Acquisition and Transmission of *Agrobacterium* by the Whitefly *Bemisia tabaci*

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Whiteflies transmit many different plant pathogens. We show here that the whitefly *Bemisia tabaci* can also acquire *Agrobacterium tumefaciens* from liquid cultures and from crown galls, and transmit it to plants. Cloned tomato yellow leaf curl virus (TYLCV) DNA and *A. tumefaciens* tumor-inducing functions were used as reporter genes. Whiteflies were fed through membranes on cultures of *Agrobacterium* At::pTY4 containing a dimeric copy of an infectious TYLCV genomic clone between the T-DNA borders of a binary vector. One hour of feeding was sufficient for the insects to be able to transmit TYLCV to tomato test plants. Infectious *Agrobacterium* was recovered from whiteflies that fed on At::pTY4 cultures, indicating that bacteria was acquired and remained intact in the insects. Whiteflies also acquired the virulent *A. tumefaciens* strain C58 from crown galls and induced tumors in tomato test plants. PCR and Southern blot analyses indicated that the target plant tissues were transformed. These results show that an insect can transfer foreign genes to plants by acquiring and delivering transforming bacteria.

Whiteflies are vectors of numerous pathogens causing important damage to ornamental and agricultural plants (Costa 1976; Bird and Maramorosch 1978; Muniyappa 1980; Duffus 1987). These insects are spreading into new geographic areas, attacking previously unaffected plants species and developing new, more devastating, biotypes (Bedford *et al.* 1992; Perring *et al.* 1993). Although the biology and epidemiology of whiteflies have been studied extensively (Byrne and Bellows 1991; Gerling 1990), the way these insects acquire and transmit pathogens is not well understood.

Whiteflies transmit numerous disease agents, including viruses, fungi, and bacteria (Costa 1976). The interaction between whiteflies and bacteria or fungi has been much less studied than that of whiteflies and viruses, because of a lesser impact as agricultural pests. At least three species of whiteflies transmit viruses from seven groups: geminiviruses, closteroviruses, carlaviruses, potyviruses, nepoviruses, luteoviruses, and an unclassified DNA-containing rod-shaped virus (Duffus 1987). Transmission of viruses by whiteflies range from non-persistent to semi-persistent and persistent (Duffus 1987). The best studied group is the geminiviruses and the most studied vector is the tobacco whitefly, *Bemisia tabaci* Gennadius. Only geminiviruses are transmitted in a persistent-circulative way by the insect, i.e., they can be maintained and transmitted by the vector for several days to life. Acquisition and retention of two *B. tabaci*-transmitted geminiviruses have been studied using molecular tools: squash leaf curl virus (SLCV; Polston *et al.* 1990) and tomato yellow leaf curl virus (TYLCV; Zeidan and Czosnek 1991).

We have shown previously that the whitefly, *B. tabaci*, can feed through membranes on sucrose solutions containing TYLCV particles and transmit the TYLCV disease to test plants (Czosnek *et al.* 1988a). In this paper we report that the whitefly *B. tabaci* can also acquire genetic information from cultures of *Agrobacterium* and from crown galls, and transmit this information to test plants. The TYLCV genome and the *A. tumefaciens* Ti plasmid tumor-inducing functions were used as reporter genes. This study may help to extend our knowledge of the variety of interactions between whiteflies and pathogens.

RESULTS

Acquisition and transmission of TYLCV by whiteflies fed through membranes on *Agrobacterium* cultures containing a dimeric copy of an infectious TYLCV genomic clone.

The ability of whiteflies to acquire *Agrobacterium tumefaciens* was investigated by feeding insects through membranes with bacterial cultures. Nine groups of whiteflies (15 insects per group, each group in a separate tube) were fed on *A. tumefaciens* (At::pTY4) containing a dimeric copy of an infectious TYLCV genomic clone in the binary vector pCGN1547 (plasmid pTY4; Navot *et al.* 1991). After 18 hr of access feeding, the presence in the insect body of TYLCV DNA carried by the *Agrobacterium* binary vector was determined by Southern blot analysis. Total DNA was extracted from 10 whiteflies from each group, and one-tenth as subjected to gel electrophoresis and hybridized with a full-length genomic TYLCV DNA probe (Fig. 1). In seven of the nine groups of insects (lanes 1–5, 7 and 8), the probe reacted solely with a discrete doublet band of DNA comigrating with plasmid pTY4 (lane Ti), indicating that this plasmid was apparently not degraded in the insect body. The binary vector was not detected in insects which fed through membranes on sucrose solutions alone or on cotton plants (lanes 10, 11). Insects feeding for the same time period on the same *Agrobacterium* culture acquired variable amounts of bacteria (lane 2 vs. lane

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7). This is similar to our previous observations showing that whiteflies feeding for the same time period on the same TYLCV-infected tomato leaf acquire different amounts of virions (Zeidan and Czosnek 1991).

