Journal of Invertebrate Pathology **78,** 220–225 (2001)
doi:10.1006/jipa.2001.5060, available online at http://www.idealibrary.com on IDEAL®

Discovering New Insect Viruses: Whitefly Iridovirus (Homoptera: Aleyrodidae: *Bemisia tabaci*)[1]

W. B. Hunter,*,[2] C. P. Patte,† X. H. Sinisterra,* D. S. Achor,‡ C. J. Funk,§ and J. E. Polston†

**USDA-ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, Florida 34945; †Gulf Coast Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 5007 60th Street E., Bradenton, Florida 34203; ‡Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, Florida 33850; and §USDA-ARS, Western Cotton Research Laboratory, 4135 E. Broadway Road, Phoenix, Arizona 85040*

E-mail: Whunter@ushrl.ars.usda.gov

Received May 4, 2001; accepted November 8, 2001

Adult whiteflies, *Bemisia tabaci* (Gennadius), collected from the field were screened for viral pathogens using a cell line from the silverleaf whitefly, *B. tabaci*, B biotype (syn. *B. argentifolii*). Homogenates from the field-collected whiteflies were applied to cell cultures and checked for cytopathic effects (CPE). Cells were observed to develop cytoplasmic inclusions and to have a change in morphology. Cells displaying CPE were observed using a transmission electron microscope and found to be infected with a virus. The virus particles had an icosahedral shape and an approximate size of 120–130 nm. The virus was observed in defined areas of the cytoplasm adjacent to the cell nucleus. Analysis using polymerase chain reaction, Southern blot hybridization, and DNA sequencing confirmed that the virus discovered infecting the whitefly cell cultures was an iridovirus. Sequence analysis showed that the amplimer (893 bp) had a 95% homology to the invertebrate iridescent virus type 6 major capsid protein gene. Discovery of new viruses of whiteflies may provide renewed interest in using pathogens in the development of innovative management strategies. This is the first report of an iridescent virus isolated from whiteflies, *B. tabaci*, collected from the field.

***Key Words: Bemisia;* biological insecticide; Hemiptera; Homoptera; iridovirus; lacewings; leafhoppers; pathogen; virus; whitefly.**

INTRODUCTION

Problems associated with pesticide use in agriculture have created an interest in the discovery of new biological control agents for the management of insect pests (Cahill *et al.*, 1996; Hunter-Fujita *et al.*, 1998; McLaughlin *et al.*, 1972). The whitefly, *Bemisia tabaci* (Gennadius), is a major pest on a wide variety of crops due to its feeding and acting as a vector for plant viruses. The *Bemisia* vector-species complex causes economic losses worldwide and throughout the southern half of the United States (Polston and Anderson, 1997; Polston *et al.*, 1999). Biological control methods are needed for this pest, but no entomopathogenic viruses have yet been discovered for this insect. Virus-like particles have been reported to occur within whiteflies, but their pathogenicity was not determined (Báo *et al.*, 1996; Costa *et al.*, 1996). Begomoviruses have been reported to bind to whitefly cells (Hunter and Polston, 2001); however, the only other report of a potential viral pathogen of whiteflies is a Begomovirus, tomato yellow leaf curl virus (TYLCV), a whitefly-transmitted, plant-infecting virus that has been suggested to have a slight pathogenic effect on whiteflies (Rubinstein and Czosnek, 1997). Discovery of new pathogens that might aid in the efforts of whitefly control are needed. Some of the problems facing the discovery of new pathogens are being able to locate, identify, and maintain pathogens that might otherwise be lost or overlooked in the wild due to the death and rapid decay of infected individuals. We developed a method whereby whitefly populations could be sampled for the presence of potential pathogens by screening homogenates on a whitefly cell culture. Herein, we report on a possible viral pathogen that was recovered from whiteflies collected from the field and identified using light and electron microscopy, Southern hybridizations, DNA sequencing, and polymerase chain reaction analysis.

[1] The mention or use of products within does not imply nor guarantee endorsement by the USDA to the exclusion of other similar products which may also be suitable.

[2] To whom correspondence should be addressed. Fax: (561) 462-5986. E-mail: Whunter@ushrl.ars.usda.gov.

