

Rapid Detection of Tomato Yellow Leaf Curl Virus in Squashes of Plants and Insect Vectors

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ABSTRACT

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DNA sequences of tomato yellow leaf curl virus (TYLCV), a geminivirus, were detected specifically and sensitively by hybridization of infected plant tissues squashed onto a nylon membrane (squash blot) with a specific DNA probe. No treatment of the sample was necessary before squashing and hybridization. TYLCV DNA could be detected in squash blots of tomato leaves, roots, stems, flowers, and fruits. Viral sequences also were

detected in single whiteflies that fed on infected plants. Squash blots were used to detect tobacco mosaic virus and potato virus Y, two RNA viruses, in infected tobacco plants. The squash-blot method was applied for screening TYLCV infection in the field in Israel and for diagnosing TYLCV in Turkey.

Additional keywords: virus diagnosis.

To predict and monitor plant virus epidemics, adequate procedures for rapid and specific virus detection are needed. Nucleic acid hybridization is a widely used diagnostic tool, mostly for the detection of viroids in vegetatively propagated plants (22). Simple procedures requiring a minimum of purification steps have been developed for detecting nucleic acids present in high copy numbers in plants. For example, viral nucleic acids have been detected in clarified cell sap (2), and highly repeated genomic DNA sequences were visualized in squashed segments of root tips (12). Similarly, plant pathogens have been detected in insect extracts and squashes (3,13).

We have developed a procedure by which virus nucleic acids can be detected in infected tissues squashed onto a nylon membrane (squash blot) following hybridization with specific probes. Detection of tomato yellow leaf curl virus (TYLCV) was studied as a model. We recently have isolated TYLCV (7), a circular single-stranded DNA geminivirus that shares many features with other viruses of this group (14). TYLCV causes a disease that affects cultivated tomato crops (*Lycopersicon esculentum* Mill.) in the eastern Mediterranean basin and in North Africa, resulting in reduced yields. Symptoms similar to those of the TYLCV disease

also have been found in Southeast Asia, Taiwan, Central Africa, and Mexico (15). The virus is transmitted by the whitefly *Bemisia tabaci* Genn. (5), usually during summer and autumn.

The detection of RNA viruses with the squash-blot method was tested with tobacco plant tissues infected with tobacco mosaic virus (TMV) and potato virus Y (PVY).

MATERIALS AND METHODS

Materials. NA-Agarose, the pTZ18 cloning vector, and restriction enzymes were obtained from Pharmacia, Inc., Piscataway, NJ. Nylon membranes (Hybond-N) and radionucleotides were obtained from Amersham Corp., Arlington Heights, IL. Kodak XAR-5 film was used for autoradiography.

Plants and insects. TYLCV and TYLCV DNA were isolated as previously described (7). Tomato squashes were done with healthy plants and TYLCV-infected plants (*L. esculentum*) that either were inoculated in the greenhouse by viruliferous whiteflies (*B. tabaci*) or naturally infected in the field. Insect blots were done with female whiteflies kept on TYLCV-infected *Datura* plants (*Datura stramonium* L.) for an acquisition period of 18 hr in an insect-proof greenhouse (6), collected, and immediately frozen at -20 C. Tobacco leaf squashes were done using healthy, TMV-, and PVY-infected plants (*Nicotiana tabacum* L. 'Samsun').

Preparation of a TYLCV-specific DNA probe. Total DNA was isolated from TYLCV-infected tomato plants using the CTAB extraction method (23). DNA was electrophoresed in a 1.5% agarose gel (16), and a longitudinal segment was Southern transferred (20) to a nylon membrane. TYLCV genomic DNA (7) (self-primed or primed with random plant DNA fragments) was radiolabelled with [³²P]-dCTP using DNA polymerase I. The membrane was hybridized (4) with the probe, and TYLCV DNA species were localized after autoradiography. The supercoiled double-stranded replicative form of the TYLCV genome was isolated by electroelution from the agarose gel (16), cut with *Hpa*II, and cloned into the *Acc*I site of pTZ18. Sequencing indicated that this 2,787 base pair (bp) TYLCV full-length clone is homologous to DNA 1 (14) of the whitefly-transmitted geminiviruses (Navot et al, unpublished). A 1.7-kilobase (kb) *Hpa*II/*Sph*I subclone lacking the gene encoding the viral coat protein was used as the TYLCV-specific probe (designated pTZ18-hs11).

