA/number of insects tested). TYLCV detection rates in insects feeding on plants C and D were similar to those in insects feeding on plants A and B, despite the difference in virus concentration. Viral DNA was detected in insects after 30 min of access to infected plants; 12% of the insects that fed on plants A and B (five/40) and 17% of the insects that fed on plants C and D (seven/40) contained detectable TYLCV DNA. The frequency of detection increased with the length of the

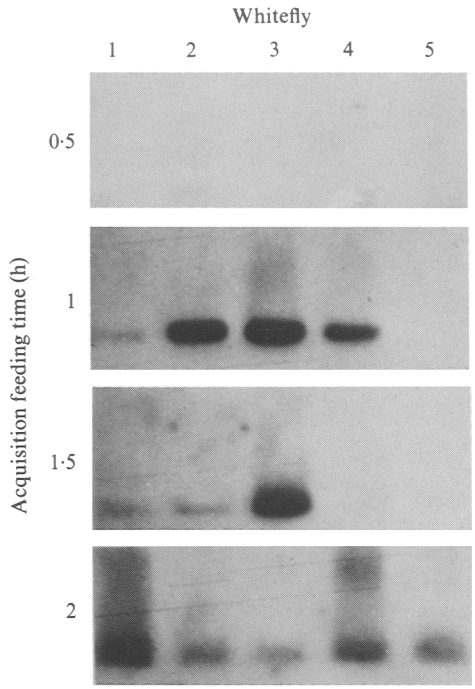

Fig. 4. TYLCV DNA associated with whiteflies after feeding on the youngest leaf (L1) of plant A. Five insects (numbered 1 to 5) were collected after 0·5, 1, 1·5 and 2 h access and were analysed for the presence of TYLCV DNA by Southern blotting.

access period. TYLCV DNA was detectable in all insects after 8 h of access to all infected plants. Only those insects which had access to the youngest leaf (L1) of plants A and B all acquired detectable TYLCV DNA within 2 h.

The amount of TYLCV DNA acquired by whiteflies during identical periods of access to the same virus source

Insects that had access to the same tissue for the same period of time acquired extremely variable amounts of TYLCV DNA. Fig. 4 shows the viral DNA associated with whiteflies that had access to the youngest leaf (L1) of plant A. After 30 min of feeding, TYLCV DNA was detected in one of the five whiteflies assayed. This insect (no. 2) had acquired about 1 million viral genomes, a value close to the limits of detection of the assay (1 pg of viral DNA is equivalent to 600000 TYLCV genomes). After 1 h of feeding, TYLCV DNA could be detected in four of the five insects tested; insect no. 1 had acquired about eight million, no. 4 about 60 million and the other two about 200 million and 400 million TYLCV genomes. After 1·5 h of feeding, viral DNA was detectable in all the insects tested. Insects no. 4 and 5 had acquired about 1 million, no. 2 and 1 about 8 and 40 million, and no. 3 close to 500 million TYLCV genomes. After 2 h of feeding, viral DNA was detected in all five insects tested,

Table 1. *Effect of the length of the access period and the feeding tissue on TYLCV DNA detection*

Source tissue			Insects with detectable TYLCV DNA Acquisition feeding time (h)										
Virus	Plant	Leaf*	0	0·5	1	1·5	2	3	4	6	8	10	12
High†	A	L1	0‡	1	4	5	5	5	5	5	5	5	5
		L2	0	0	0	1	3	3	3	5	5	5	5
		L3	0	0	0	1	3	2	3	5	5	5	5
		L4	0	0	1	1	3	3	2	4	5	5	5
	B	L1	0	1	3	4	5	5	5	4	5	5	5
		L2	0	1	1	3	3	3	2	4	5	5	5
		L3	0	0	1	1	3	2	4	4	5	5	5
		L4	0	2	1	0	3	4	4	4	5	5	5
Low§	C	L1	0	1	1	2	1	3	3	3	5	5	5
		L2	0	2	1	3	2	4	3	2	5	5	5
		L3	0	1	1	0	2	3	2	4	5	5	5
		L4	0	0	1	2	2	2	2	1	5	5	5
	D	L1	0	2	0	2	1	2	3	3	5	5	5
		L2	0	1	1	1	1	1	4	3	5	5	5
		L3	0	0	0	2	2	2	2	2	5	5	5
		L4	0	0	4	1	2	3	2	2	5	5	5

* L1 to L4, leaves numbered from the youngest (L1) to the oldest (L4).
† About 5 ng TYLCV DNA/μg plant chromosomal DNA.
‡ Each result shows the number of insects with detectable TYLCV DNA of a total of five insects sampled.
§ About 0·05 ng TYLCV DNA/μg plant chromosomal DNA.

each one having acquired between 50 (no. 3) and 400 (no. 1) million TYLCV genomes. The insects feeding on the other leaves of plant A acquired varying amounts of TYLCV DNA (not shown). Similar results were found when insects had access to the other source plants (not shown).