0022-2011/01 $35.00

MATERIALS AND METHODS

Cells and Inoculation

A whitefly embryo cell line, developed from *B. tabaci* by Hunter and Polston (2001) was established by culturing late-stage embryos in medium consisting of 40.4% 62 mM L-histidine buffer (pH 6.2), 40.4% Schneider's *Drosophila* medium (Life Technologies, Inc., USA), 4% Medium 199, 10× (Life Technologies, Inc.), 2% CMRL medium-1066 (Life Technologies, Inc.), 1% penicillin–streptomycin solution (10,000 units per ml), and 12.2% fetal bovine serum (FBS; 26140-079; Life Technologies, Inc.). The medium used was after Kimura (Kimura, 1984). Cells were incubated at room temperature and were passaged at a 1:2 ratio every 7–10 days. Adherent cells were released from the tissue culture flasks using a nonenzymatic, cell dissociation solution (Sigma, USA).

Adult whiteflies collected from the area around Homestead, Florida were taken back to the laboratory on caged tomato plants. The whiteflies were prepared as follows: 20–30 whiteflies were rinsed with 70% ethanol and transferred to 1.5-ml Eppendorf centrifuge tubes. Samples were centrifuged with an Eppendorf microcentrifuge for 2 min at 14,000 rpm. The ethanol was replaced with 1 ml of L-histidine buffer (pH 6.2) and centrifuged again. The buffer was then replaced and centrifuged two more times, after which a few microglass beads were added plus 500 μl of L-histidine buffer and the whiteflies homogenized with a plastic pestle. The homogenate was then centrifuged for 3 min at 14,000 rpm. Under sterile conditions the supernatant was transferred to a sterile 1.5-ml centrifuge tube. One milliliter of fresh, fetal bovine serum (FBS)-free, medium was added to the supernatant, and the solution was mixed by inverting tubes 15–20 times. The solution was then dispensed through a sterile 0.45-μm syringe filter onto cell cultures in a 25-cm^2 flask, which had an additional 1 ml of fresh medium. The inoculum was left on cells for 8 h and then the medium was replaced with 4 ml of fresh medium containing FBS. The cells were observed daily with an inverted light microscope for evidence of cytopathic effects (CPE). If cells were observed to develop CPE or a change in morphology then they were processed and observed using a transmission electron microscope. Culture medium from infected cells was used as future inoculum after passed through a 0.45-μm filter.

Electron Microscopy

Samples from whitefly cell cultures were collected using dissociation solution to release adherent cells. Tissue culture cells were collected by centrifugation (3 min at 2000 rpm), resuspended in 1% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.2, and incubated 3 h at room temperature. The cells were then centrifuged and washed twice in the above buffer and either embedded in 10% gelatin or centrifuged into a tight pellet (3 min at 14,000 rpm). The pellets or gelatin pieces were placed in 3% glutaraldehyde in 0.1 M potassium phosphate buffer overnight in the refrigerator and then postfixed in 2% osmium tetroxide in the same buffer for 2–4 h at room temperature. Afterward the cells were washed four to six times in phosphate buffer, dehydrated in acetone, and embedded in Spurr's resin (Spurr, 1969). Thin sections were made on an LKB Huxley ultramicrotome (LKB-Produkter AB, Bromma, Sweden), mounted on uncoated 200-mesh copper grids, and stained with uranyl acetate (Stempak and Ward, 1964) and lead citrate (Reynolds, 1963). They were viewed and photographed with a Philips 201 transmission electron microscope (Philips Scientific & Analytical Equipment, Eindhoven, The Netherlands).

Molecular Analyses

DNA was extracted using an AquaPure Genomic DNA isolation kit, as per instructions (Bio-Rad Laboratories, Richmond, CA). Consensus primers were designed for PCR and sequencing based on a conserved region within the capsid protein gene from three Iridoviruses: IIV1, IIV6, and IIV22 (GenBank Accession Nos. M33542, M99395, and M32799, respectively) (Webby and Kalmakoff, 1998). Amplification by PCR was conducted with the consensus primers P1FOR (5′ ACY TCW GGK TTY ATC GAT ATC GCC ACT 3′) and P2REV (5′ TTR ATW GCA TGA GAG AAR CGA ATA TC 3′), corresponding to IIV6 major capsid protein nucleotide positions 25–52 and 892–917, respectively (synthesized by Life Technologies, Inc.). The PCR mix was 1 μl of DNA, 2 μl of primers (50 μM each), 3 μl of 25 mM $MgCl_2$, 45 μl of Supermix (Life Technologies, Inc.). Cycles were run in an automated Peltier Thermal Cycler (PTC 200; MJ Research).