Squash blotting of tomato tissues and whiteflies. Tomato leaves and flowers were squashed onto a dry nylon membrane using a hard object (a glass rod or pen, for example). Stems either were cut longitudinally or sliced serially from the apex to the crown and imprinted on the membrane. Fruits were cut open and imprinted. Frozen whiteflies were sprinkled on the nylon membrane, each individual separated from the others, and squashed with a glass rod. Squash blots were hybridized (4) for 16 hr with about 0.1 μg of TYLCV-DNA probe radiolabelled by nick translation (18) with [³²P]-dCTP (about 10⁸ dpm/μg DNA). The blots were washed at 65 C for 2 × 20 min in 150 mM NaCl, 15 mM trisodium citrate (1 × standard saline citrate [SSC]) and exposed at -75 C to X-ray film with two intensifying screens for 5 to 18 hr (standard hybridization, washing, and autoradiography conditions).

Squash blotting and hybridization of tobacco leaves inoculated with RNA virus. Leaves from TMV-infected tobacco plants were squashed onto a nylon membrane presoaked with 0.5% sodium dodecyl sulfate (SDS) and 100 μg/ml proteinase K (to prevent degradation of RNA by plant ribonucleases). The blot was hybridized with a [³²P]-dCTP radiolabelled TMV-RNA reverse transcript (11). Leaves from PVY-infected tobacco plants, squashed as described above, were hybridized with a 4-kbp PVY cDNA fragment (5' end of the PVY genome) radiolabelled by nick translation with [³²P]-dCTP. Leaves from uninoculated tobacco plants were squashed and hybridized with both probes as described above. All squash blots were hybridized (4) for 16 hr, washed at 65 C for 2 × 20 min in 1 × SSC, and autoradiographed for 18 hr.

RESULTS

Preparation of a TYLCV-specific probe. We isolated and cloned the putative replicative form of the TYLCV genome from a TYLCV-infected tomato plant. This full-length clone is homologous to DNA 1 of the whitefly-transmitted geminiviruses (14). The gene encoding the virus coat protein was deleted; the remaining 1.7-kbp fragment was subcloned and used as the probe. Southern blots of healthy and TYLCV-infected tomato plant DNA were hybridized with this fragment (Fig. 1). Autoradiographic analysis indicated that the probe detects double- and single-stranded DNA forms of the viral genome in the infected plants, similar to those described in plants infected by other geminiviruses (10,21). One of these forms corresponds to the 2,800-nucleotide TYLCV genomic DNA that can be visualized as an ethidium bromide-stained band after agarose gel electrophoresis of TYLCV-infected tomato plant DNA. No hybridization was found with DNA from healthy plants (Fig. 1).

Use of squash blots to detect TYLCV-infected tomato plants. TYLCV nucleic acids can be detected directly in squashes of the tested tissues without any pretreatment of the samples. Tomato leaves were squashed onto a dry nylon membrane and hybridized with the TYLCV probe. A strong signal was obtained with TYLCV-infected plant tissues. Tissues from uninfected plants did not react at all (Fig. 2).

Squash-blotted material is very stable and can be kept at ambient temperature (free in the air) for several months without

losing its hybridization capacity. The blots sustain boiling (for removing probes), alkali treatment (to make double-stranded DNA available to hybridization), and repeated freezing and thawing, without losing their hybridization capacity. Storage of blots for more than 1 wk in 100% relative humidity at 37 C reduced significantly but not completely the hybridization signal (not shown).

The squashed materials were irradiated for 3 min with an ultraviolet transilluminator whenever samples were sent to other laboratories. Irradiation did not affect the hybridization capacity

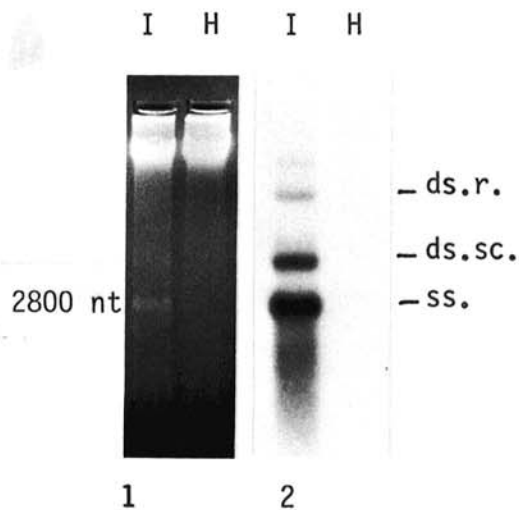


Fig. 1. Autoradiograph of tomato yellow leaf curl virus (TYLCV) DNA species in total DNA isolated from TYLCV-infected tomato. The probe was a cloned 1.7-kilobase pair viral fragment (pTZ18-hs11) radiolabelled by nick translation with [³²P]-dCTP. Five micrograms of DNA from infected (I) and healthy (H) plants was electrophoresed in a 1.5% agarose gel, blotted, hybridized with the viral probe, and autoradiographed. 1, Gel stained with ethidium bromide; 2,800 nucleotides (nt) is the estimated length of the single-stranded viral genomic DNA. 2, Autoradiogram of the blotted gel. ss. = single-stranded viral DNA; ds.sc. = double-stranded supercoiled viral DNA; ds.r. = double-stranded relaxed viral DNA.

