United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Plant Protection
and Quarantine

July 1996

New Pest Response Guidelines

Whitefly Borne Geminiviruses Family Geminiviridae

Developed by: Plant Protection Laboratories

Prepared by: Jeffrey N. L. Stibick

Date: July 1996

CONTENTS

PURPOSE AND DISCLAIMER

Purpose

These New Pest Response Guidelines provide information concerning actions for mitigating the impact of any of the geminiviruses borne by the sweetpotato whitefly (SPWF), more recently known as the silverleaf whitefly (SLW).

These Guidelines are to be used as an aid for States when developing State action plans. The procedures described in these New Pest Response Guidelines were developed by Plant Protection and Quarantine (PPQ), Plant Protection Laboratories (PPL) staff through discussion, consultation, or agreement with other Animal and Plant Health Inspection Service (APHIS) staff, the Agricultural Research Service (ARS), and university advisors.

Disclaimer

This document is not intended to be complete and exhaustive. The information given herein was taken from some of the available literature and synthesized into a specialized document intended to assist further work, as stated above. Some key articles were not available at the time this was written, nor have all pertinent specialists and other members of the research community been consulted for their advice.

GENERAL INFORMATION

Action Statement

The information contained in this document is intended for use as guidance in designing a program to detect and respond to an infection of any of the geminiviruses vectored by whiteflies (whitefly borne geminiviruses [WBGV]). These New Pest Response Guidelines provide information on implementing detection and control procedures for any phase of a WBGV detection, control, containment, or eradication program. Specific emergency program action must be based on information available at that time.

Initial Program Procedures

The following steps should serve to initiate program efforts and should be kept in mind throughout the beginning stages.

Step 1—Identification and Detection:

It will be most important to determine the identification and detection procedures that will be used throughout the program. Options that may be used are given under Identification Procedures and Addenda 4 and 8 of this document.

Step 2—Determining the Scope of the Problem:

It will be necessary to determine the extent of the infestation and the difficulties faced by program managers during a good survey and to identify the biological (Addendum 7, Life History) and practical realities in advance of any active program to control, suppress, or eradicate a given geminivirus.

Step 3—The Response: No Action to Eradication:

The effectiveness of the various control options should be considered, including regulatory action (see Regulatory Procedures), available options for control or suppression of the vector population, and destruction or treatment of the hosts (see Control Procedures and Addendum 5). From this information, and in the light of available information and resources, a decision must be made either to take no action (when a program is impractical, i.e., the infested area is too large for practical control measures, the technology is not available to achieve the objectives, proper funding is not available) or to control, suppress, or eradicate the viral population, if possible (see Control Procedures, Selection of Options and No Action, for decision options).

Background Information

Subgroup III geminiviruses are all transmitted by the sweetpotato whitefly (SPWF) with several possible exceptions. Interest in these viruses has increased as a result of the appearance of the tomato mottle virus (TMoV) in Florida, which caused an epidemic of serious proportions (Polston et al., 1993). This epidemic was preceded by an outbreak of three diseases in Arizona and California in 1981, caused by a sudden populational increase of biotype A of SPWF. This biotype proliferated in large numbers because of insecticide use and caused losses in excess of $100 million (Meyerdirk et al., 1986). A newly recognized biotype, biotype B, invaded Florida and has subsequently been found in most areas of the world. Biotype B is considered by some to be a previously unrecognized species of *Bemisia*, now named *B. argentifolii*,

Background Information (continued)

the silverleaf whitefly (SLW; Bellows et al., 1994). This position remains in dispute, although there are some biological and morphological differences between the biotypes. The vector potential of the silverleaf whitefly for transmitting the geminiviruses has not been fully determined.

Three of the above diseases are caused by subgroup III geminiviruses, and intensive study led to the discovery of others (i.e., Polston and Bois, 1994). These viruses are nonenveloped viruses whose particles are in the form of two icosahedra and consist of two molecules of circular double-stranded and separately encapsidated DNA (Francki et al., 1991). They induce characteristic cytoplasmic inclusions in the form of aggregates of particles and ring-shaped fibrillar bodies (as they appear in Electron Microscope (EM) sections), with segregation of the nucleoli of affected cells.