The amplification protocol was as follows: denature cycle of 95°C for 10 min, 94°C for 2 min, 41°C for 2 min, 72°C for 5 min, followed by 30 cycles at denaturing at 94°C for 1 min, annealing at 41°C for 1 min, extension at 72°C for 3 min with a final cycle at 94°C for 1 min, 41°C for 1 min, 72°C for 5 min, hold at 4°C. A 20-μl sample of each reaction mixture was fractionated by electrophoresis in a 1% agarose gel in 1× TAE buffer and the fragments were stained with ethidium bromide. The ladder used was a wide-range DNA marker (16 fragments, 50–10,000 bp) (Sigma). The gel-purified 893-bp DNA fragment was automatically sequenced with an ABI Prism 310 genetic analyzer (PE Applied Biosystems, Foster City, CA) using the Dye Deoxyterminator-Tag cycle sequencing technique, as per instructions (PE Applied Biosystems).

Preparations for Southern Blot Hybridization

DNA Extractions. DNA was extracted from whitefly cells in a 25-cm^2 flask as follow. Cells were suspended with cell dissociation solution, pelleted, and resuspended in 300 μl DD solution (100 mM Tris–HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.2% 2-mercaptoethanol, 1% PVP 40) in a 1.5-ml tube. A small scoop of acid-washed glass beads, 12.5 μl of 10 mg/ml proteinase K (Boehringer Mannheim Corp., Indianapolis, IN), and 2 μl 10 mg/ml RNAse was added to the cell suspension. The cells were then ground with a small pestle and incubated at 37°C for 30 min. After incubation the cell solution was centrifuged and the supernatant transferred to a clean 1.5-ml tube and mixed with 500 μl phenol:chloroform:isoamyl alcohol solution (25:24:1). The mixture was centrifuged and the supernatant was collected and transferred to a clean 1.5-ml tube. The phenol:chloroform:isoamyl alcohol extraction was repeated three more times. After the last extraction 0.1 volumes of 3 M sodium acetate and 2.5–3 volumes of ethanol were added to the transferred supernatant. This was incubated for 1–16 h at −20°C and then centrifuged, and the pelleted DNA was rinsed with 75% ethanol and resuspended in 50 μl TE (10 mM Tris–HCl (pH 7.5), 1 mM EDTA).

DNA amplification for Southern blot hybridization. DNA was amplified using modified primers from Stohwasser *et al.* (1993) in a GeneAmp PCR System 9700 (PE Applied Biosystems). The modified sense primer (5′ GGT WWC GAT AAY ATG ATT GGW AAT GT 3′) and the modified antisense primer (5′ TKG TTK TAT TTC KWA CAG CAA AGA AWA R 3′) (Operon Tech., Inc., Alameda, CA) amplified a ~700-bp fragment in the AgIV iridovirus major capsid protein gene from the velvet bean caterpillar (*Anticarsia gemmatalis* Hübner) (Lepidoptera). The PCR mixture consisted of 3 μl extracted DNA, 5 μl Buffer II (Promega Corp., Madison, WI), 5 μl 25 mM $MgCl_2$, 1 μl DNTPs (Promega Corp.), 1 μl 50 mM Spermidine, 1 μl each 50 pM/μl primer, 0.2 μl *Taq* DNA polymerase, and 32.8 μl H_2O (Life Technologies, Inc.). The method used was an initial denaturing step at 94°C for 5 min followed by 30 cycles of denaturing at 94°C for 20 s, annealing at 42.5°C for 20 s, and extension at 72°C for 20 s, ending with an elongated extension step of 72°C for 7 min.

Southern blot hybridization. Amplified DNA was run on a 1% agarose gel in TAE (4.84 gm/L Tris–base, 1.142 ml/L Glacial acetic acid, 2 ml/L 0.5 M EDTA (pH 8.0)) for 1 h at 100V. The gel was soaked in 150 mM HCl for 15 min, rinsed with water, soaked an additional 45 min in 500 mM NaOH and 1.5 M NaCl, rinsed again with water, and soaked in 3 M NaCl and 1 M Tris (pH 7.5) for 60 min, changing the solution after 30 min. It was finally soaked for 15 min in 6× SSC (20× SSC: 350 gm/L NaCl, 176 gm/L sodium citrate, pH 7.0–7.5). The DNA was transferred to Hybond-N^+ (Amersham Pharmacia Biotech, Piscataway, NJ) nylon membrane after prewetting with water and then 1 M NaCl using a technique from Sambrook *et al.* (1989). After the overnight transfer the membrane was auto-crosslinked twice in a UV Stratalinker 1500 (Stratagene, La Jolla, CA), washed with water, and stored at 4°C until used.