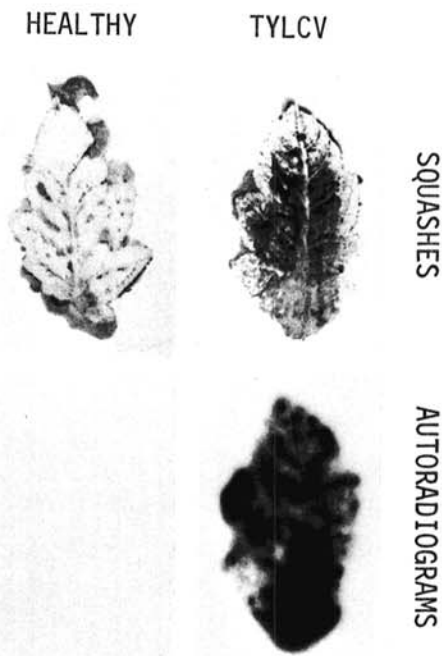


Fig. 2. Autoradiograph of tomato yellow leaf curl virus (TYLCV)-infected tomato leaf squash. Top row: photograph of leaf squash blot from TYLCV-infected and healthy tomato plants. Bottom row: corresponding autoradiograms, following hybridization with the cloned TYLCV-DNA probe.

of the samples.

TYLCV-DNA probe hybridizes mainly with viral genomic single-stranded DNA present in infected plant squashes. To determine the nature of the hybridizing material, TYLCV-infected leaves were squashed onto a membrane and the squash blots were incubated with nucleases before hybridization (Fig. 3). Both DNAase I and nuclease S1 completely prevented hybridization, whereas treatment with DNAase-free RNAase A slightly decreased the hybridization signal. These results indicated that single-stranded TYLCV genomic DNA is the major viral DNA class hybridizing with the TYLCV-DNA probe (see also Fig. 1).

Quantitation of detectable virus in TYLCV-infected leaves. Infected leaf tissues were diluted with healthy tissues in defined proportions (infected tissues constituted 100, 50, 20, 10, 5, 2, and 1% of the mixture, by weight). Twenty milligrams of the mixtures was squashed on a 0.3-cm² dot matrix (sufficient to contain the entire sample). Known amounts of cloned full-length viral DNA were used as the standard (0.12-cm² spot). The membranes were hybridized simultaneously with the TYLCV probe (Fig. 4).

The amount of TYLCV DNA detectable in plant tissues was estimated by comparing visually the hybridization signals of infected leaf squashes with that of known amounts of virus DNA (1 picogram [pg] of cloned viral DNA is equivalent to 300,000 genome copies) (Fig. 4). For example, the signals obtained with squashes from two infected plants P and P' (20 mg of tissue in a 0.3-cm² spot) were comparable to those obtained with 100 and 20 pg of cloned viral DNA (in a 0.12-cm² spot) (between 75 and 18 million viral genome copies, after standardization to a 0.3-cm² spot). Hybridization of a leaf mixture containing 20% of infected tissue corresponded to 15 million viral genome copies; hybridization of leaf mixtures containing 5% of infected tissues corresponded to 3.5 million viral genome copies. Thus the method allows detection of virus amounts corresponding to a few percent of the amount present in a plant with typical disease symptoms.

Although the determination of TYLCV amounts is very approximate, it can be roughly estimated that the number of virus genome copies in a leaf from an infected plant is in the range of one million per milligram of tissue.

Specificity of hybridization with the TYLCV probe. The

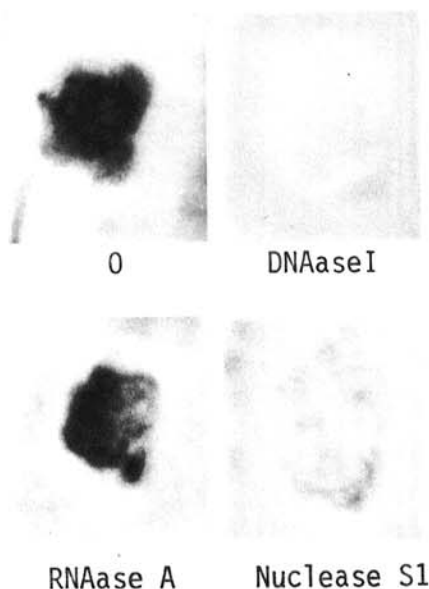


Fig. 3. Nuclease analysis of the material hybridizing with the tomato yellow leaf curl virus (TYLCV) probe. Leaves from a TYLCV-infected tomato plant were squashed onto a membrane. The membranes were incubated for 1 hr at 37 C with either DNAase I (100 µg/ml, Worthington), DNAase-free RNAase A (100 µg/ml, Sigma Chemical Co., St. Louis, MO) or nuclease S1 (100 units/ml, Pharmacia, Inc., Piscataway, NJ). After nuclease treatment, the membranes were washed with 0.5% sodium dodecyl sulfate and sterile water, hybridized with the cloned TYLCV-DNA probe, and subjected to autoradiography.