The amount of *Agrobacterium* acquired by whiteflies and the efficiency with which *Agrobacterium* DNA was extracted from the insects was estimated using reconstitution experiments (Fig. 2). DNA from a single whitefly was mixed with 5, 12, or 50 pg plasmid pTY4 DNA (lanes 1′–3′). In parallel, a single whitefly was mixed with 1, 2.5, or 10 µl of an *Agrobacterium* At::pTY4 culture (at the concentration of 1 OD_{600nm}/ml, equivalent to 5×10^8 cells/ml) and the DNA was extracted from the mixtures (lanes 1–3). The various DNA samples were subjected to gel electrophoresis, together with 50 ng of DNA from *Agrobacterium* strains LBA4404 (lane At) and At::pTY4 (lane At′), and with the DNA equivalent of one whitefly membrane-fed for 24 hr on *Agrobacterium* At::pTY4 (lane Wf). The DNA samples were hybridized with a full-length genomic TYLCV DNA probe (panel A) and with *Agrobacterium* LBA4404 DNA (panel B). The TYLCV probe reacted with a doublet band of DNA (as in Fig. 1) conspicuous in the samples that contained mixtures of whitefly and pTY4 DNA, DNA extracted from mixtures of whitefly and *Agrobacterium* At::pTY4, DNA from *Agrobacterium* At::pTY4, and DNA from insects membrane-fed on a culture of *Agrobacterium* At::pTY4 (but not DNA from *Agrobacterium* LBA4404). The LBA4404 DNA probe reacted with a single band of bacterial chromosomal DNA in all samples containing DNA from *Agrobacterium* strains At::pTY4 and LBA4404, including the insects membrane-fed on *Agrobacterium* At::pTY4. The reconstitution experiment indicated that the extraction of bacterial DNA from mixtures of insects and bacteria and from insects membrane-fed on *Agrobacterium* cultures was efficient. By comparing the intensity of the hybridization signals (lanes 1–3, 1′–3′, and Wf), and by taking into account that there are less than 10 copies of binary plasmid pTY4 per bacterial cell, we estimated that the amount of *Agrobacterium* At::pTY4 associated with a single whitefly (lane Wf) was in the range of 10^5 cells per insect. *Agrobacterium* At::pTY4 was apparently intact in the insect body since both its chromosomal DNA and its plasmid pTY4 (see also Fig. 1) were apparently not degraded in whiteflies membrane-fed with the *Agrobacterium* culture.

The time-course of *Agrobacterium* acquisition and transmission was investigated. Whiteflies were fed for 1, 2, 4, 8, 12, and 24 hr on At::pTY4. One group of 15 insects per tube was used for each feeding period. After feeding, total DNA was extracted from 10 whiteflies per group and one tenth was used for PCR analysis (Fig. 3). TYLCV DNA could be amplified from the DNA of insects after 1 hr of feeding on At::pTY4 (lane 3), yielding a 410-bp amplification product (panel A). A PCR product of similar size was obtained with DNA samples from whiteflies fed on a TYLCV-infected tomato plant (lane 10) and from a TYLCV-infected tomato (lane 9). TYLCV DNA was not amplified from the DNA of insects which fed on *Agrobacterium* At::pPCV002 (lane 2), which did not contain TYLCV DNA sequences (Koncz and Schell 1986), or on sucrose solutions alone (lane 1). The viral origin of the amplified DNAs was confirmed after hybridization with a full-length TYLCV genomic clone (panel B). Both the PCR-amplified DNA (lanes 3–10) and the viral-related DNA forms (genomic ssDNA and replicative dsDNA forms) from the infected plant (lane 9) hybridized with the cloned viral DNA probe.

The ability of whiteflies to transmit TYLCV to tomato test plants after feeding through membranes on cultures of *Agro-*

