Techniques

Nucleic Acid Probes for Detection and Strain Discrimination of Cucurbit Geminiviruses

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Partial support for this work was received from the California Melon Research Advisory Board.
We wish to express our gratitude to J. Duffus for gifts of antisera and virus cultures and to S. Lazarowitz for the gift of cloned viral DNA's.
Accepted for publication 14 June 1989 (submitted for electronic processing).

ABSTRACT

Polston, J. E., Dodds, J. A., and Perring, T. M. 1989. Nucleic acid probes for detection and strain discrimination of cucurbit geminiviruses. Phytopathology 79: 1123-1127.

A nucleic acid spot hybridization assay was developed for detection of cucurbit geminiviruses in plant tissue extracts. With a probe composed of four unique full-length DNA geminiviral genome components cloned from squash infected with two variants of squash leaf curl virus (SLCV), viral nucleic acid could be detected in various infected hosts representing nine genera from four plant families. The least amount of virus that could be detected by the spot hybridization assay was 300–400-fold less than that by enzyme-linked immunosorbent assay (ELISA). The limit of detection of purified SLCV was 3.2 pg of viral nucleic acid (17 pg of virus). A unique restriction fragment from each of the four genome components was identified (19). Two biologically different strains could be differentiated with these cloned fragment probes and mixtures of these two strains could be detected in experimentally infected plants. All four SLCV genome components were detected in field-collected squash plants. The genome components of both strains could be found in the same plant; however, in some plants the two genome components of one strain and only one of the other strains could be detected.

Squash leaf curl is a disease of cucurbits in the Imperial Valley of southern California occurring at high incidences in fall-planted crops (6,7,9,17,23). It is caused by a whitefly-transmitted geminivirus and is transmitted by *Bemisia tabaci* Gennadius (4). The whitefly-transmitted geminiviruses are single-stranded DNA plant viruses with bipartite genomes that possess serologically related coat proteins and share a considerable amount of nucleic acid sequence homology (14,15,18,25,27).

Two biologically distinguishable virus strains have been reported to cause squash leaf curl disease in the southwest United States. (These are being considered strains for the purposes of this manuscript, though criteria have not yet been established for the distinction between geminivirus strains and viruses.) Squash leaf curl virus (SLCV), as first described by Cohen et al (4), infects, among other hosts, bean (*Phaseolus vulgaris* L.) and squash (*Cucurbita pepo* L.) but not melon (*Cucumis melo* L.). A second virus, named in California melon leaf curl virus (MLCV) (8) and in Arizona watermelon curly mottle virus (WCMV) (3), infects bean, squash, and melon. In addition, two very closely related geminivirus genomes were cloned from leaf curl diseased squash plants growing in the Imperial Valley of California (19,20). Results of mechanical and *Agrobacterium*-mediated inoculation of cloned genomic components to bean and *Nicotiana benthamiana* Domin. revealed that one infected bean and *N. benthamiana* (designated SLCV-E, for extended host range) and one infected bean only (designated SLCV-R, for restricted host range).

The purpose of this research was to develop nucleic acid-based assays for the detection of leaf curl viruses in plant tissues, to distinguish between the two strains causing squash leaf curl

disease, and to reduce the confusion surrounding the identity of the virus(es) causing leaf curl disease of cucurbits in the Imperial Valley of California.

MATERIALS AND METHODS

Sources of virus, probe DNA, and whiteflies. Both full-length and partial clones of SLCV-E and SLCV-R were obtained from S. Lazarowitz, Carnegie Institute of Washington, Baltimore, MD. Lazarowitz cloned four unique full-length geminivirus genomic components from viral DNA extracted from squash tissue, expressing leaf curl symptoms (virus culture SLCV-3), collected in the Imperial Valley (20). Full-length viral ssDNA A and B components of SLCV-E and SLCV-R were cloned separately in pEMBL plasmids and maintained in *Escherichia coli* 71-18 (20). An equimolar mixture of all four full-length components comprised the 'mixed' probe and was used to detect geminivirus nucleic acid in infected tissue. Lazarowitz identified within each full-length component one unique restriction fragment (20). These were isolated and subcloned separately into pUC118 and *E. coli* JM109, using standard procedures (21). Each of these unique fragments hybridized only to the component from which it was obtained, as demonstrated by Southern analysis (20). Thus, each unique fragment could be used to detect individual genomic components of both virus strains. The four clones are as follows: a 600-bp *Hin*DII-*Sal*I fragment from SLCV-R component A (probe R_A), a 900-bp *Bam*HI-*Sac*I fragment from SLCV-R component B (probe R_B), a 700-bp *Bam*HI-*Sst*I fragment from SLCV-E component A (probe E_A), and a 1,100-bp *Sal*I-*Xba*I fragment from SLCV-E component B (probe E_B).