specificity of the probe for TYLCV detection was tested by attempting to hybridize leaf squash blots from tomato plants infected by other viruses with the TYLCV probe. Leaves from tomato plants infected with cucumber mosaic virus, PVY (Fig. 5), or TMV (not shown) did not hybridize with the TYLCV probe. Leaf squash blots from a plant of *Abutilon selloianum* Reg. infected with the abutilon mosaic virus (AbMV), a geminivirus (1), did not react with the TYLCV probe (not shown).

Detection of TYLCV DNA in squash-blotted infected tomato tissues. The presence of viral nucleic acid was visualized in squashes of roots, stems, leaves, flowers, and fruits, and a differential distribution of the virus in these tissues was observed.

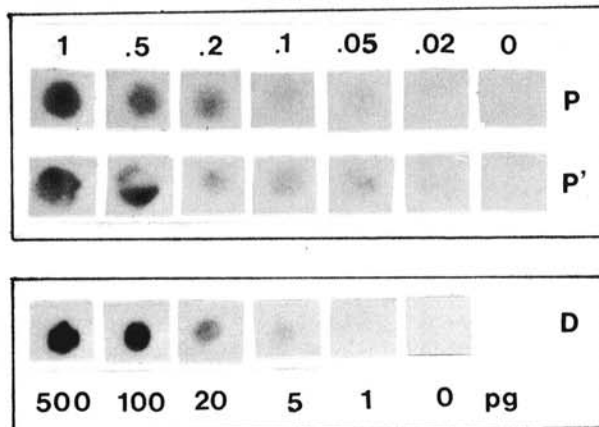


Fig. 4. Quantitation of viral DNA in tomato yellow leaf curl virus (TYLCV)-infected leaf tissues. A leaf from a TYLCV-infected plant and one from an uninfected plant were shredded with a scalpel. The tissues were mixed together in defined portions (w/w), and 20 mg of tissue mix was squashed onto a membrane using a 0.6-cm-diameter dot matrix, sufficient to contain the entire sample. Leaves from two infected plants were used (P and P'). The percentages of infected tissues in the leaf mixes were: 100 (1), 50 (.5), 20 (.2), 10 (.1), 5 (.05), 2 (.02), and 0% (0). Known amounts (in picograms [pg]) of alkali-denatured cloned TYLCV-DNA were spotted (0.4-cm-diameter spot) on the membrane and used as standard (D). One-picogram cloned viral DNA corresponds to 0.3 million copies of the viral genome. The membranes were hybridized with the radiolabelled TYLCV probe and processed for autoradiography.

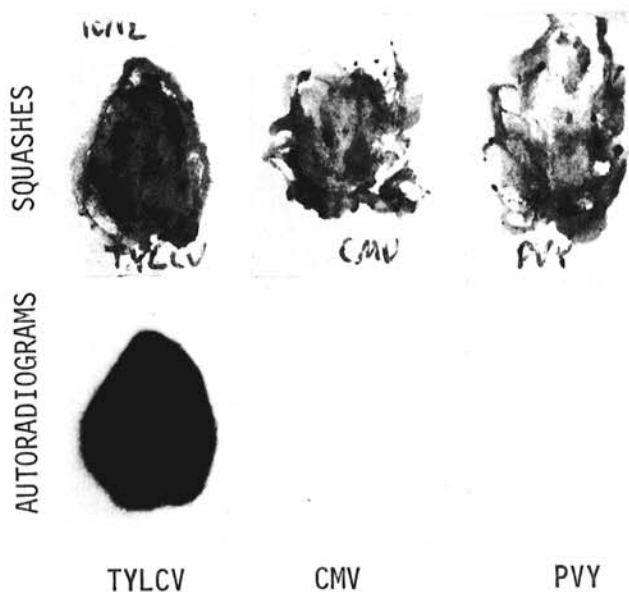


Fig. 5. Specificity of the tomato yellow leaf curl virus (TYLCV) probe for the detection of TYLCV-infected squash-blotted tissues. Leaf squash blots (top panels) from tomato plants infected with either TYLCV, cucumber mosaic virus (CMV), or potato virus Y (PVY) were hybridized with the TYLCV cloned DNA probe and autoradiographed (bottom panels).

For example, hybridization of cross or longitudinal stem sections indicate that viral nucleic acids in these tissues are found mainly in the vascular system (Fig. 6).