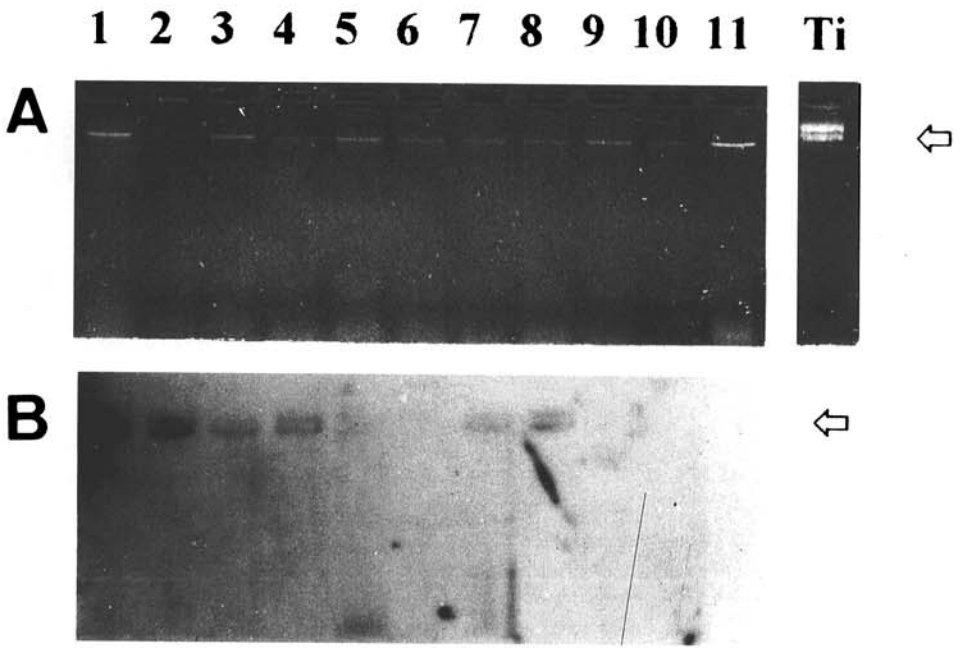

Fig. 1. Autoradiographic detection of TYLCV DNA in whiteflies fed for 18 hr through membranes with *Agrobacterium tumefaciens* At::pTY4, containing a dimeric copy of an infectious TYLCV genomic clone. Each lane represents one tenth of a DNA preparation from a pool of 10 insects. Lanes 1–9: insects fed on At::pTY4; lane 10: insects fed on sucrose only; lane 11: insects raised on cotton. Lane Ti: purified plasmid pTY4, the *Agrobacterium* Ti plasmid-derived binary vector containing the TYLCV DNA sequences. **A,** Ethidium bromide-stained gel. **B,** Autoradiogram probed with a full-length genomic TYLCV DNA probe. Arrow: position of pTY4 doublet electrophoresed in the same gel.

bacterium At::pTY4 for 1, 4, 8, or 12 hr was studied (Fig. 4). Four groups of 20 insects per tube were used for each feeding period. Four groups of whiteflies were also fed for 24 hr on sucrose alone, and four groups on *Agrobacterium* At::pPCV002. Each group of 20 insects was then caged with one tomato test plant. Two weeks after caging, DNA prepared from the two youngest leaves was analyzed by Southern blot hybridization with a full-length viral genomic DNA probe. Thirteen of the 16 plants caged with whiteflies fed on *Agrobacterium* At::pTY4 contained detectable TYLCV DNA by 2 wk. Insects fed on At::pTY4 for only 1 hr were able to inoculate test plants (lanes 1–4). Whiteflies that fed on sucrose or on *Agrobacterium* not containing TYLCV DNA did not transmit TYLCV (lanes S and E). The amounts of viral DNA in the inoculated plants were extremely variable, possibly because the insects acquired variable amounts of At::pTY4 (see Fig. 1). The plants which contained high levels of viral DNA at the time of the assay, developed symptoms about 3 wk after whitefly-mediated agroinoculation. By 6 wk, all the plants inoculated with whiteflies fed on *Agrobacterium* At::pTY4 showed symptoms undistinguishable from those induced by natural infection with TYLCV in the field.

Whiteflies which fed on a culture of *Agrobacterium* At::pTY4 harbored infectious bacteria that were recovered from the insects and used to agroinoculate test plants (Fig. 5). Whiteflies that fed for 24 hr on *Agrobacterium* At::pTY4 were macerated in M9 minimal medium and the mixture was used to inoculate M9 medium supplemented with rifampicin and gentamycin (antibiotics to which At::pTY4 is resistant). After 96 hr, the culture was centrifuged, and the pellet was suspended in water and injected into 10 tomato test plants. Eight weeks thereafter, symptoms were clearly visible in four of the 10 plants. Hybridization of plant total DNA with a TYLCV full-length genomic clone indicated that these plants contained large amounts of viral DNA (lanes 1,5,7,9). *Agrobacterium* At::pTY4 was not recovered from whiteflies membrane fed with sucrose alone.

Transmission of tumorigenic *Agrobacterium* to plants by whiteflies fed on crown galls.