The geminivirus culture described by Cohen et al (4) and designated in this study SLCV-1, was obtained from J. Duffus, U.S. Agricultural Research Station, Salinas, CA. The geminivirus culture SLCV-2, was obtained from squash plants used to maintain a culture of *B. tabaci* established in 1981 from insects collected in the Imperial Valley. The virus was maintained in squash by whitefly transmission. *B. tabaci* used in the studies were obtained from virus-free cultures started with insects collected from the Imperial Valley and maintained on sweet potato (*Ipomoea batatas* L.), cotton (*Gossypium hirsutum* L.), or *P. vulgaris*. Cultures were reared in greenhouses at 25–35 C.

Virus purification. Virus was purified from mechanically inoculated plants of *P. vulgaris* 'Topcrop' (10). Plants were inoculated 7–10 days after planting, maintained in an Environmental growth chamber (model M31-15, Chagrin Falls, OH) at 32 C, 14-hr daylength and harvested 12–14 days after inoculation.

Sample preparation. Plant samples were prepared for blotting by grinding tissue to a powder in a mortar in the presence of liquid nitrogen and then grinding the powder in TE buffer (0.01 M Tris, 0.001 M EDTA, pH 8.0) at a 1:4, w:v, ratio. The homogenate was strained through Miracloth and then 3 M sodium hydroxide was added to 0.1 M. The extract was incubated for 10 min at room temperature and then a 0.1 volume of 3 M sodium acetate, pH 5.0, was added (21).

Nucleic acid spot hybridization procedure. Samples were spotted in 25-μl aliquots onto Zeta-probe (Bio-Rad Laboratories, Richmond, CA) membrane soaked in TAE buffer (0.04 M Tris, 0.02 M Na acetate, 0.001 M EDTA, pH 7.8) by using a blotting manifold (Bio-Rad) and vacuum. After spotting, the membranes were baked for 2 hr at 80 C in a vacuum oven, and either prehybridized immediately or sealed in a plastic bag and refrigerated until prehybridization. Blots were prehybridized and hybridized in a solution, a modification of Reed and Mann (24), containing 50% formamide, 4×SSPE (4×SSPE = 0.72 M sodium chloride, 0.04 M disodium phosphate, 0.004 M EDTA), 1.0% (w/v) nonfat dry milk dissolved in water, 1.0% SDS, and 0.5 mg/ml of denatured salmon sperm DNA. Blots were sometimes stored for short periods at 4 C in the prehybridization fluid until hybridization was started. All blots were hybridized individually in heat sealed plastic bags with 0.1-ml solution per square centimeter. Blots were allowed to hybridize with the mixed probe at 42 C and with the component-specific probes at 47 C.

Plasmid DNAs containing viral inserts were extracted from cells of *E. coli* (5) and labeled by nick translation with alpha [^{32}P] CTP and a nick translation system (New England Nuclear Research Products, Dupont Co., Boston, MA) (21). The specific activity of labeled probe was approximately 1.0×10^8 cpm/μg of DNA. Labeled DNA was boiled for 5 min, quick cooled, and added to the membrane in prehybridization fluid to a final concentration of $0.7–3 \times 10^6$ counts per milliliter.

Blots hybridized with the component-specific probes were rinsed as follows: 2×SSC, 0.1% SDS, 21 C for 5 min; 2×SSC, 0.1% SDS, 65 C, 15 min; 1×SSC, 65 C, 15 min; twice with 0.1×SSC, 0.1% SDS, 65 C, for 15 min each, and 0.1×SSC, 1.0% SDS, 65 C, for 30 min. Blots of plant tissue hybridized with the mixed probe were rinsed either at the above conditions of high stringency or at a lower stringency, 4×SSC, 0.1% SDS, 55 C, twice for 10 min followed by 2×SSC, 0.1% SDS, 55 C four times, 10 min each, depending on the level of specificity required. Blots were air-dried and then exposed to X-Ray film (Kodak XR-1) with intensifying screens.