In SPWF, geminiviruses are transmitted in a persistent manner. The virus may be retained when the vector moults, depending on the geminivirus involved. Optimum-acquisition feeding is generally greatest for 1-day-old SPWF, and inoculation-access feeding is generally greatest for 3-day-old SPWF. The virus can be lost or it may be transmitted in a persistent manner up to the life of the adult insect. The virus does not multiply in the vector and is not transmitted directly to progeny (Brunt et al., 1990). A helper virus is not required for transmission. The virus is not transmitted by touching of plants, by seed, or by pollen; it can be transmitted by grafting or mechanical inoculation.

WBGV are distributed worldwide in tropical and subtropical areas, corresponding to the distribution of SPWF.

Life Cycle Information of Vector

Vector development is temperature dependent. Egg, nymphal, and adult development of the SPWF is influenced by air temperatures and may also be influenced by the host. There is a minimum temperature threshold below which no measurable development takes place.

For SPWF *Bemisia tabaci* (Gennadius), this threshold is 50.0 °F (10 °C) in air (Zalom et al., 1985). A temperature model that is designed to use modified air temperature data for all arthropod stages can be used to predict the entire life cycle. A number of degrees accumulated above the developmental threshold for a life stage is called day degrees. One day degree (DD) is 1 day with the average temperature 1° greater than the threshold for development.

An upper threshold of 89.96°F (32.2°C) must be factored into the equation (if necessary). For the model depicted inTable 1-1, 600.8 DD in Fahrenheit (316 °C) must be accumulated before one life cycle has been completed.

Life Cycle Information of Vector (continued)

***Table 1-1*: Day degree calculations**

Formula:

Minimum Daily		Maximum Daily		Total		Average Daily		Thresholds		Day Degrees
Temp °F	+	Temp °F	=	Temp °F	=	Temp °F	=	Temp °F		-
				# of DD				2		

Example: (Air Temperature Model with 50.0 and 89.96 °F thresholds)

Minimum Daily		Maximum Daily		Total		Average Daily		Thresholds		Day Degrees
54 °F	+	74 °F	=	128 °F	=	64 °F	-	50 °F	=	14.0 DD
				2						

The average daily temperature did not rise above the upper threshold. Had that happened, the DD gain would have been 39.96 DD (the difference between 50 and 89.96).

Program actions are governed in part by vector life cycle data. Control or eradication treatments, length of survey activities, and regulatory functions are affected primarily by the length of time it takes for the vector to complete its life cycle.

Temperature data are available from the National Oceanic and Atmospheric Administration, the U.S. Department of Commerce, private, State, university, or industry sources, or from remote site weather monitoring stations run by any of the above. Unforeseen delays in completion of the life cycle must be anticipated.

IDENTIFICATION PROCEDURES

Correct and proper identification is the key to determining whether a program will be attempted and, if so, the extent, direction, and magnitude of the program. It will also help determine program changes and program failures. The decision to discontinue a program will very likely be the result of a determination that program efforts are not succeeding, based on identifications of perceived viral spread or finds.

Identification Characters

General Description of the Geminiviridae—Subgroup III:

Particles—Geminate particles, about 18×30 nm in length, consisting of two incomplete icosahedra, with T = 1 surface lattice with a total of 22 capsomers. The nucleic acid consists of two molecules of circular single-stranded DNA, each MW = $7–8\times10^5$ (2.4–2.8 kb). There are open reading frames on both the viral strand and its complement (Francki et al., 1991).

Inclusions—Large, azure-A stained blue-violet nuclear inclusions, aggregates of particles in vascular tissues, and ring-shaped fibrillar bodies are characteristic (Christie et al., 1986).

Nucleolus—The nucleoli of affected cells are segregated (Christie et al., 1986).

See Figures 2-1 through 2-2.

Collection of Specimens

As many specimens as possible of suspect plant samples should be collected for screening or identification by the local designated identifier.

Suspect whitefly vectors are collected for identification at the sample site.

Preservation and Shipment of Samples:

The following procedures, with some modifications, were developed for the Canadian/U.S.A. Potato Y virus (PVY^n) Management plan. In general, this procedure may be followed for leaf samples. Different procedures may be necessary for other plant parts and for vectors. Field procedures also may differ depending on the identification technique used.