Since whiteflies were able to acquire *Agrobacterium* from cultures and to transmit it to plants, we investigated the question of whether whiteflies could acquire *Agrobacterium* from crown galls and transmit the crown gall disease to tomato test plants. Tomato plants at their four-leaf stage were injected with a culture of *Agrobacterium* C58 by making a 0.5-cm-long incision in the stem, about 3 cm above the soil level. The plants started to developed tumors at the site of the injection about 1 mo after inoculation. Two months after inoculation, the crown galls were removed from the plant and caged with 200 whiteflies in a muslin-covered 50-ml Falcon tube. Half of the insects were collected after 4 hr of access feeding, the remaining 4 hr thereafter. The whiteflies were pooled and four groups of 20 insects each were caged with four tomato test plants. About 4 wk later, tumors started to appear along the central stem of two of the four plants. The fully developed tumors induced by the insects were similar in size and in form to the source crown galls (data not shown). No tumors ap-

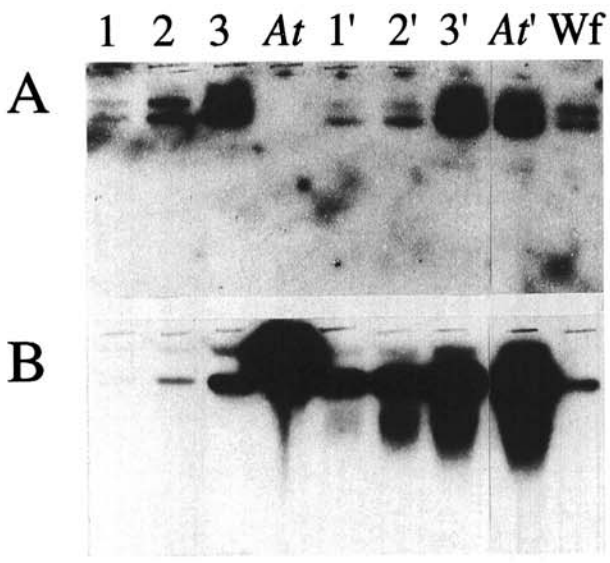

Fig. 2. Autoradiographic detection of pTY4 binary vector and of *Agrobacterium* chromosomal DNA in *Agrobacterium* At::pTY4 associated with whiteflies. Lanes 1–3: DNA extracted from a single whitefly mixed with 5, 12, or 50 pg pTY4 DNA. Lanes 1′ to 3′: DNA extracted from mixtures of a single whitefly with 1, 2.5, or 10 µl of a culture of *Agrobacterium* At::pTY4 (at the concentration of 1 $OD_{600nm/ml}$). Lanes At and At′: 50 ng of DNA from *Agrobacterium* strains LBA4404 and At::pTY4, respectively. Lane Wf: DNA amount equivalent to one whitefly membrane-fed for 24 hr with a culture of *Agrobacterium* At::pTY4 (at the concentration of 10 $OD_{600nm/ml}$). After gel electrophoresis and blotting, the samples were hybridized with a radiolabeled full-length genomic TYLCV DNA probe **(A)** and with radiolabeled total DNA from *Agrobacterium* LBA4404 **(B).**

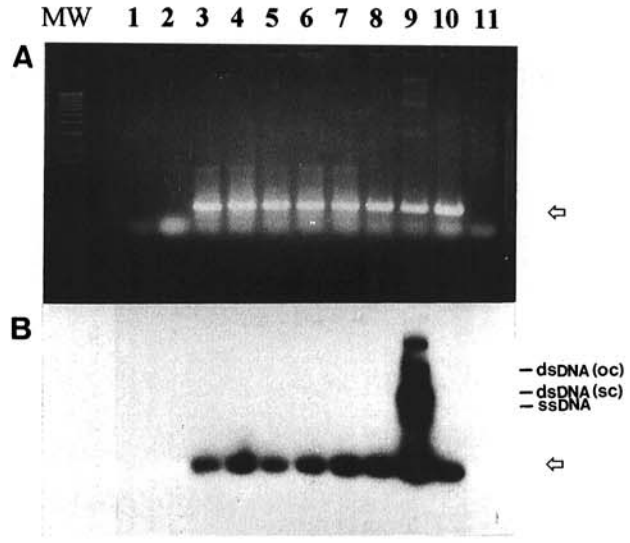

Fig. 3. PCR-amplification of TYLCV DNA from whiteflies membrane-fed with a culture of *Agrobacterium tumefaciens* At::pTY4 for 1, 2, 4, 8, 12, or 24 hr (lanes 3 to 8, respectively). Whiteflies were also fed for 24 hr with sucrose (lane 1), with an *Agrobacterium* culture (At::pPCV002) not containing TYLCV DNA sequences (lane 2), or with a TYLCV-infected tomato plant (lane 10). Lanes 9 and 11 contain PCR reactions with DNA extracted from a TYLCV infected and a noninfected tomato plant, respectively. Lanes 1–8 and 10 contain PCR products obtained with one tenth of the total DNA extracted from a pool of 10 insects. **A,** Ethidium bromide stained gel of PCR products after gel electrophoresis. **B,** Same gel after blotting and hybridization with a TYLCV DNA probe (4-hr exposure). The arrow points to the 410-bp PCR-TYLCV product. MW: molecular weight markers ('1-kb ladder,' from Gibco-BRL, Gaithersburg, MD).

peared on plants caged with insects which were not fed with the C58 tumors.