The membrane was probed with a DNA fragment amplified from purified velvet bean caterpillar iridovirus (provided by Simon Scott, Clemson University) (Kinard *et al.*, 1995). The DNA fragment was amplified from the iridovirus major capsid protein gene using modified primers from Stohwasser *et al.* (1993) in a GeneAmp PCR System 9700 (PE Applied Biosystems). Fragment was labeled using High Prime DNA Labeling Kit (Roche Molecular Biochem., Indianapolis, IN) with [α-^{32}P]dCTP (specific activity 3000 Ci/mmol) (Amersham Pharmacia Biotech, Piscataway, NJ). The hybridization was carried out according to Polston *et al.* (1989).

RESULTS AND DISCUSSION

Cells inoculated with homogenates made from whiteflies collected in the field were observed to develop cytoplasmic inclusions and to change morphology. When observed using a transmission electron microscope, these cells showed virus particles (Fig. 1). The virus observed was nonenveloped, icosahedral, and similar in size to iridoviruses (Devauchelle, *et al.*, 1985; Williams, 1996). Examination of virions within infected cells allowed their size to be calculated to between 120 and 130 nm in diameter (Fig. 1d). As in other iridovirus infections, virus particles were observed in defined areas of the cytoplasm adjacent to the nucleus (Webby and Kalmakoff, 1998; Williams, 1996). Infected cells developed vesicles and other cytopathic effects similar to those described in Funk *et al.*, 2001, (Fig. 1e).

We attempted to amplify viral DNA using primers to iridoviruses as previously described and used by Webby and Kalmakoff (1998). These primers failed to amplify the viral DNA from whitefly extracts, which suggested either that the virus that we found in these whiteflies may not have been an iridovirus or that the virus was different from evaluated by Webby and Kalmakoff (1998).

To investigate further we focused on the major capsid protein genes from several iridoviruses which had been sequenced and found to contain high homologies (Cameron, 1990; Stohwasser *et al.*, 1993; Tajbakhsh *et al.*, 1990; Tidona *et al.*, 1998). The PCR primers that we designed were different from those used for both diagnostics and other sequencing projects (Cameron, 1990; Stohwasser *et al.*, 1993; Williams, 1993; Williams and Cory, 1994). Our primers for PCR analysis were made to span a conserved region within the

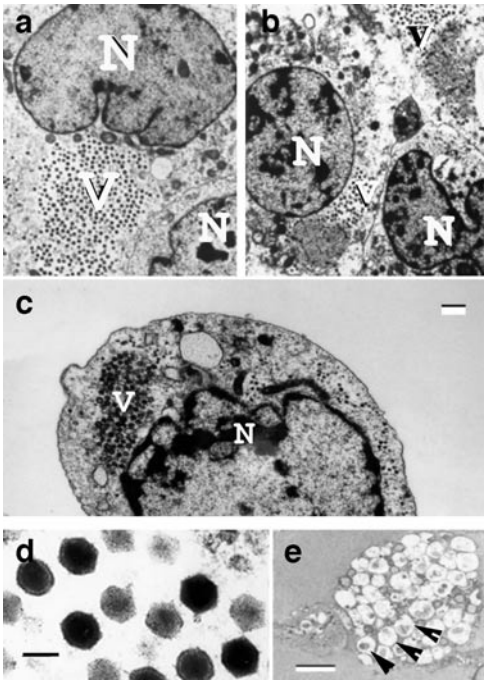

FIG. 1. Insect cells infected with an iridovirus discovered in whiteflies. (a) Iridovirus infection in leafhopper cell line, *Circulifer tenellus;* (b) iridovirus infection in lacewing cell line, *Ceraeochrysa cubana;* (c) iridovirus infection in whitefly cell line, *Bemisia tabaci,* B-biotype, iridovirus (V) in the cell cytoplasm adjacent to nucleus (N); (d) iridovirus in whitefly cell higher magnification, virions are ~120 nm; (e) whitefly cell infected showing increased vessicle formation (bars in (c) = ~600 nm, (d) = 120 nm, (e) = 6 μm).