Samples were considered positive when the spot on the autoradiograph was darker than the negative controls. Quantitative measurement of reactions was accomplished using a densitometer (DU-50 spectrophotometer with a Gel Scan Accessory, Beckman Instruments, Palo Alto, CA) to measure the maximum absorbance of light at 560 nm. Maximum absorbance of each autoradiograph spot was determined by using the following settings: a 1.0-mm horizontal slit width, 0.2-mm vertical slit width, and 10 readings/mm.

Procedure for SLCV ELISA. Antisera to SLCV was obtained from J. Duffus, U. S. Agricultural Research Station, Salinas, CA. Gamma globulin was purified by chromatography on DE 22 cellulose and conjugated with alkaline phosphatase (1). Direct, double-antibody sandwich ELISA was performed using conditions similar to those established for bean golden mosaic virus (2). Flat bottomed polystyrene Immulon 1 (Dynatech Lab, Inc., Alexandria, VA) microtiter plates were coated with 1 μg/ml of gamma globulin for 3 hr at 4 C. Plant samples were extracted in buffer (1:3, w/v) with a mortar and pestle as described for the hybridization assay. Samples (0.2 ml) were added to the coated wells and incubated overnight at 4 C. Conjugated gamma globulin (1 mg/ml), diluted 1:800 with PBS-Tween, was added to the wells and plates were incubated at room temperature for 4 hr. Substrate at 1 mg/ml concentration was added and incubated for 0.5–1 hr before the reaction was stopped with 3 M sodium hydroxide. Measurements of absorbance at 405 nm were made with a Bio-Tek EIA reader (Model EL 307, Bio-Tek Instruments, Inc., Burlington, VT). Reactions were considered positive when the A_{405nm} values were greater than the mean of the negative controls (a mean of at least three values) on the same plate, plus three times the standard deviation of the sample population (13,28).

Procedure for whitefly manipulation. Whiteflies were transferred using glass-tipped aspirators. All transmission studies involving single insects used whole plant cages to cover the test plants during acquisition and inoculation periods. In some cases whiteflies were reared on virus-infected plants housed in wood framed and nylon netted cages large enough to contain several plants. Details of specific experiments are described in the Results section.

RESULTS

Detection of SLCV with mixed probe. Several different extraction methods were examined for detection of geminiviral DNA in plant tissue using the mixed probe in a spot hybridization assay. The method described by Robinson et al (26), grinding tissue in TE buffer and adding NaOH and $NaCH_3COO^-$, was superior to or equal in sensitivity to grinding in TE buffer alone. The use of Pronase (0.12 vol of 10 mg/ml stock, 30 min of incubation 37 C, centifuged 4,000 rpm 5 min) either before or in place of the NaOH and $NaCH_3COO^-$ decreased the sensitivity of the assay.

The spot hybidization assay was able to detect as little as 17

pg of virus when blots were autoradiographed for 107 hr (Table 1). This is equivalent to 3.2 pg of viral DNA, assuming SLCV is 19% nucleic acid, as shown for another geminivirus, bean golden mosaic virus (11). Exposures of 24 hr detected only 680 pg of virus (129 pg of viral DNA). Nonspecific binding of the purified uninoculated bean and the mixed probe occurred at only the lowest dilutions. The relative sensitivities of this assay and ELISA were examined. Purified SLCV-2 was diluted twofold in either PBS, for the ELISA, or in TE buffer, for the spot hybridization assay. Uninfected Topcrop tissue processed as if for a virus purification was used as a control. The comparison was conducted twice, with three sample replications per blot or plate, each time using recently purified virus. The least amount of virus that could be detected by the spot hybridization assay was 300–400-fold less than that by ELISA, depending on the length of exposure of the autoradiogram (Table 1). The limit of detection of ELISA was in the range of 50–100 ng of virus, while the limit of detection of the spot hybridization assay was 0.20–0.009 ng. The detection of SLCV-2 by ELISA and nucleic acid spot hybridization were compared over a range of purified virus dilutions, using data from experiment 2 of Table 1 (Fig. 1). The nucleic acid spot hybridization assay was able to detect virus at dilutions beyond the sensitivity of ELISA.