The acquisition and transfer of tumorigenic *Agrobacterium* by whiteflies, from the source tumor to the test plants, was analyzed by PCR (Fig. 6). Primers specific for the *Agrobacterium* C58 isopentenyl transferase (*ipt*) T-DNA gene were used to identify the transferred bacterium, because this gene is usually deleted in engineered *Agrobacterium* strains, such as *Agrobacterium* At::pTY4. Tumors were removed from the test plants when they were about 5 mm in diameter and total DNA was extracted. DNA was also extracted from the source crown galls and from the whiteflies which fed on them for 8 hr. A 430-bp DNA fragment corresponding to the size of the *ipt* gene target was amplified from DNA extracted from *Agrobacterium* C58 (lane 6), from the primary source tumor (lane 1), from insects which fed on it (lane 2), and from the tumors induced on test plants by these insects (lanes 3, 4). T-DNA was not amplified from whiteflies which were not fed with the *Agrobacterium* C58-induced crown galls (lane 7), or from test plants not caged with these insects (lane 5). These results indicated that the tumors that appeared on the test plants were generated by whiteflies fed on crown galls induced by *Agrobacterium* C58.

To confirm that T-DNA was transferred to test plants by whiteflies that fed on *Agrobacterium* C58-induced crown galls, DNA isolated from the source and the induced tumors was digested with *Hin*dIII and hybridized with the *Hin*dIII fragment 23 of the Ti plasmid. Similar hybridization patterns were obtained for DNA isolated from the source and from the induced tumors (data not shown).

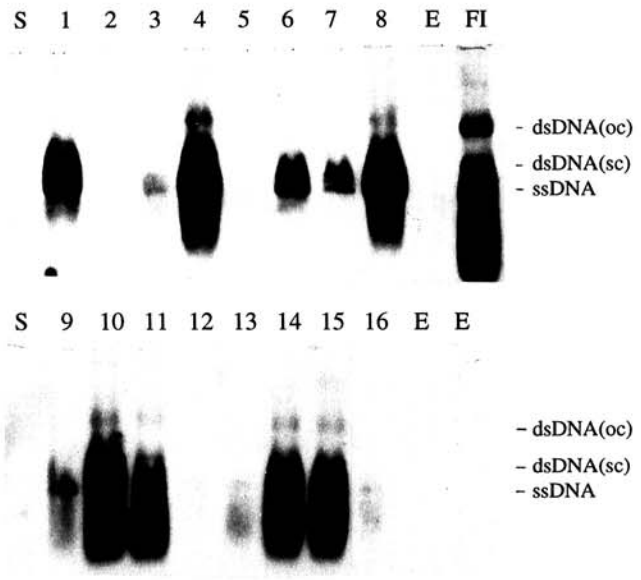

Fig. 4. Autoradiographic detection of TYLCV DNA in tomato plants inoculated by whiteflies fed through membranes with *Agrobacterium tumefaciens* At::pTY4. DNA was extracted from the two youngest leaves of tomato plants 2 wk after being caged with whiteflies fed with a culture of At::pTY4 for various periods of time. The DNA preparations were submitted to gel electrophoresis, blotted, and hybridized with a virus-specific DNA probe. Each lane represents a single plant. Lanes 1–4: plants inoculated with insects fed for 1 hr with At::pTY4; lanes 5–8: plants inoculated with insects fed for 4 hr; lanes 9–12: plants inoculated with insects fed for 8 h; lanes 13 to 16: plants inoculated with insects fed for 12 hr. S: Plants caged with whiteflies fed for 24 hr on sucrose; E: Plants fed with *Agrobacterium* At::pPCV002 which does not contain TYLCV DNA sequences. FI: TYLCV field-infected plant. ssDNA: Single-stranded viral genomic DNA; dsDNA(oc) and dsDNA (sc): Double-stranded viral DNA replicative forms (oc: open circular, relaxed; sc: supercoiled).

DISCUSSION

The ability of whiteflies to act as virus vectors has focused research on virus-whitefly relationships although bacterial and fungal pathogens can also be transmitted by these insects (Costa 1976). In this communication we show that whiteflies can acquire and transmit genetic information carried by *Agrobacterium tumefaciens*.

Whiteflies acquired *Agrobacterium* from liquid cultures. Whitefly mouth parts utilized for sucking consist of a large rostrum with a groove containing the stylet used to pierce the plant leaf (in our case, the membrane). The stylet reaches out from the rostrum at the beginning of feeding and retracts into

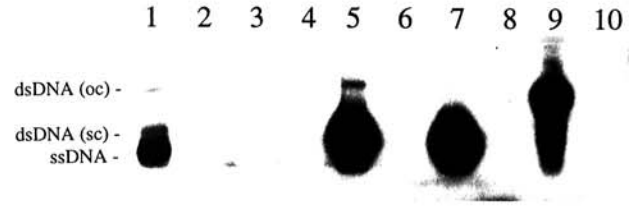

Fig. 5. Autoradiographic detection of TYLCV DNA in tomato plants inoculated with *Agrobacterium* recovered from whiteflies that were membrane-fed for 24 hr with a culture of At::pTY4. Each lane represents a single, different plant. ssDNA: Single-stranded viral genomic DNA; dsDNA (oc) and dsDNA (sc): Double-stranded viral DNA replicative forms (oc: open circular, relaxed; sc: supercoiled).