The survey, in this case, will be biased toward sampling plants that have symptoms.

Shipment of Leaves for Laboratory Diagnosis:

1. The terminal three leaves will be sampled from each selected plant. Preferably, leaves should be young. Only plants with suspected geminivirus infections should be sampled. Leaves of infected plants have symptoms such as vein clearing; golden or green mosaic; and leaf stunting, crinkling, curling, or other distortions. Plants with questionable symptoms as well as healthy plants (for comparison) should also be collected, and the symptoms should be carefully described on the sampling form. See Figures 2-3 through 2-4.

Collection of Specimens (continued)

2. Plant samples should be bagged individually in resealable bags that keep each sample separate (*no compositing*).

3. The leaf samples should be cooled (BUT NOT FROZEN) to 5 °C as soon as possible. This should be done within several hours of picking (particularly on warm days). If ice packs (–15 to –20 °C) are used, they should be insulated with two or three layers of paper or other packing material and placed in the middle or top of the cooler. Two 6" ×6" ice packs per cooler are usually sufficient. Avoid packing the leaves too tightly.

If samples are to be transported or stored for extended periods of time (greater than 1–2 days), it is better to dry the leaves and rehydrate them when ready to assay or extract to identify the virus.

4. If the leaves are to be shipped to the laboratory by courier, the leaves should be held overnight in refrigerated storage. For shipment, the bags of leaves should be packed loosely in Styrofoam containers and placed in cardboard boxes. An ice pack should be included, but it should be sufficiently insulated with paper so as not to freeze any leaves.

If shipment is interstate, a permit may be required for interstate movement. The need for the permit must be stated, and proper procedures must be followed.

5. A complete list of contents should be placed on the top of the samples or with the bill of lading (if an overnight courier is used) and signed (if possible) by the collector.

6. A field log of sampling dates, samples submitted, etc., is recommended to ensure sample continuity from the field to the laboratory.

7. Shipment of samples should be postponed if it is apparent that the package will be held in transit over a holiday or a weekend.

8. Regular communication (e.g., phone or fax) between collectors and the destination laboratory is strongly recommended to optimize the use of testing resources.

9. Initial identification should be confirmed with more than one technique (if possible). If confirmatory testing (after screening test) is to be performed at another laboratory, the leaf samples should be placed in good-quality bags and then packed and shipped as above.

Identification Techniques

The identification technique(s) used for a given program should be appropriately sensitive, accurate, rapid, and suitable for the specific situation. To achieve these ends, any single technique or combination of techniques may be used. In most cases, initial identification of a possible find should be followed by specific identification, to ensure the accuracy of the process.

Identification Techniques (continued)

Before identification can proceed, it is necessary to assess the quality of the sample. The following classification scheme may be used as a guide:

Good—Sample tissue contains no broken down tissue and the entire sample is in good condition.

Fair—Sample tissue is almost completely intact with some breakdown evident.

Poor—Sample tissue contains some breakdown, but intact tissue is present from each sample and can be bioassayed.

Very Poor—Sample tissue is largely broken down with no intact tissue from each sample (such samples should NOT be bioassayed).

Procedures for Identification:

The following are various procedures for identification. The technique selected for a given whitefly borne geminivirus (WBGV) may depend on program needs and goals.

1. Identification—Trap Plant
   a. Host(s)

   Discovery of known symptoms in a host is a good indicator, especially if the vector(s) is present.

   b. Inclusion Bodies

   The discovery of characteristic inclusion bodies in samples of known hosts can be a good indicator.

   The use of a good light microscope to study or confirm any of the above observations may, under program conditions, be used to verify finds, if it is certain that no other viral pest is likely to be confused with the virus in question (Christie et al., 1986).

Protocol for the Azure-A Staining Method for the Detection of WBGV Inclusions:

- Strip epidermal tissue or cut paradermal sections.
- Place in vial with staining basket containing a mixture of 0.2 M Na_2PO_4 in Double Distilled H_2O (1 part) and 0.1 percent Azure-A in 2 methoxyethanol (9 parts). Be sure to mix well, and do not use a volume that more than half fills the staining basket.