Samples of plants infected with different viruses were spotted onto membranes, hybridized to the mixed probe at 42 C, and rinsed under lower stringency conditions to determine the specificity of the mixed SLCV-DNA probe. The following viruses were detected with this assay: bean golden mosaic virus (Puerto Rico isolate), cotton leaf crumple virus (California isolate), euphorbia mosaic virus (Puerto Rico isolate), melon leaf curl virus (California isolate), mungbean yellow mosaic virus (India isolate), rhynchosia mosaic virus (Puerto Rico isolate), and squash leaf curl virus (California isolates SLCV-1 and SLCV-2). Zucchini yellow mosaic virus and lettuce infectious yellows virus, commonly found in the field with cucurbit geminiviruses, were not detected using this assay when samples known to be infected with these viruses were tested. No reactions were observed with any uninfected plant tissues representing 15 families and 26 genera. The following plants were experimentally inoculated with SLCV-2 by *B. tabaci*, and viral DNA was detected in bean (*P. vulgaris*), winter and summer squashes (*Cucurbita pepo*), cantaloupe and honeydew melon (*Cucumis melo* L. var. *reticulatus* and *inadorus*, respectively), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus vulgaris* Schrad.), *N. benthamiana*, white flowered gourd

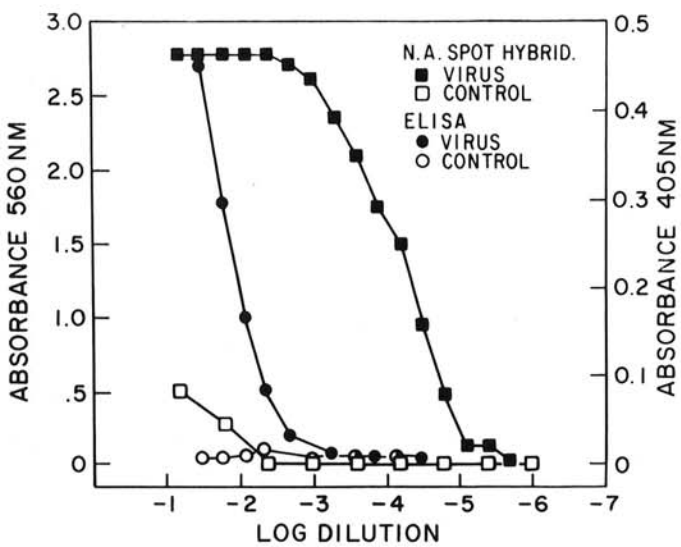

Fig. 1. Comparison of detection of dilutions of purified squash leaf curl virus (SLCV)-2 by enzyme-linked immunosorbent assay (ELISA) and nucleic acid spot hybridization. Each point represents the mean of three replications. The control treatments for ELISA and the nucleic acid spot hybridization assay are noninfected Topcrop tissue treated as if for a virus purification and diluted as if purified virus.