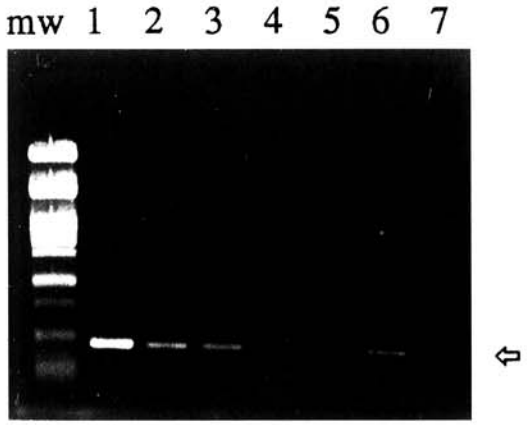

Fig. 6. PCR analysis of tumors induced in tomato test plants by whiteflies fed on crown galls induced after inoculating source tomato plants with *Agrobacterium* C58. The PCR products were submitted to gel electrophoresis containing ethidium bromide. Lane 1: DNA from crown gall obtained 2 mo after inoculating source tomato plants with *Agrobacterium* C58; lane 2: DNA from whiteflies fed on the source crown gall of lane 1; lanes 3 and 4: DNA from tumors obtained after caging tomato test plants with whiteflies fed with source crown galls (each lane represents tumors from a different plant); lane 5: DNA from plant not inoculated with whiteflies; lane 6: DNA from *Agrobacterium* C58; lane 7: DNA from whiteflies fed on TYLCV infected plant. mw: Molecular weight markers, lambda phage DNA digested with *Pst*I. The arrow indicates the 430-bp amplified DNA fragment.100 ng of plant DNA and one tenth of pooled DNA from 10 whiteflies were used.

it when feeding ends. Therefore, whiteflies feeding on *Agrobacterium* At::pTY4 cultures acquired *Agrobacterium* solely with their stylet and ingurgitated the bacteria. The amount of bacterium that remained on the external surface of the stylet must have been negligible. Reconstitution experiments indicated that the number of bacteria associated with an insect after 24 hr feeding on *Agrobacterium* cultures was in the range of 10^5. Whiteflies acquired intact *Agrobacterium* from the culture. The bacteria remained intact in the insect body since *Agrobacterium* could be recovered from whitefly homogenates, cultivated in selective medium,and injected into tomato test plants, inducing typical TYLCV symptoms. Likewise, the *Agrobacterium* binary vector pTY4 carrying the TYLCV DNA sequences was found apparently undegraded in the insect. Whiteflies fed on cultures of *Agrobacterium* At::pTY4 transmitted TYLCV to test plants with an efficiency comparable to that of insects fed for similar time periods on infected tomato plants.

The fact that *B. tabaci* can acquire *Agrobacterium* is not surprising. Whiteflies, as well as other plant virus-transmitting insects such as aphids and mealybugs, contain symbiotic gram-negative bacteria, which are distinct from *A. tumefaciens* (Baumann *et al.* 1993; Campbell 1993). While *Agrobacterium* acquisition by whiteflies is likely to be a passive process, retention and transmission of the bacteria may involve specific mechanisms, as in the case of viruses (Pollard 1955; Duffus 1987). The interaction between *B. tabaci* and viruses depends on the type of the virus, and is either circulative (geminiviruses), semi-persistent (closteroviruses), or non-persistent (carlaviruses). We are presently comparing the parameters of acquisition, retention, and transmission of *Agrobacterium* by whiteflies with those of TYLCV (Zeidan and Czosnek 1991) to find out whether the association between the whitefly and the bacterium is similar to that between the insect and the virus.