Detection of TYLCV DNA in squash blots of viruliferous whiteflies. The squash-blot technique makes possible the detection of viral nucleic acids in insect vectors. Female whiteflies kept on a TYLCV-infected or a healthy *Datura* plant for 18 hr (6) were individually squashed onto the nylon membrane and hybridized with the viral probe. Viral sequences were detected by autoradiography in each of the whiteflies that fed on the infected plant only. The amount of virus carried by the insects was estimated by spotting known quantities of cloned TYLCV DNA on the same membrane (from 100 to 1 pg DNA, equivalent to 30 to 0.3 million copies of the viral genome). Although the period of feeding on the infected plant was the same for all the whiteflies, the intensity of the hybridization signals showed considerable variability from one individual to the other. By visually comparing intensities of hybridization with standards, we estimated that the insects carried between one and 100 million copies of the viral genome (Fig. 7).

Detection of TMV and PVY RNA in squashes of infected tobacco leaves. The squash-blot method can be used, with minor modifications, to detect RNA viruses. Leaves from uninfected tobacco and TMV- and PVY-infected tobacco plants were squash blotted onto a nylon membrane previously saturated with SDS and proteinase K and dried (to denude the viral RNA and protect it from nucleases). TMV was detected after hybridization of squashes with a radiolabelled TMV cDNA. PVY was detected after hybridization with a radiolabelled PVY cDNA clone. Strong and specific signals were obtained with the tissues infected by these two RNA viruses, whereas no signal was obtained with the uninfected tissues (Fig. 8).

For RNA detection, pretreatment of the membrane with SDS and proteinase K is obligatory (8 M guanidine-HCl also is adequate). Samples squashed on an untreated membrane hybridized poorly (not shown).

Use of squash blots to screen for infected plants in the field. Squashes can be done easily in the field and assayed for the presence of TYLCV DNA sequences in the laboratory. We sampled 164 tomato plants in the field. A few millimeters of a young leaf of each plant was squashed directly onto a 0.5 × 0.5 cm

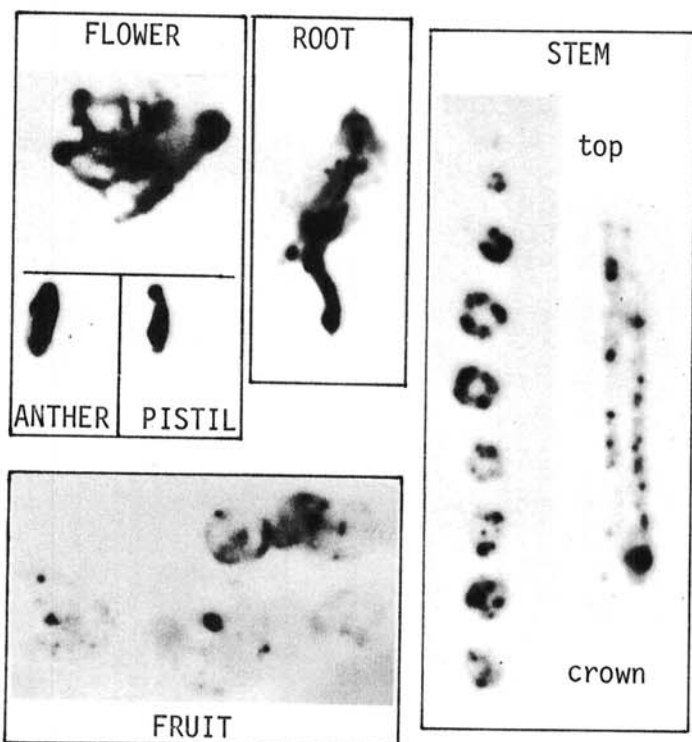


Fig. 6. Autoradiograph of tomato yellow leaf curl virus (TYLCV) DNA sequences in squashes of different tissues from a TYLCV-infected plant.

square so that all 164 samples could fit on a 20 × 2.5 cm membrane. This was done within 2 hr. As a positive control, we added a squash from a plant inoculated in the greenhouse with viruliferous whiteflies. The membrane then was hybridized with the radio-labelled viral probe, and three TYLCV-infected plants could be detected by autoradiography (Fig. 9). Although disease symptoms were not apparent in these three plants at the time of sampling, they developed within 3 wk.

Diagnosis of a tomato yellow leaf curl disease epidemic in Turkey. Squash blots are very stable; they can be mailed from one country to another for sampling and analysis. In November 1987, we sent a membrane containing leaf squashes of healthy control and TYLCV-inoculated tomato from Israel to Turkey. Two months later, leaves from tomato plants grown in glasshouses in the Mersin region were squashed on this membrane. The samples included plants with yellow leaf curl symptoms as well as plants without any symptoms. The membrane was returned to us and hybridized with the TYLCV probe in February 1988 (Fig. 10). The results showed that, out of the 12 samples, eight were infected with TYLCV. The samples positive for TYLCV were from plants with and without symptoms. All negatives were from symptomless plants. Similarly, we have used the squash-blot method to diagnose the TYLCV disease in Senegal, Mali, Cape Verde, Taiwan, and Thailand.