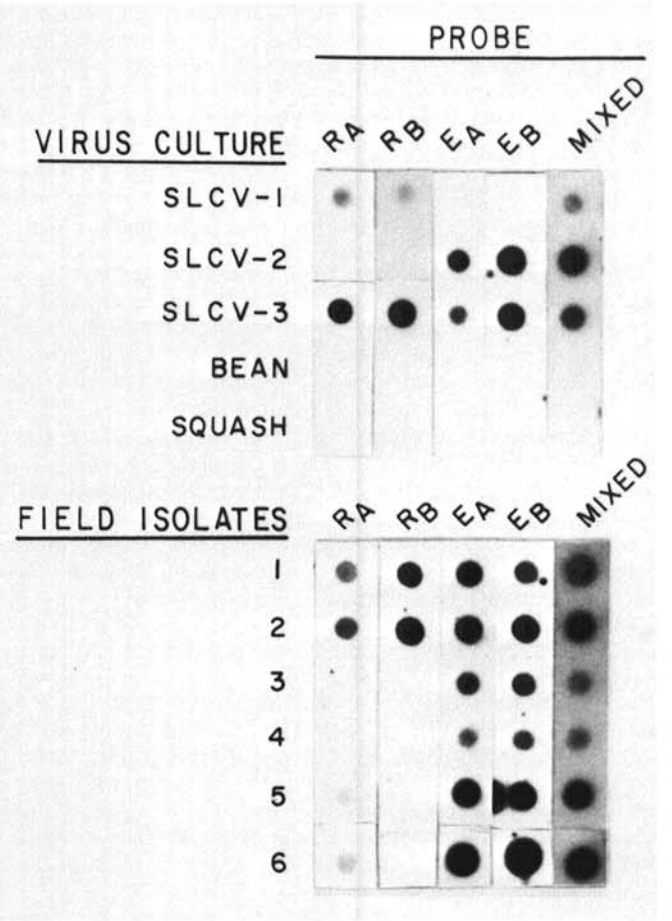

Fig. 2. Autoradiograph showing specificity of squash leaf curl virus (SLCV) component-specific clones in a nucleic acid spot hybridization assay. SLCV-1, a virus isolate described by Cohen et al (3), SLCV-2, a virus isolate similar in host range to melon leaf curl virus and watermelon curly mottle virus, SLCV-3, a virus isolate from which DNAs used as probes were isolated. Plant extracts were prepared as described in Materials and Methods section, and were spotted onto five different membranes, each of which was hybridized to a different probe. Hybridization and rinsing conditions are as described in Materials and Methods section. Membranes were exposed to X-ray film at −80 C for 19–28 hr. Genome component-specific probes are R_A, R_B, E_A, and E_B. The mixed probe is nonspecific for genome components.

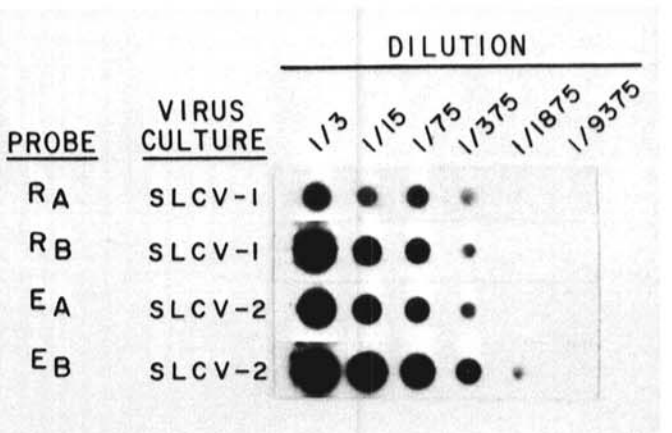

Fig. 3. Relative sensitivities of the component-specific probes: Comparison of probe R_A with probe R_B, and probe E_A with probe E_B using a series of fivefold dilutions of bean tissue infected with squash leaf curl virus (SLCV)-1 and SLCV-2, respectively. Plant extracts were prepared as described in Materials and Methods section, and were spotted onto five different membranes each of which was hybridized to a different probe. Hybridization and rinsing conditions are as described in Materials and Methods section. The specific activities of all four probes were 2.0×10^8 cpm/μg DNA. Blots were exposed to film at −80 C for 20 hr.

(*Lagenaria siceraria* (Mol.) Standl.), *Datura stramonium* L., *Euphorbia heterophylla* Desf., and *Rhynchosia minima* DC. No viral DNA was detected in pigeon pea (*Cajanus cajan* (L.) Millsp.), pepper (*Capsicum annuum* L.), cotton (*G. hirsutum*), *Macroptilium lathyroides* L., *Malvastrum coromandelianum* Regel, *N. rustica* L., *N. glutinosa* L., and mungbean (*Phaseolus aureus* Roxb.) inoculated with SLCV-2.

Detection of SLCV with genome component-specific probes. The component-specific probes were able to distinguish between the two squash leaf curl viruses in a spot hybridization assay (Fig. 2). Under conditions of high stringency, probes R_A and R_B hybridized only to the narrow host range strain, SLCV-1. Probes E_A and E_B hybridized only to the broad host range strain, SLCV-2. These component-specific probes did not hybridize under conditions of high stringency to DNA of two other geminiviruses tested, cotton leaf crumple virus (California isolate), or bean golden mosaic virus (Puerto Rico isolate). All four probes hybridized to the tissue from which the clones were originally derived, virus culture SLCV-3. These clones did not hybridize with uninoculated melon, squash, or bean samples.

The abilities of the component-specific probes to detect genomic components were determined by hybridization of probes to a fivefold dilution series of infected tissues; probes RA and RB to SLCV-1 and probes EA and EB to SLCV-2 (Fig. 3). Each probe was able to detect viral components in plant homogenate diluted as far as 1:375.

Six samples of squash were collected from different field plants and were analyzed with this assay (Fig. 2). Two samples reacted with all component specific and mixed probes, two samples reacted with the mixed probe and with two of the component specific probes (probes E_A and E_B), and two reacted with the mixed probe and three of the four component-specific probes (probes R_A, E_A, and E_B).