The amount of TYLCV DNA acquired by whiteflies during identical periods of access to tissues with high or low virus content

During the same access period, whiteflies feeding on tissues with low virus content acquired amounts of TYLCV DNA similar to those acquired by insects feeding on tissues with high virus content. Fig. 5 shows the TYLCV DNA from insects following a period of access to plants A and C of 6 h. All insects which had access to plant A except one (no. 4 on L4) acquired detectable amounts of viral DNA, ranging from 2 million (no. 3 on L4) to 600 million viral genomes (no. 5 on L1). Although TYLCV DNA was detected in only half of the whiteflies feeding on plant C, these insects acquired amounts of TYLCV DNA similar to those acquired by insects feeding on plant A (from about 50 million to 400 million TYLCV genomes).

The 880 insects tested during the acquisition experiment were divided into four groups based on the amount of TYLCV DNA they acquired: group 1, DNA undetectable; 2, from 600000 (limit of detection) to 20 million viral genomes; 3, from 20 million to 100 million viral genomes; 4, more than 100 million viral genomes. Fig. 6 shows the distribution of the insects between the four groups and the variation in the numbers in each group with the differing periods of access to the two groups of source plants (A and B, and C and D); no differentiation was made between the leaves. The number of insects with undetectable viral DNA decreased with increasing access periods. TYLCV DNA was undetectable in 50% of insects tested after a 2 h access period; after 8 h, all the insects had acquired detectable TYLCV DNA. The number of insects in each group reached a maximum level depending on the length of the access period. After 3 h, more than one-third of the insects tested had acquired between 600000 and 20 million TYLCV genomes, whereas TYLCV DNA remained undetectable in 40% of the insects. After 8 h, close to 60% of the whiteflies had acquired between 20 and 100 million viral genomes; after 12 h, 90% of the insects had acquired more than 100 million viral genomes, with many of them having as many as 600 million TYLCV genomes. Plants C and D were as good a source of virus as plants A and B, which contained 100-fold the amount of viral DNA (Fig. 6).

The maximum amount of TYLCV acquired by whiteflies

Autoradiographic analysis of viral DNA in insects indicated that whiteflies acquired up to 600 million

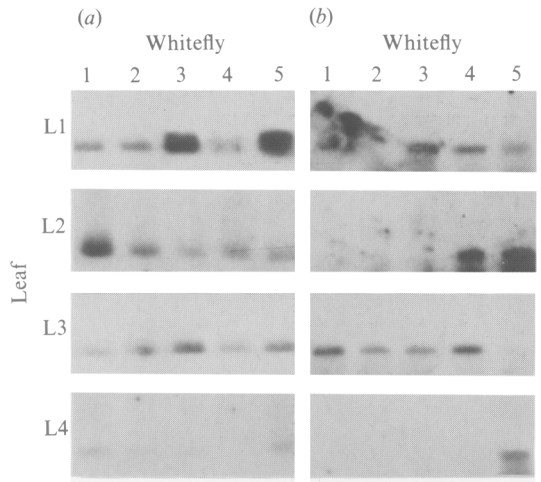

Fig. 5. TYLCV DNA associated with whiteflies after a 6 h period of access to plants A (*a*) and C (*b*). Five insects (numbered 1 to 5) were collected from each leaf and analysed for their TYLCV DNA content by Southern blotting.

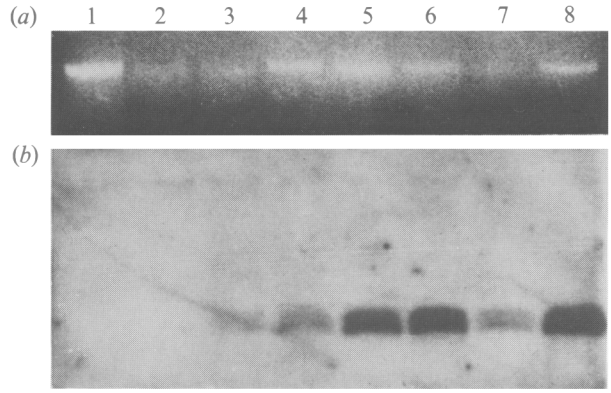

Fig. 7. Acquisition of TYLCV DNA during 48 h of access feeding. Whiteflies were placed on a TYLCV-infected tomato plant and groups of 20 (five insects from each leaf) were collected after 0, 1, 4, 8, 16, 24, 36 and 48 h (lanes 1 to 8). DNA was extracted and one-twentieth was analysed by Southern blotting and hybridized with the TYLCV DNA probe. (*a*) Insect chromosomal DNA in an ethidium bromide-stained gel; (*b*) autoradiographic detection of TYLCV DNA.