DISCUSSION

The squash-blot procedure that we have described provides a specific, rapid, and simple means of using molecular hybridization techniques to detect infected plant tissues.

The diagnosis of TYLCV-infected tissues by hybridization was performed directly on tissues squashed onto a membrane (Fig. 2). No pretreatment of the samples (for example, preparation of saps, lysates, nucleic acids) was necessary. Nylon membranes are the support of choice for squashed samples; nitrocellulose membranes are too fragile. It was not necessary to bake or UV-irradiate membranes after squashing to fix plant nucleic acids. Incubation of the squashed samples with nucleases before hybridization indicated that most of the hybridizable viral nucleic acids in the infected tissues is single-stranded DNA (Fig. 3; see also Fig. 1). Incubation of squash blots with either SDS and proteinase K (to denude viral nucleic acids) or with alkali (to denature double-stranded DNA) before hybridization did not significantly increase hybridization signals (not shown). Therefore the hybridizable viral DNA either is not encapsidated in the plant cells or is released from

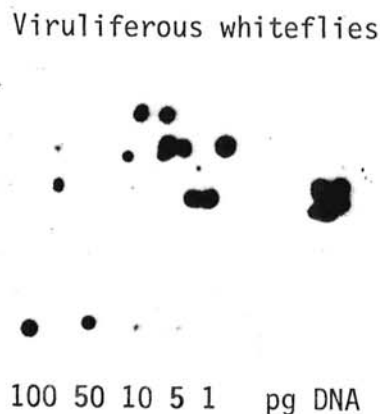


Fig. 7. Detection and quantification of tomato yellow leaf curl virus (TYLCV)-DNA sequences in viruliferous whiteflies. Female whiteflies kept on a TYLCV-infected *Datura* plant for an 18-hr virus acquisition period were collected, frozen at -20°C , individually squashed onto a membrane, and hybridized with the viral probe. Quantification was done by spotting known amounts of alkali-denatured TYLCV DNA (100–1 picograms [pg]) onto the same blot, in a range equivalent to 30 to 0.3 million copies of the viral genome. Each spot is the autoradiographic signal given by an individual insect; nonviruliferous whiteflies do not hybridize at all.

the capsid during squashing as a result of a mechanical or a proteolytic effect.

With our method, we estimated that, in a leaf with typical symptoms, TYLCV DNA is present in the range of one million genome copies per milligram of infected tissue. Dilution experiments showed that with this method it is possible to detect as few as 50,000 genome copies per milligram of tissue (0.15 pg of virus DNA) (less if autoradiographic exposure is extended to several days) (Fig. 4). Sensitivity of the method can be compared favorably with that of spot and Southern blot hybridizations. We have shown previously that TYLCV DNA can be detected in plant lysates and in plant DNA spotted on a membrane and hybridized with a TYLCV probe (8). To reach levels of detection similar to the level provided by squash blots (in the range of 10 pg of virus DNA per 20 mg of tissue squashed in a 0.3-cm² spot), the spotted samples should contain at least 1 μg of plant DNA. Such DNA amounts are