DISCUSSION

A nucleic acid hybridization assay was developed that could detect SLCV-2 nucleic acid in plant tissues from greenhouse and field collected plants. When the mixed probe was used the limit of detection was in the range of $1–2 \times 10^1$ pg virus ($1–2 \times 10^0$ pg of nucleic acid), which is similar to other reported values (16,22). This was 2 to 3 magnitudes less than of the absolute amount of virus detected at the endpoint of ELISA. The assay was specific for geminivirus DNA and showed only very slight reaction to highly concentrated extracts of uninfected bean tissue after extended exposures (greater than 100 hr).

One of the virus cultures used in this study, SLCV-2, has a host range similar to that reported for MLCV (8) and WCMV (3), infecting bean, squash, melons, and *N. benthamiana*, but not cotton. In addition to these shared plant hosts, SLCV-2 could infect the following plants not tested with MLCV or WCMV: white-flowered gourd (*L. siceraria*), *Datura stramonium*, *Euphorbia heterophylla*, and *Rhynchosia minima*.

TABLE 1. Comparison of sensitivity of detection of purified SLCV-2 by enzyme-linked immunosorbent assay (ELISA) and by nucleic acid hybridization assay with varying exposure times

		Nucleic acid hybridization assay			
Experiment	ELISA	18 hr[a]	24 hr	38 hr	107 hr
1[b]	98[c]	0.76[d]	···	0.38	···
2	76	···	0.68	···	0.017

[a] Exposure of blot to film in hours.

[b] Purified virus was diluted with TE buffer (0.01 M Tris, 0.001 M EDTA, pH 8.0), virus concentrations were established with an extinction coefficient of 6.0 $(mg/ml)^{-1}cm^{-1}$ at 260 nm (12). Each experiment used a different virus purification.

[c] ELISA values represent the endpoints of detection (ng of virus) of dilution series of purified SLCV-2 of known concentration. The endpoint was calculated from the mean A_{405nm} value of three samples as determined by a Bio-Tek EIA reader.

[d] Hybridization values represent the endpoint of detection (ng of virus) of a dilution series of purified SLCV-2 of known concentration. The endpoint was calculated from the mean of the maximum A_{560nm} values of three samples as determined by a DU-50 spectrophotometer.

The use of cloned unique restriction fragments of viral genome components allowed the development of an assay that could detect SLCV genomic components present in plant tissue extracts. The component specific probes could distinguish between two biologically different strains causing leaf curl disease of cucurbits. These two virus strains have coat proteins that could not be distinguished by ELISA, using SLCV-1 polyclonal antisera, or by symptomology in common hosts (J. E. Polston, *unpublished data*). In addition, the homologies of probes R_A and R_B to SLCV-1, and E_A and E_B to SLCV-2, suggest that SLCV-R and SLCV-1 are the same strain, and that SLCV-E and SLCV-2 are the same strain. This conclusion is further supported by host ranges of the strains.

Field-collected squash plant extracts were tested with the component-specific probes in the nucleic acid spot hybridization assay. Some plants contained detectable levels of all four viral components, indicating that mixed infections of these two strains do exist and can be resolved with the assays developed. Some plants contained detectable levels of the broad host range strain genome components. Unexpectedly, other plants contained detectable levels of only three components. This finding will require further study to better understand the incidence and significance of geminivirus mixed infections of more than two viral components.

The nucleic acid spot hybridization assay is useful for detection of SLCV in epidemiological studies. It can be adapted to the processing of large numbers of samples, can be used with a wide range of plant tissues and can possess the desired level of specificity including the detection of either single or multiple DNA genome components through the use of tailored probes. This assay may be the one of choices for epidemiology studies because of the low levels of virus present in field-infected plants, and because of the antigenic similarities present among the whitefly transmitted geminiviruses.