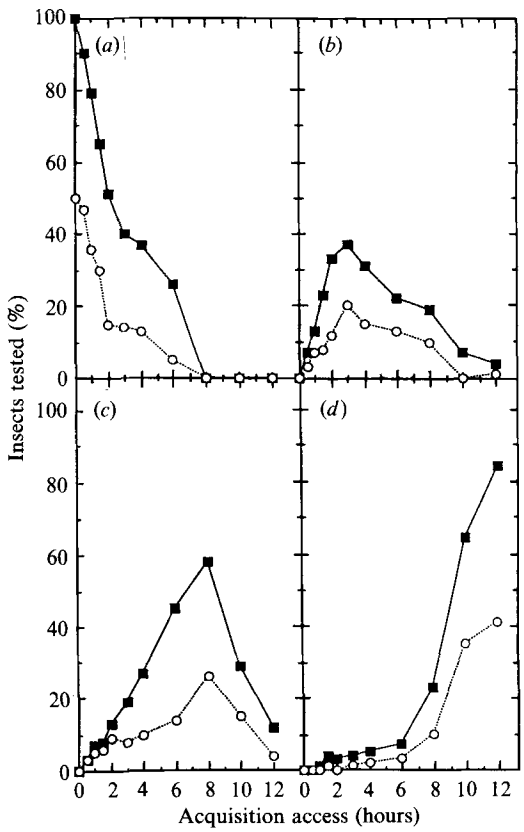

Fig. 6. Variation in the amount of TYLCV DNA associated with whiteflies after various periods of access to infected tomato plants. Five insects were collected from each leaf of plants A, B, C and D at 0·5, 1, 1·5, 2, 3, 4, 6, 8, 10 and 12 h after they had been placed on these plants. TYLCV DNA associated with each insect was quantified after Southern blot analysis (see also Table 1). The insects were divided into four groups according to the amount of TYLCV DNA they had acquired (see Results). (*a*) Group 1, (*b*) group 2, (*c*) group 3 and (*d*) group 4. ■, Insects that fed on the leaves of all four plants; ○, insects that fed on the leaves of plants C and D only.

TYLCV genomes. To investigate whether a whitefly can acquire a finite number of virus particles, insects had access to an infected plant for periods up to 48 h.

About 200 whiteflies were placed on a TYLCV-infected tomato plant at its four-leaf stage. Care was taken that approximately the same number of insects was placed on each leaf. Groups of 20 whiteflies were removed 1, 4, 8, 16, 24, 36 and 48 h later; each group included five insects collected from each leaf. Nucleic acid was extracted and one-twentieth (equivalent to one insect) was analysed by Southern blotting (Fig. 7). Viral DNA was detected in the pool of insects collected after 4 h of feeding, but not in that collected after 1 h. The amount of viral DNA acquired by insects increased with the length of the access period, levelled off after 8 to 16 h, reaching quantities equivalent to approximately 600 million viral genomes (about 1 ng viral DNA), and did not increase further when insects had access to the infected plant for more than 16 h (standardised to equal amounts of insect chromosomal DNA).

Retention of TYLCV DNA by whiteflies

Whiteflies which had access to a TYLCV-infected tomato plant for 12 h were placed on an uninfected tomato plant and collected 1, 2, 4, 8, 12, 18, 24, 36, 48, 72, 96, 120, 168, 240 and 312 h later. At each time, five insects were removed from the feeding population (one from each leaf and the stem). Fig. 8 shows the TYLCV DNA in two of the five insects collected at each time. TYLCV DNA was detected in almost all the insects during the 13 days of the experiment. The amount of TYLCV DNA started to decrease 2 to 12 h after the insects were placed on the tomato plant, followed by an