Identification Techniques (continued)

- Allow to stand at room temperature for 10 minutes. Then place vial in a water bath at 60 °C for an additional 1 or 1½ minutes.
- Transfer to 95 percent ethanol for two or three changes of about ¼ minute each.
- Mount in Euparal on slide and cover with cover glass.
- Press with lead weight and allow to stand for a few minutes before trying to observe in the microscope. The ring-shaped inclusions will be faintly stained blue-green in Azure-A (Matthews, 1993).

When properly stained, most inclusions can readily be detected with a light microscope. Light microscopic recognition of inclusion types offers a reliable and practical method for identifying virus diseases at the virus group level and can often lead to a specific diagnosis when the virus host range is considered (Christie & Edwardson, 1986).

2. Advanced Laboratory Techniques

The following include techniques completed in the laboratory. Unless it is possible to fully verify a find through inclusion bodies, one or more of these methods may be used as the final authority for a find. At this time, it is felt that symptoms are not reliable for making a diagnosis.

Polymerase chain reaction (PCR) and molecular hybridization (MH) could be used with probes to identify geminiviridae in general or, in some cases, with a specific probe to identify the virus of concern (Gilbertson et al., 1991; Polston et al., 1989).

a. Enzyme-Linked Immunosorbent Assay

This procedure, called ELISA for short, is currently the easiest laboratory detection method for most geminiviruses and is generally applicable for most virus detection with available virus-specific or group-specific antisera. A sample of the plant part most likely to contain the virus must be collected from hosts, especially from parts showing suspect symptoms, and sent to the laboratory along with full collection data (Klein & Wyatt, 1989).

In general, a small sample of the specimen is ground in a buffer and incubated in a specimen microtiter plate for a few hours before adding an enzyme-labeled monoclonal antibody. The sample is incubated for a few more hours with the final addition of a color-forming substrate subsequently quantitated in an ELISA plate reader.

Identification Techniques (continued)

In some cases, woody specimens may have to be established or grafted to plant hosts for weeks or months from germplasm to increase the pathogen titer to determine the presence of a given virus in a diagnostic array. This particular procedure is not recommended for a program if samples from suspect host plants can be processed and determined within a reasonable time frame (Bravo-Almonacid et al., 1992).

b. Squash Blot Molecular Hybridization (Bravo-Almonacid et al., 1992).

The use of genomic libraries is essential to this technique. Clones can be obtained from appropriate researchers or can be prepared from the samples (with time and effort). Double-stranded DNA is obtained by plasmid purification and restricted with endonucleases to liberate the inserts. These inserts are subjected to electrophoresis in agarose gels, and cDNA viral fragments are obtained. These fragments are in turn purified by electroelution and labeled with radioisotope or nonradioactive labels through a random oligonucleotide priming method. The samples are then subjected to molecular hybridization with these fragments. Using a specific clone, even a small amount of nucleic acid can be detected. For instance, with PVY^n about 1 pg of viral DNA can be detected after an 8-hour radiographic treatment. For squash leaf curl virus (SLCV), the limit of detection is 3.2 pg of viral DNA (Polston et al., 1989). This procedure is therefore more sensitive than ELISA for virus detection.

c. Polymerase Chain Reaction

The most sensitive identification uses PCR technology. This technique amplifies a virus' unique nucleic acid sequence and makes enough additional copies of it for quick and reliable detection. Amplification takes as little as 3–5 hours, and the results are available within 1–2 days.

d. Direct Tissue Blot Immunoassay

The direct tissue blot immunoassay (DTBI) is an immunoassay technique that uses direct blotting of plant or animal tissue onto nylon (preferred; Navot et al., 1989) or nitrocellulose membranes. The assay is specific, sensitive, reliable, and rapid. Large numbers of samples may be assayed in this way. The technique precisely locates nucleic acids present in plant hosts or animal tissue. The blots can be carried out in the field with just a few instructions and then transported to diagnostic laboratories for processing. If blots are stored properly, the antibodies can still be detected for at least a month after the sample is taken. Blots can be stored for long periods after processing (Bravo-Almonacid et al., 1992; Hsu et al., 1993).