Whitefly-mediated agroinoculation, by combining the features of inoculation by whiteflies and by *Agrobacterium*, may enhance the efficiency of inoculation, extend the host range of viruses, and alter vector specificity. The high efficiency and specificity of whitefly-mediated inoculation is due to the fact that the virus is deposited directly into the phloem tissues (Pollard 1955). Agroinoculation, on the other hand, delivers large amounts of viral DNA which spreads rapidly into the plant vascular system (Grimsley and Bisaro 1988). In addition, agroinoculation has been shown to deliver viruses to plants which are usually recalcitrant to *Agrobacterium*-mediated transformation (Grimsley *et al.* 1987) and to overcome natural plant resistance mechanisms to viruses (Kheyr-Pour *et al.* 1994). It would be of interest to find out whether whiteflies can acquire and deliver *Agrobacterium* containing infectious cloned DNA from leafhopper-transmitted geminiviruses, thereby breaking the vector specificity of geminiviruses dictated by the virus capsid protein (Briddon *et al.* 1990). It would also be of interest to find out whether the noninoculative whitefly *Trialeurodes vaporariorum* (Antignus *et al.* 1993) is able to transmit TYLCV following feeding on *Agrobacterium* At::pTY4.

Not only can whiteflies acquire *Agrobacterium* from liquid cultures and transmit to test plants information contained in the T-DNA, but these insects can also acquire the bacteria from crown galls and induce tumors in test plants. PCR and Southern blot analyses clearly indicated that the origin of the secondary tumor was due to the transfer, by whiteflies, of information contained in the *Agrobacterium* T-DNA of the source crown gall. Moreover, T-DNA sequences could be amplified from the whitefly carriers. We therefore have here a case where an insect can be instrumental in the transformation of plant tissues.

Our finding that whiteflies are able to acquire *Agrobacterium* from cultures and from tumors raises the question of the possible participation of insects in the transfer and the dissemination of genes from plant to plant.

MATERIALS AND METHODS

Maintenance of virus cultures, whiteflies, and plants.

Whiteflies (*B. tabaci*) were reared on cotton plants (*Gossypium hirsutum* L. 'Akala') grown in insect-proof wooden cages at 26° C as previously described (Zeidan and Czosnek 1991). All insects were of the B type (Cohen 1993). Virus cultures were maintained in tomato plants (*Lycopersicon esculentum* Mill. 'FA144'). Uninfected plants were grown in an insect-proof growth chamber.

Plasmids and *Agrobacterium* strains.

Plasmid pHind23 contained a 3.4-kbp *Hin*dIII fragment (*Hin*dIII fragment 23 that contains the nopaline synthase gene and spanning the right border of T-DNA) from the virulent *A. tumefaciens* nopaline Ti plasmid pTiT37 cloned into pBR325 (Bevan *et al.* 1983).

Plasmid pTY4 contained a head-to-tail dimer of an infectious clone of the TYLCV genome inserted between the borders of the *Agrobacterium* binary vector pCGN1547; pTY4 was introduced into *Agrobacterium tumefaciens* LBA4404 to produce *Agrobacterium* At::pTY4 (Navot *et al.*, 1991). *Agrobacterium* At::pPCV002 contained the binary vector pPCV002 in *Agrobacterium tumefaciens* C58 (Koncz and Schell 1986); without TYLCV DNA sequences. The wild-type *Agrobacterium* C58 (Holsters *et al.* 1980) was also used. For membrane feeding experiments, 48-hr cultures of *Agrobacterium* were pelleted, washed twice with water, and re-suspended in 15% sucrose.

Feeding of whiteflies through membranes.

Whiteflies were collected by mouth aspiration into 5 ml Gilson tips as described previously (Zeidan and Czosnek 1991). The tips were immediately closed with Parafilm at their thinner aperture. The larger aperture was covered with a Parafilm sheet stretched to about four times its surface. A 15% sucrose solution (100 µl) containing yellow food dye (Czosnek *et al.* 1988a) and 10 OD_{600nm} units of *Agrobacterium* were deposited on the membrane and covered with a glass coverslip to avoid evaporation. The tips were placed in a rack, about 50 cm under a 40W standard electric bulb. Within 10 min, most of the insects were feeding through the membrane.

Recovery of viable *Agrobacterium* from whiteflies.

A group of 50 whiteflies were fed through membranes with At::pTY4. After 24 hr, the insects were collected and macerated in 0.5 ml of M9 minimal medium (Sambrook *et al.* 1989). The homogenate was used to inoculate 10 ml of M9

containing 100 μg/ml of rifampicin (resistance carried by the *Agrobacterium* chromosome) and 15 μg/ml of gentamicin (resistance carried by plasmid pTY4). After 48 hr in culture, 90 ml of M9 supplemented with the two antibiotics were added. After an additional 48 hr, the culture was centrifuged for 10 min at 10,000 *g* and the pellet suspended in 0.5 ml of sterile water.

Whitefly-mediated inoculation of tomato test plants.

Whiteflies which had access to plant tissues or to sucrose solutions were placed on uninfected tomato plants at the four-leaf stage in an insect-proof cage and remained on the plant for the duration of the experiment.

Preparation of DNA from insects, plants, and bacteria.