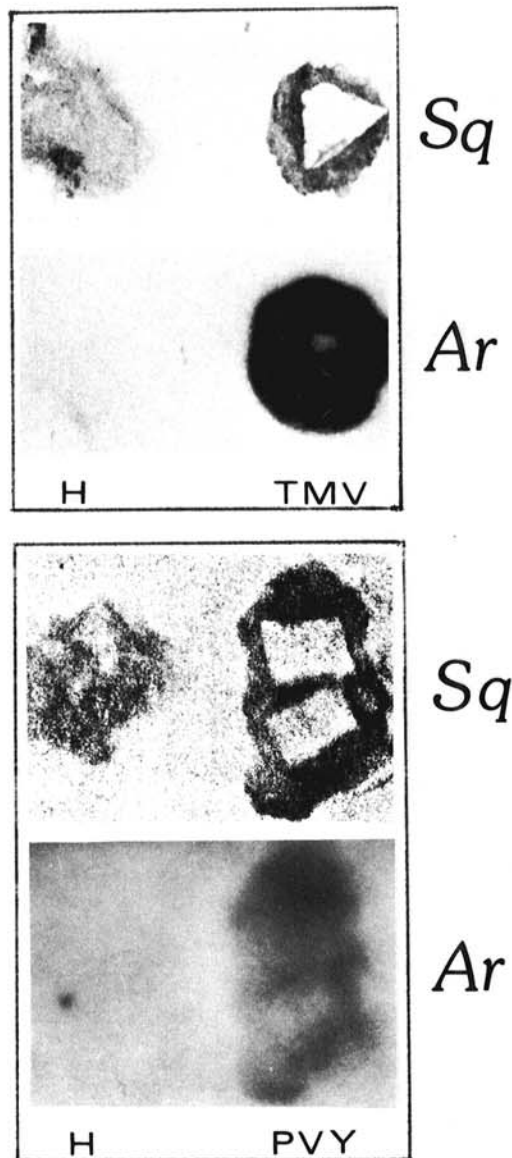


Fig. 8. Detection of tobacco mosaic virus (TMV) and potato virus Y (PVY) RNA sequences in squash blots of TMV- and PVY-infected tobacco leaves. Tissues were squashed onto a nylon membrane presoaked in 0.5% sodium dodecyl sulfate, 100 μg/ml proteinase K and hybridized with the virus-specific probes. Top panel, first row: photograph (Sq) of leaves from healthy uninoculated (H) and TMV-infected (TMV) tobacco plants; second row: autoradiogram (Ar) following hybridization of squashes with a TMV-RNA reverse transcript. Bottom panel, first row: photograph (Sq) of leaf squashes from healthy uninoculated (H) and PVY-infected (PVY) tobacco plants; second row: autoradiogram (Ar) following hybridization of squashes with a PVY cDNA clone.

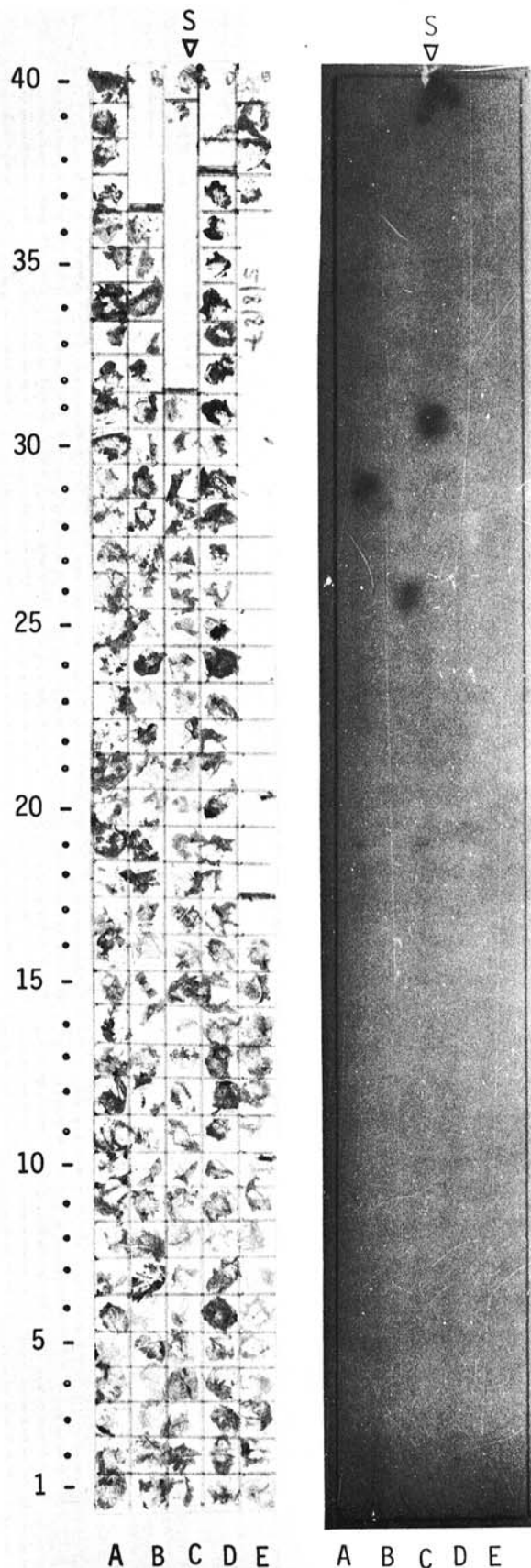


Fig. 9. Detection of tomato yellow leaf curl virus (TYLCV)-infected plants from 164 tomato plants sampled directly in the field. A leaf from each plant was squashed on a 0.5 × 0.5 cm square that was a part of a grid drawn on a 20 × 2.5 cm membrane (left panel). The membrane was hybridized with the TYLCV-specific probe, and the TYLCV-infected plants (A29, B26, and C31) were detected by autoradiography (right panel). S indicates TYLCV-infected control plant.

obtained after processing 200–500 mg of leaf tissue (8,23). Therefore, because samples do not need to be processed before detection, the squash-blot method minimizes losses of hybridizable viral DNA.