Identification Techniques (continued)

Field Procedures (Navot et al., 1989):

Plants—Leaves, flowers, and other plant parts may be squashed onto a dry nylon membrane, using a hard object such as a glass rod or pen. Stems are cut longitudinally or sliced serially from the apex to the crown and squashed. Fruits are cut open and imprinted on the membrane. See Addendum 4, Nylon Membrane Preparation and Squashing Procedures, for further information.

Whiteflies—Carry live to the laboratory for immediate freezing at -20 °C. When frozen, vector bodies may be squashed on a nylon membrane as above. An alternate and superior procedure, made possible because of the small size of whiteflies, is to grind them with the pointed end of a 0.4-mL microfuge tube in a 1.5-mL microfuge tube with 0.025 mL of TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0) (Polston et al., 1990).

SURVEY PROCEDURES

The survey effort is to determine the extent of viral spread and the means by which the virus is being spread. Aside from determining where the sweetpotato whitefly (SPWF) (silverleaf whitefly [SLW]) may have spread the virus, human and other natural means of dispersal also must be considered. Such pathway dispersal must be factored into an active survey if it is not adequately covered under Regulatory Procedures.

Vectors

Bemisia tabaci biotypes are the primary vectors. No other vectors are known, but it is possible that such vectors could be found, especially in a newly invaded area. For that reason, it may be necessary to determine whether any local whitefly vectors also could transmit the target virus. Aside from direct squash blot assay from field-collected possible vectors (see Addendum 8, Identification Techniques), trials may be necessary to determine local transmission of whitefly borne geminivirus (WBGV) (i.e., see Webb and Kok-Yokomi, 1993). In the meantime, the following parameters shall govern vector aspects of the survey:

1. If the vector is a biotype of SPWF and it is determined that no other vector(s) or suspect vector(s) are present, then the survey will be based on that vector.

2. If an SPWF biotype is present and other suspect vectors are also present, the SPWF biotype vector(s) takes priority in survey activities until competent investigation eliminates or confirms one or more additional suspect vectors.

3. If a vector is not known, then suspect vectors shall be monitored until competent investigation eliminates or confirms one or more suspect vectors. (Presently, three species of WBGV are known to occur in the absence of a vector, under circumstances that are not entirely clear.)

Once the vectors are verified, they may be rated in effectiveness. However, because even an inefficient vector can transmit WBGV, all vectors must be monitored for the purposes of an active program.

Detection Survey

Cross transit surveys are recommended for a rapid detection survey for WBGV. This type of survey also will be used in support of a delimiting survey (see Delimiting Survey below). The survey proposed here is biased toward the primary host(s) of concern and in areas where the WBGV, if introduced, might be expected to be found first. Owing to the possibility of air dispersal of the vector, a special survey may be warranted for certain high-risk downwind areas.

There are three types of high-risk areas to cover in this survey:

1. Commercial Host Production Areas—Those areas where commercial hosts are grown.

2. Downwind Areas—Those areas where winds may reasonably be expected to carry the vector(s) from areas where the WBGV already exists.

Detection Survey (continued)

Local movement by whiteflies is the most likely means of natural dispersal. Whiteflies are weak fliers and generally move by surface air along the ground in individual adult flights up to about 20 miles or so. There is the possibility of long distance movement, but no anecdotal evidence is available at present (Byrne & Bellows, 1991; Byrne et al., 1994).

3. Residential Areas—Major cities and towns where residents and visitors may be expected to travel to and from areas where the WBGV already exists.

Delimiting Survey

When one or more WBGV finds are confirmed in an area, a delimiting survey should be implemented immediately to determine the population distribution. Using the site of the detection as the epicenter (focal point), the survey should use the following methods to delimit the prevalence of the pathogen.