Total DNA was isolated from whiteflies as described by Zeidan and Czosnek (1991) with slight modifications. Whiteflies were placed in an Eppendorf tube containing 0.4% SDS-μg/ml proteinase K (10 insects in 200 μl) and ground using a glass rod. Following 1 hr incubation at 55° C, the mixture was extracted twice with phenol-chloroform-isoamylalcohol (25:24:1). Isopropanol (0.6 vol.) was added to the aqueous phase and the nucleic acid precipitate was suspended in sterile water.

Plant DNA was prepared by adapting the procedure described by Czosnek *et al.* (1988b). Tomato leaves were frozen in liquid nitrogen and ground to a fine powder. Boiling extraction buffer (containing 1% hexadecyltrimethyl-ammonium bromide [CTAB], 100 mM Tris-HCl pH 8.0, 20 mM ethylene-diaminetetraacetic acid [EDTA], 1.4 M NaCl and 1% 2-mercaptoethanol) was added (2 ml/g of tissue). The mixture was boiled for 2 min and incubated for 15 min at 55° C. An equal volume of chloroform-isoamylacohol (24:1) was added, and the aqueous phase was extracted. Isopropanol (0.6 volume) was added to the aqueous phase and the nucleic acid precipitate was suspended in sterile water.

Total *Agrobacterium* DNA was prepared as described by Herrera-Estrella and Simpson (1988). A 48-hr culture at 28° C of *Agrobacterium* was centrifuged for 15 min at 8,000 *g*. The pellet suspended in sterile water was incubated at 37° C for 1 hr with 1.5% sarkosyl and 1 mg/ml lysosyme. After phenol extraction, the aqueous phase was extracted twice with chloroform and the DNA precipitated by the addition of NaCl (0.25 M final) and 2 volumes of ethanol.

Plasmid pTiC58 was isolated from 10 ml of culture of *Agrobacterium* C58. The culture was centrifuged for 15 min at 8,000 *g*. The pelleted cells were suspended in 0.5 ml of 5 M NaCl and incubated at 22°C with 1% sarkosyl for 10 min. The cells were pelleted and suspended in 0.5 ml of 50 mM glucose, 10 mM EDTA, 25 mM Tris-HCl pH 8.0, 3 mg/ml lysosyme. One milliliter of 0.2 N NaOH-0.1% SDS and 0.8 ml of 3 M K-acetate, pH 4.8, were added and the mixture was centrifuged for 5 min at 10,000 *g*. Nucleic acids in the supernatant were precipitated by the addition of isopropanol (0.6 vol) and suspended in sterile water.

Southern blot detection of TYLCV DNA.

Nucleic acids from bacteria, individual whiteflies and from plants (about 0.5 μg of plant chromosomal DNA) were submitted to electrophoresis in a 1% agarose gel using Tris-phosphate-EDTA buffer pH 6.8 (Sambrook *et al.* 1989), alkali blotted, hybridized either with a full-length genomic TYLCV DNA clone (plasmid pTYH20.7, Navot *et al.* 1991), plasmid pHind23 or *Agrobacterium* LBA4404, and autoradiographed as described (Zeidan and Czosnek 1991). Blots were exposed to X-ray films for 24 hr unless specified otherwise in the legend of the figure.

Amplification of TYLCV DNA and T-DNA by the polymerase chain reaction (PCR).

The target DNAs consisted either of plant DNA (0.5–0.1 μg), *Agrobacterium* DNA (0.5 μg), or the amount of DNA equivalent to a single whitefly (about 30 ng). Two units of Taq polymerase (New England Biolabs, Berverly, MA) and 0.2 mM of each primer were used per reaction. A Techne PHC-2 thermocycler was utilized.

A 410-bp TYLCV DNA fragment was PCR-amplified from plant and insect DNA using two primers deduced from the nucleotide (nt) sequence of the TYLCV genome (Navot *et al.* 1991). They were 5′ATACTTGGACACCTAATGGC3′ (nt 61–80, viral strand) and 5′AGTCACGGGCCCTTACAA3′ (nt 456–473, complementary strand). A 430-bp T-DNA fragment was amplified using two primers deduced from the nucleotide sequence of the *Agrobacterium* isopentenyl transferase (*ipt*) gene of Ti plasmid Bo542 (Strabala *et al.* 1989). They were 5′GATCGCGTCCAATGCTGT (nt 97-114, coding strand) and 5′ATCGATATCCATCGATCC3′ (nt 509–526, complementary strand). The cycling protocol was as follows (in parenthesis the conditions specific for TYLCV DNA amplification): The first cycle consisted of DNA denaturation for 5 min (10 min) at 95° C, annealing of primers for 10 sec at 50° C (60° C), addition of Taq polymerase at 50° C (60° C), extension for 2 min at 72° C. Forty (30) cycles were performed as follows: 1 min (50 sec) at 95° C, 1 min (55 sec) at 60° C, 2 min at 72° C. The last cycle was completed by 5 min (7 min) extension at 72° C.

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