Diagnosis of viruses in plants relies mainly on the enzyme-linked immunosorbent assay (ELISA). This method depends on the availability of specific antibodies against the virus coat protein. Serological studies have indicated that several whitefly-transmitted geminiviruses are antigenically related (19,24). This is due to sequence homologies between their coat proteins (17). ELISA is usually less sensitive than DNA hybridization. In the case of bean golden mosaic virus, another whitefly-transmitted geminivirus, where both antiserum and DNA probe are available, ELISA was sensitive in detecting the virus at the nanogram range whereas hybridization (Southern blot) could detect virus in the picogram range (9). Because antiserum against TYLCV coat protein is not available yet, we can only infer that future diagnostics of TYLCV by ELISA may be less specific and sensitive than DNA hybridization.

In the diagnostic procedure based on molecular hybridization with cloned DNA probes, specificity of detection depends on the sequence of the probe. For TYLCV diagnostics, we have used cloned genomic TYLCV DNA from which the gene that encodes the coat protein has been deleted. It did include the gene that encodes the putative replicase, which is more diverged among geminiviruses than the coat protein and the 200-nucleotide region unique to each geminivirus (14). For routine TYLCV diagnostics, this probe is adequate because no other geminiviruses infect tomato in our region. In the case where a plant may be infected by two geminiviruses, virus-specific probes can be prepared from the 200-nucleotide unique sequence.

The squash-blot method can be applied to the detection of other geminiviruses (AbMV, for example; H. Jeske, *personal communication*). The method can be modified slightly to enable diagnosis of double-stranded DNA and RNA viruses. For the detection of double-stranded DNA viruses, preincubating the squash blot in alkali may be necessary before hybridization with a specific probe. As we have demonstrated for the diagnosis of TMV- and PVY-infected tissues, a prerequisite for the detection of RNA viruses is the preincubation of the membrane with RNAase inhibitors such as SDS and protease K.

Squash blotting is a tool for rapid, large-scale diagnostics of

TYLCV and other viruses. A small piece of tissue is sufficient for analysis, and an entire field can be sampled on the spot in a short time by untrained personnel (Fig. 9).

TYLCV can be detected in squashes of whiteflies. Single-stranded TYLCV genomic DNA is the only viral DNA form found in viruliferous whiteflies (Czosnek et al, *unpublished*). Because the virus circulates in the insect's body, it is assumed that the viral genome is kept intact only if it is encapsidated. Therefore, it is likely that the viral genome is denuded (mechanically or enzymatically) during squashing. TYLCV concentration in whiteflies can be estimated using TYLCV DNA standards (Fig. 7). The comparison between the amounts of virus detectable in the viruliferous whiteflies (in the range of one million per microgram of tissue) with those found in the infected leaf (in the range of one million per milligram of tissue) indicated that the insect concentrates the virus in its body in several orders of magnitude (Figs. 4 and 7).

Squash-blotting samples are surprisingly stable. Squash blots kept for 6 mo at ambient temperatures did not show any reduction in their hybridization capacity. We have shown that blots can be mailed from one country to another for sampling and analysis (Fig. 10). Therefore the method can be used for large-scale epidemiological studies conducted either by monitoring the spread of viruliferous insect vectors or by identifying virus-infected plants. Squash blots also can be used in quality control and in monitoring plant resistance to viral diseases in the course of breeding programs.

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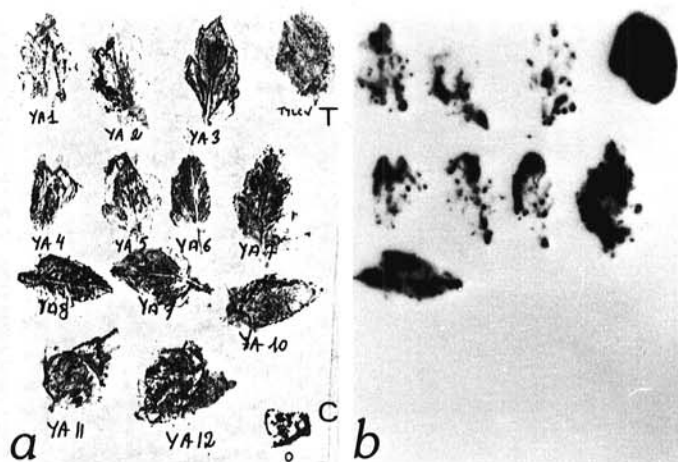


Fig. 10. Diagnosis of tomato yellow leaf curl virus (TYLCV)-infected plants from Turkey. A membrane already containing squashes from control (C) and TYLCV-infected (T) tomato plants applied in Israel was used as a surface on which tomato leaves were squashed in Turkey. The samples, from glasshouse cultures in the Mersin region, included symptomless plants as well as plants showing symptoms similar to those induced by TYLCV (YA1–YA12). The membrane was mailed back to Israel for autoradiographic analysis with the TYLCV-specific probe. A, Photograph of leaf squashes. B, Autoradiogram following hybridization with the TYLCV probe. All samples hybridizing with the TYLCV probe were from plants exhibiting various degrees of yellow leaf curling.

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