LITERATURE CITED

1. Avrameas, S., and Ternynck, T. 1969. The cross-linking of proteins with gluteraldehyde and its use for the preparation of immunoadsorbents. Immunochemistry 6:53-66.
2. Bajet, N. 1983. Detection of bean golden mosaic virus in host tissues and infectivity of its genome in bean mesophyll protoplasts. Ph.D. dissertation. University of Illinois, Urbana-Champaign, IL. 131 pp.
3. Brown, J. K., and Nelson, R. M. 1986. Whitefly-borne viruses of melons and lettuce in Arizona. Phytopathology 76:236-239.
4. Cohen, S., Duffus, J. E., Larsen, R. C., Liu, H. Y., and Flock, R. A. 1983. Purification, serology and vector relationships of squash leaf curl virus, a whitefly-transmitted geminivirus. Phytopathology 73:1669-1673.
5. Cooksey, D. A., and Moore, L. W. 1982. Biological control of crown gall with an Agrocin mutant of *Agrobacterium radiobacter*. Phytopathology 72:919-921.
6. Dodds, J. A., Lee, J. G., Nameth, S. T., and Laemmlen, F. F. 1984. Aphid and whitefly-transmitted cucurbit viruses in Imperial County, California. Phytopathology 74:221-225.
7. Duffus, J. E., and Flock, R. A. 1982. Whitefly-transmitted disease complex of the Desert Southwest. Calif. Agric. 36:4-6.
8. Duffus, J. E., Liu, H. Y., and Johns, M. R. 1985. Melon leaf curl virus—A new gemini virus with host and serological variations from squash leaf curl virus. (Abstr.) Phytopathology 75:1312.
9. Flock, R. A., and Mayhew, D. 1981. Squash leaf curl, a new disease of cucurbits in California. Plant Dis. 65:75-76.
10. Goodman, R. M. 1977. Single-stranded DNA genome in a whitefly-transmitted plant virus. Virology 83:171-179.
11. Goodman, R. M., and Bird, J. 1978. Bean golden mosaic virus. No. 192 in: Descriptions of Plant Viruses. Commonw. Myclol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England.
12. Goodman, R. M., Shock, T. L., Haber, S., Browning, K. S., and Bowers, G. R., Jr. 1980. The composition of bean golden mosaic virus and its single-stranded DNA genome. Virology 106:168-172.
13. Gordon D. T., Nault, L. R., Gordon, N. H., and Heady, S. E. 1985. Serological detection of corn stunt spiroplasma and maize rayado fino virus in field-collected *Dalbulus* spp. from Mexico. Plant Dis. 69:108-111.

14. Harrison, B. D. 1985. Advances in geminivirus research. Annu. Rev. Phytopathol. 23:55-82.
15. Howarth, A. J., and Goodman, R. M. 1986. Divergence and evolution of geminivirus genomes. J. Mol. Evol. 23:313-319.
16. Hull, R. 1984. Rapid diagnosis of plant virus infections by spot hybridization. Trends Biotechnol. 2:88-91.
17. Johnson, M. W., Toscano, N. C., Reynolds, H. T., Sylvester, E. S., Kido, K., and Natwick, E. T. 1982. Whiteflies cause problems for southern California growers. Calif. Agric. 36:24-26.
18. Lazarowitz, S. 1987. The molecular characterization of geminiviruses. Plant Molec. Biol. Rep. 4:177-192.
19. Lazarowitz, S. 1989. Molecular characterization of two bi-partite geminiviruses causing squash leaf curl virus: Role of transactivation and defective genomic components in determining host range. Virology (In press).
20. Lazarowitz, S., and Lazdins, Inara B. 1989. Infectivity and complete nucleotide sequence of the cloned genomic components of SqLCV-E, a bipartite squash leaf curl geminivirus with a broad host range phenotype. Virology (In press).
21. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
22. Maule, A. J., Hull, R., and Donson, J. 1983. The application of spot hybridization to the detection of DNA and RNA viruses in plant tissues. J. Virol. Meth. 6:215-224.
23. Nameth, S. T., Dodds, J. A., Paulus, A. O., and Laemmlen, F. F. 1986. Cucurbit viruses of California: An ever-changing problem. Plant Dis. 70:8-12.
24. Reed, K. C., and Mann, D. A. 1985. Rapid transfer of DNA from agarose gels to nylon membranes. Nuc. Acid Res. 13:7207-7221.
25. Roberts, I. M., Robinson, D. J., and Harrison, B. D. 1984. Serological relationships and genome homologies among geminiviruses. J. Gen. Virol. 65:1723-1730.
26. Robinson, D. J., Harrison, B. D., Sequeira, J. C., and Duncan, G. H. 1984. Detection of strains of African cassava mosaic virus by nucleic acid hybridization and some effects of temperature on their multiplication. Ann. Appl. Biol. 105:483-493.
27. Sequeira, J. C., and Harrison, B. D. 1982. Serological studies on cassava latent virus. Ann. Appl. Biol. 101:33-42.
28. Shapiro, S. S., and Wilk, M. B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52:591-611.