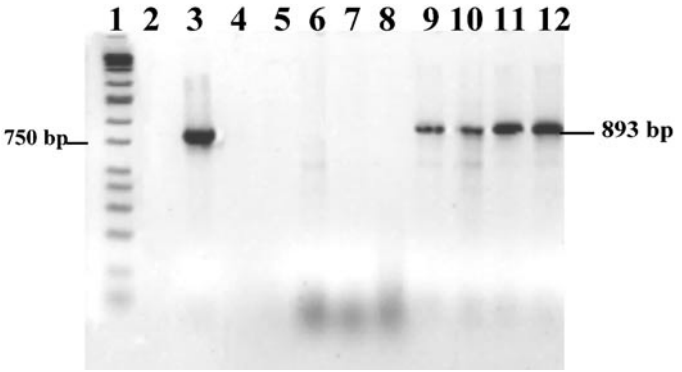

FIG. 2. PCR amplification using consensus primers to the major capsid protein gene designed to three iridoviruses. Cell samples were inoculated with homogenate made from adult whiteflies caught in the field. Lanes 1, ladder wide-range DNA marker (16 fragments, 50–10,000 bp); 2, blank; 3, purified IIV6 virus; 4, blank; 5, water; 6, adult whitefly (DNA from five adults extracted from a colony); 7, whitefly cell culture control; 8, leafhopper cell culture control; 9, leafhopper cell culture 15 days postinoculation with virus; 10–12, whitefly cells 15 days postinoculation with virus.

capsid protein genes posted in the GenBank database. The sequences of three insect iridoviruses were used to design the new primers: IIV1 originally isolated from a crane fly (Tipulidae: Diptera) species, *Tipula paludosa* Meigen (Xeros, 1954); IIV6 isolated from the rice stem borer (Pyralidae: Lepidoptera), *Chilo suppressalis* Walker (Fukaya and Nasu, 1966); and IIV22 isolated from a *Simulium* sp. (Simuliidae: Diptera) (Cameron, 1990). An 893-bp PCR product was generated with these primers and sequenced. Analysis of the sequence indicated that the amplimer was 95% homologous to the major capsid protein gene of IIV6 and 93% homologous to a sequence of the major capsid protein gene from the *Gryllus bimaculatus* iridovirus (Gryllidae: Orthoptera). This sequence similarity provides evidence that the virus belongs in the iridovirus classification.

As an alternative approach, we took an existing consensus primer set (Stohwasser *et al.*, 1993) and amplified a fragment of the AgIV major capsid protein gene, a characterized iridovirus from the velvet bean caterpillar, to use as a probe for Southern blotting. When the whitefly DNA was transferred to a nylon membrane and probed with the labeled amplified DNA from AgIV, a positive band was detected in virus-inoculated samples (Kinard *et al.*, 1995) (Figs. 2 and 3). All these results support the conclusion that there was an iridovirus infecting the whitefly cells.

Although we determined that the whitefly cells were infected with an iridovirus, we did not know whether it was a previously described iridovirus or a new discovery. The fact that the whitefly iridovirus DNA was not amplified by primers designed to characterize 18 diverse iridoviruses (Webby and Kalmakoff, 1998) suggests that this isolate may be a new iridovirus. Recent evidence has indicated that the whitefly cell line can support replication of iridescent virus IIV6 (Funk *et al.*, 2001), but the major capsid protein gene sequence derived from the whiteflies indicated that the whitefly isolate differs from the IIV6 (Kinard *et al.*, 1995). Further molecular characterization, such as sequencing