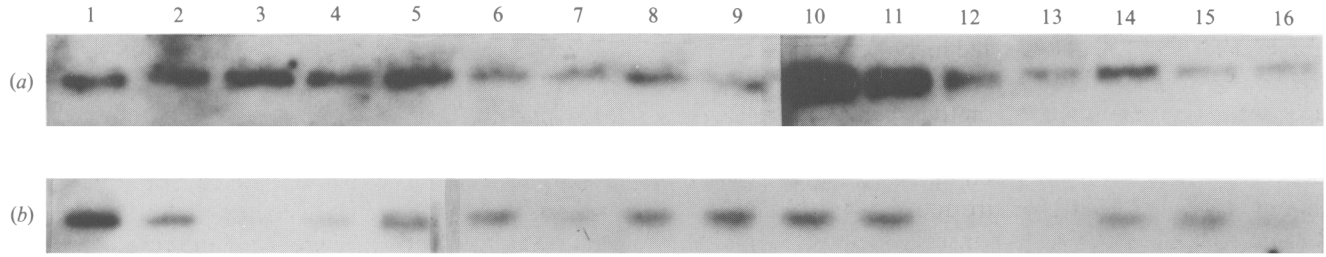

Fig. 8. Retention of TYLCV DNA over a period of 13 days (312 h). Whiteflies that had access to a TYLCV-infected tomato plant for 12 h were placed on an uninfected tomato plant. Five insects were collected after 0, 1, 2, 4, 8, 12, 18, 24, 36, 48, 72, 96, 120, 168, 240 and 312 h (lanes 1 to 16) (one from each leaf and the stem). DNA in each insect was analysed by Southern blotting. Rows (*a*) and (*b*), autoradiographic detection of TYLCV in each of two of the five insects collected.

increase observed 6 to 36 h later. Additional variations in the amount of TYLCV DNA with no obvious relationship to the infection cycle were observed. Similar variations were observed in the five sets of insects. During the time the viruliferous insects had access to the tomato plant, inoculation took place and virus DNA was detected in leaves 7 days after the beginning of the experiment (not shown). Disease symptoms appeared 2 weeks later.

Discussion

Nucleic acid can be extracted from a single whitefly and, using known amounts of DNA, we estimate that a whitefly contains about 50 ng of chromosomal DNA. The integrity of insect DNA preparations was always assessed by examining its chromosomal DNA. Prolonged storage of insects at −20 °C did not affect this parameter.

TYLCV DNA can be detected in an individual whitefly. The viral DNA associated with the insect appears as a unique DNA species, comigrating with the viral genomic ssDNA present in infected plants, and with virion DNA (Czosnek *et al.*, 1989). No virus-related dsDNA, the putative TYLCV DNA replicative form, could be detected in the whitefly when either cloned TYLCV dsDNA or cloned virus positive-sense DNA was used as a probe, indicating that TYLCV does not replicate in its insect vector.

Whiteflies placed on tomato leaves remain still for at least 24 h; sampling does not disturb feeding insects. These observations allowed us to study the influence of the feeding tissue on the acquisition of TYLCV without having to use leaf cages (Ber *et al.*, 1990). Two plants with clear symptoms (A and B) and two plants with mild symptoms (C and D) were chosen as the virus source. Plants A and B contained at least 100-fold the amount of viral DNA of plants C and D.

Generally, the frequency of TYLCV detection in individual insects increases with the length of the access period until viral DNA is detected in all insects tested. TYLCV DNA was detected in 15% of the insects tested as early as 30 min after access to the infected tissue, irrespective of the source plant tissue; this might represent the minimum time required for the insect stylet to penetrate the leaf epidermis, contour the parenchyma cells, reach the TYLCV-rich phloem cells (Pollard, 1955) and acquire enough virus to be able to transmit the disease efficiently (Cohen & Nitzany, 1966).

Although the whitefly *B. tabaci* is the vector of many geminiviruses, TYLCV is detected much earlier than SLCV (Polston *et al.*, 1990); TYLCV DNA is detectable in 50% of the insects within 2 h of access to infected tomato plants, whereas 48 h is necessary to achieve this detection rate in insects feeding on SLCV-infected bean plants. Although all insects acquired TYLCV DNA within 8 h, the frequency of detection of SLCV reaches its optimum after 120 h, when SLCV DNA is detected in only 60% of whiteflies tested (Polston *et al.*, 1990). The differences observed in the rate of TYLCV and SLCV acquisition by the same insect vector might be due to the texture of the source tissue, the distribution of virions in the infected plant, or the feeding habit of the insect on tomato and bean plants. The fact that SLCV has a bipartite genome (Lazarowitz, 1991) and TYLCV a monopartite genome (Navot *et al.*, 1991) does not seem to be the cause of the observed difference in their pattern of acquisition. In the infected plant, SLCV DNAs A and B are represented equally (Lazarowitz, 1991). Therefore, the SLCV gene products which have the potential to play a role in recognition of the virus by the insect should be present in plants in sufficient amounts to ensure efficient virus acquisition.