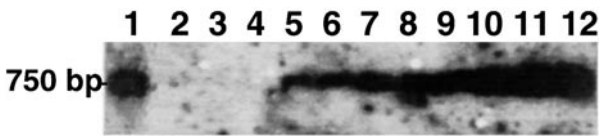

FIG. 3. Southern blot of cell line DNA vs whitefly egg and adult DNA. Lane 1, purified iridovirus, AgIV, from *A. gemmatalis;* lane 2, *B. tabaci* egg DNA from colony; lane 3, cell line *Circulifer tenellus* leafhopper DNA; lane 4, *Ceraeochrysa cubana,* lacewing cell culture DNA; lane 5, cell line *Circulifer tenellus* DNA inoculated with medium from primary infected cell line; lane 6, cell line *Ceraeochrysa cubana* DNA inoculated with medium from primary infected cell line; lanes 7–12, cell line *B. tabaci* DNA inoculated with medium from primary infected cell line. Gel of amplified DNA was amplified with modified consensus primers from Stohwasser *et al.* (1993), ran on a 1% agarose gel, and then transferred to nylon. The nylon blot, when probed with purified iridovirus from *A. gemmatalis,* produced a positive band of the predicted 750-bp size in lane 1, positive control, and lanes 5–12. Cells were inoculated with medium from primary infected cells for 1 h and then maintained in fresh medium until 10 days postinoculated.

the genome, is being conducted to answer these questions. Other insect iridoviruses, like IIV6, have been reported to be of particular economical importance since they can infect many important insect pests (Ward and Kalmakoff, 1991; Williams, 1996). They have also reported that cell lines infected with iridoviruses release virus by budding, extrusion of vacuoles, or cell lysis. This led us to evaluate the possibility of infecting two other insect cell cultures, a leafhopper cell line (Wayadande and Fletcher, 1998) and a lacewing cell culture (W. B. Hunter, unpublished). We found that by taking the medium from virus-infected cell cultures and sterilizing the medium through a 0.45-μm filter, this inoculum could produce viral infections in these other cell lines (Figs. 1a and 1b).

Although modes of transmission and persistence of iridoviruses in nature are still unknown (Williams, 1996) in whiteflies, the modes of transmission may depend on being passed through the vast amounts of excreta or honeydew produced. Possibly, when other whiteflies land on the virus-contaminated leaves, they may pick up virus *per os* either through labial dabbing or through grooming behaviors, which might allow entry through the trachea. However, these hypotheses need further testing before we truly understand the mode(s) of transmission for this iridovirus in whiteflies.

One of the major benefits in using cell cultures to screen for pathogens is the ability to detect pathogens that cause patent infections (infections that result in insect or cell death) and those which cause covert infections (infections that do not cause rapid insect death). Many covert infections in insect pests go unnoticed and unexploited due to the lack of obvious symptoms (Williams, 1993; Mariana *et al.*, 1999). The availability of novel tools for the detection of covert infections and the discovery and characterization of new virus pathogens to whiteflies infuses a renewed interest in using insect viruses in the development of innovative molecular tools and/or management strategies.

ACKNOWLEDGMENTS

Thanks go to Dr. J. Fletcher and Dr. A.Wayadande, Oklahoma State University, for use of a leafhopper cell line; Dr. S. Scott, Clemson University, for providing an iridovirus-amplified DNA fragment; Dr. C. Betz for assistance with primer design; and Drs. E. Davidson, D. Schuster, J. Price, and H. Costa for critical comments. This research was supported in part by the Fred C. Gloeckner Foundation and the Bedding Plants Foundation, Inc.

REFERENCES

Báo, S. N., Kitajima, E. W., Callaini, G., and Darai, R. 1996. Virus-like particles and rickettsia-like organisms in male germ and cyst cells of *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Invertebr. Pathol.* **67,** 309–311.

Cahill, M., Gorman, K., Kay, S., and Denholm, I. 1996. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Entomol. Res.* **86,** 343–349.

Cameron, I. R. 1990. Identification and characterization of the gene encoding the major structural protein of the insect iridescent virus type 22. *Virology* **178,** 35–42.

Costa, H. S., Westcot, D. M., Ullman, D. E., Rosell, R. C., Brown, J. K., and Johnson, M. W. 1996. Virus-like particles in the mycetocytes of the sweetpotato whitefly, *Bemisia tabaci* (Homoptera, Aleyrodidae). *J. Invertebr. Pathol.* **67,** 183–186.

Devauchelle, G., Attias, J., Monnier, C., Barray, S., Cerutti, M., Guerillon, J., and Orange-Balange, N. 1985. *Chilo* iridescent virus. *Curr. Top. Microbiol. Immunol.* **116,** 37–48.

Fukaya, M., and Nasu, S. 1966. A Chilo iridescent virus (CIV) from the rice stem borer *Chilo suppressalis* Walker (Lepidoptera, Pyralidae). *Appl. Entomol. Zool.* **1,** 69–76.