The developmental stage and the virus content of the feeding tissues influence the efficiency of TYLCV DNA detection. The frequency of TYLCV detection increases with time, but this increase is less regular when insects

fed on plants C and D than when they fed on plants A and B. TYLCV DNA is detected in all insects feeding on the youngest leaf of plants A and B after 2 h, but only after 8 h when the insects fed on the other leaves of these plants, or on the leaves of plants C and D. With equal periods of access, insects feeding on plants with low amounts of TYLCV can acquire quantities of viral DNA similar to those acquired by insects feeding on plants containing 100-fold the amount of virus. These observations might reflect variations in virus concentration in the cells from which insects feed. It is possible that individual cells in TYLCV source tissues contain either many or few virions, as shown for abutilon mosaic virus-infected tissues (Horns & Jeske, 1991). According to this hypothesis, plants at an early stage of infection would have relatively few cells with a high virus content and give a weak hybridization signal. Plants showing severe symptoms would contain a larger proportion of cells of high virus content and give a strong hybridization signal. The amount of virus acquired by an insect will depend on the proportion of cells with high virus content in the feeding tissue and on the virus content of the cell(s) the insect feeds from. The combination of a thin epidermis with a high concentration of virus-rich feeder cells may explain why insects feeding on the youngest leaf of plants A and B acquire virus more rapidly than those feeding on other tissues.

The acquisition of TYLCV is not just a passive ingestion of virus-containing cell sap. The concentration of TYLCV in the body of an insect is at least 1000-fold that in the plant sap from which it feeds (Navot *et al.*, 1989). However, it seems that there is a limit to the amount of TYLCV DNA one insect can accumulate which is in the range of 600 million genomes (1 ng DNA); this limit can be approached after feeding for 1 to 2 h. Since the genome makes up 20% of the virus mass (Harrison, 1985), an insect can acquire about 5 ng of virus, about 0·015% of its body weight (30 μg) (Byrne *et al.*, 1988). A similar limit seems to exist for the acquisition of SLCV (Polston *et al.*, 1990), strongly suggesting the existence of a mechanism(s) regulating the number of virions associated with an insect. It is possible that *B. tabaci* possesses receptors for geminiviruses which can be saturated or that antiviral factors produced in the viruliferous whitefly (Cohen, 1969; Cohen & Marco, 1970) play a role in controlling the number of virions in the insect.

Other whitefly species, *Trialeurodes abutilonea* and *T. vaporariorum*, are able to acquire SLCV (Cohen *et al.*, 1989; Polston *et al.*, 1990), indicating that these insects may also have receptors for geminiviruses. However, because they are not able to transmit SLCV (or TYLCV) disease, virus acquisition does not imply insect infectivity. It should be noted that TYLCV is transmissible by *B. tabaci* only during the first 10 days after acquisition (Cohen & Nitzany, 1966). It is possible that the factor(s) which prevent virus transmission by *Trialeurodes* species are similar to those which are induced in *B. tabaci* following TYLCV acquisition.

Although TYLCV DNA is detectable in all insects following 8 h of access feeding, whiteflies are not immediately infective. There is an interval of at least 24 h between the start of insect feeding and the start of its ability to transmit TYLCV efficiently (Cohen & Nitzany, 1966); the latent period of SLCV is 8 h (Cohen *et al.*, 1983).

Viruliferous insects retain TYLCV DNA for many days when placed on uninfected tomato (13 days in this experiment). During the first hours of access to the plant, we observed a decrease in the amount of TYLCV DNA associated with the insects, followed by an increase. These variations might be associated with the process of inoculation feeding. Virus replication can be detected at the site of inoculation within 7 days and spreads to other tissues 11 to 13 days post-inoculation (Ber *et al.*, 1990). Between these times an equilibrium (steady state) between virus loss by inoculation feeding and its gain by acquisition feeding might be reached; similar conditions might occur in the field. Whiteflies fed on SLCV-infected plants also retain SLCV DNA for many days after they have been placed on cotton, a non-host plant, the amount of virus DNA remaining approximately constant (Polston *et al.*, 1990). These observations imply that under field conditions whiteflies feeding on infected plants can carry geminiviruses for their entire adult life (25 to 50 days; Gerling, 1990), and this may reduce their life expectancy (Cohen *et al.*, 1983).

Recognition and understanding of the factors involved in the host–virus–vector relationship may lead to efficient control of TYLCV disease and the breeding of resistant cultivars (Cohen, 1990).

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