Funk, C. J., Hunter, W. B., and Achor, D. S. 2001. Replication of insect iridescent virus 6 in a whitefly cell line. *J. Invertebr. Pathol.* **77,** 144–146.

Hunter, W. B., and Polston, J. E. 2001. Development of a continuous whitefly cell line [Homoptera: Aleyrodidae: *Bemisia tabaci* (Gennadius)] for the study of begomovirus. *J. Invertebr. Pathol.* **77,** 33–36.

Hunter-Fujita, F. R., Entwistle, P. F., Evans, H. F., and Crook, N. E. 1998. Characteristics of insect pathogenic viruses. *In* "Insect Viruses and Pest Management," pp. 1–26. Wiley, New York.

Kimura, I. 1984. Establishment of new cell lines from leafhopper vector and inoculation of its cell monolayers with rice dwarf virus. *Proc. Jpn. Acad.* **69**(B), 198–201.

Kinard, G. R., Barnett, O. W., and Carner, G. R. 1995. Characterization of an iridescent virus isolated from the velvetbean caterpillar, *Anticarsia gemmatalis. J. Invertebr. Pathol.* **66,** 258–263.

Mariana, C. F., Arredondo-Jimenez, J. I., Castillo, A., and Williams, T. 1999. Sublethal effects of iridovirus disease in a mosquito. *Oecologia* **119,** 383–388.

McLaughlin, R. E., Scott, H. A., and Bell, H. R. 1972. Infection of the boll weevil by Chilo iridescent virus. *J. Invertebr. Pathol.* **19,** 285–290.

Polston, J. E., and Anderson, P. K. 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the western hemisphere. *Plant Dis.* **81,** 1358–1369.

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

Polston, J. E., McGovern, R. J., and Brown, L. G. 1999. The appearance of tomato yellow leaf curl virus in Florida and implications for the spread of it and other geminiviruses in the U.S. *Plant Dis.* **83,** 984–988.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron dense stain for electron microscopy. *J. Cell Biol.* **17,** 208–212.

Rubinstein, G., and Czosnek, H. 1997. Long-term association of tomato yellow leaf curl virus (TYLCV) with its whitefly vector *Bemisia tabaci:* Effect on the insect transmission capacity, longevity and fecundity. *J. Gen. Virol.* **78,** 2683–2689.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. "Molecular Cloning," 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26,** 31–43.

Stempak, J. G., and Ward, R. T. 1964. An improved staining method for electron microscopy. *J. Cell Biol.* **22,** 697–701.

Stohwasser, R., Kaab, K., Schnitzler, P., Janssen, W., and Darai, G. 1993. Identification of the gene encoding the major capsid protein of insect iridescent virus type 6 by polymerase chain reaction. *J. Gen. Virol.* **74,** 873–879.

Tajbakhsh, S., Lee, P. E., Watson, D. C., and Seligy, V. L. 1990. Molecular cloning, characterisation, and expression of the *Tipula* iridescent virus capsid gene. *J. Virol.* **64,** 125–136.

Tidona, C. A., Schnitzler, P., Kehm, R., and Darai, G. 1998. Is the major capsid protein of iridoviruses a suitable target for the study of viral evolution. *Virus Genes* **16,** 59–66.

Ward, V. K., and Kalmakoff, J. 1991. Invertebrate Iridoviridae. *In* "Viruses of Invertebrates" (E. Kurstak, Ed.), pp. 197–226. Dekker, New York.

Wayadande, A. C., and Fletcher, J. 1998. Development and use of an established cell line of the leafhopper *Circulifer tenellus* to characterize *Spiroplasma citri*—Vector interactions. *J. Invertebr. Pathol.* **72,** 126–131.

Webby, R., and Kalmakoff, J. 1998. Sequence comparison of the major capsid protein gene from 18 diverse iridoviruses. *Arch. Virol.* **143,** 1949–1966.

Williams, T. 1993. Covert iridovirus infection of blackfly larvae. *Proc. R. Soc. London B.* **251,** 225–230.

Williams, T. 1996. The Iridoviruses. *Adv. Virus Res.* **46,** 345–413.

Williams, T., and Cory, J. S. 1994. Proposals for a new classification of iridescent viruses. *J. Gen. Virol.* **75,** 1291–1301.

Xeros, N. 1954. A second virus disease of the leatherjacket, *Tipula paludosa*. *Nature* **174,** 562–563.