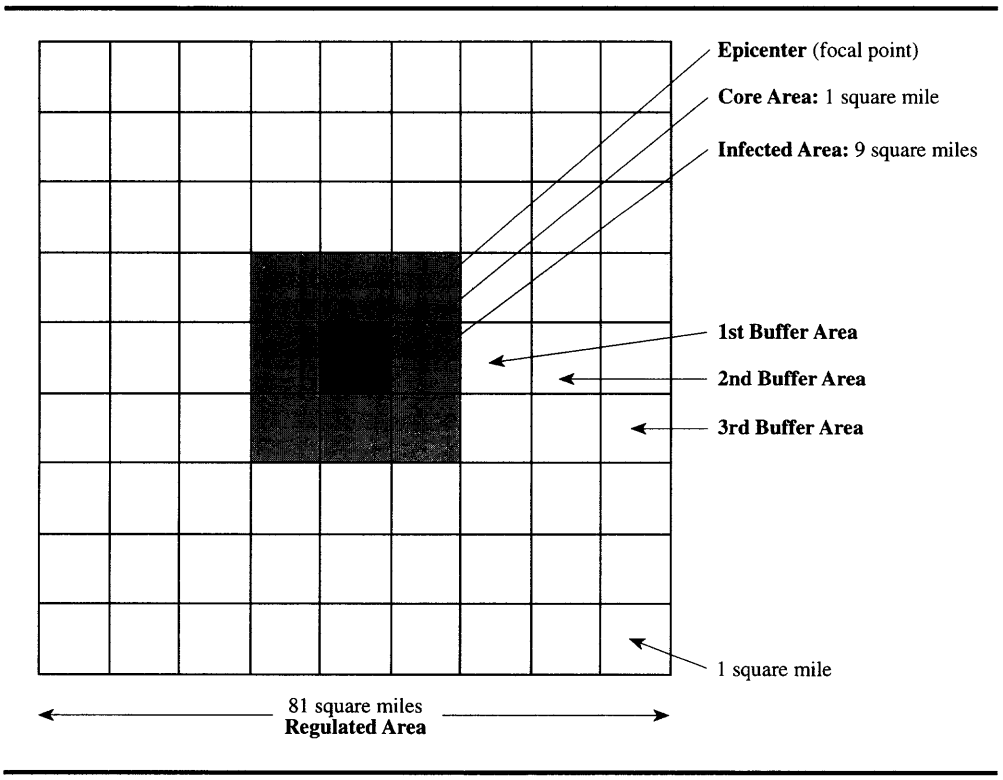


Figure 3-1: **Survey area in square miles**

Delimiting Survey (continued)

Cross Transit Survey:

Cross transit surveys are recommended for a rapid delimitation survey for the WBGV when a find is verified or suspected. The objective is to find and delimit the infected area in the shortest possible time with minimal labor and expense but with a high degree of confidence that, if the WBGV is present, it will be found.

The survey proposed here is biased in the same way as it is for the detection survey. It is biased toward the primary host(s) of concern and in areas where WBGV, if introduced, might be expected to be found first. Owing to the possibility of air dispersal of vectors, a special survey to track these vectors during the growing season may be warranted for certain areas.

There are three types of areas to cover in this kind of survey:

1. Host Production Areas—Those areas where large numbers of host material are found, as on commercial nurseries or farms where hosts are brought in for propagation and sale, grown for commercial purposes, or stored for replanting purposes.

2. Downwind Areas—Those areas where winds may reasonably be expected to carry the vector(s) from locations where the WBGV already exists.

3. Residential Areas (including agricultural communities)—Major cities and towns where residents and visitors may be expected to travel to and from areas where the WBGV already exists.

Block Survey:

In the conduct of this survey, it should be borne in mind that whiteflies reproduce quickly and move rapidly into regional areas, creating new foci of infestations along the way.

The following measures should be taken if a find is verified and the cross transit survey indicates the infected area is small and perhaps well defined:

- If appropriate, conduct a block-to-block survey in the suburban/urban areas up to 7.2 km (4½ mi) from each find.
- In rural areas, conduct a property-by-property survey up to 7.2 km (4½ mi) from each find.
- Each block or property can be scored, if WBGV is present on any combination of host species, as

  Light—WBGV only in one or a few infected hosts.
  Medium—WBGV in six or more infected hosts.
  Heavy—Entire area with numerous WBGV-infected plant hosts.

Delimiting Survey (continued)

The survey will permit the project to plot the area, extent, and nature of the infection more accurately, taking into account such variations as unequal distribution in infected hosts and the influence of temperature (i.e., summer) on the titers obtained.

Each find may be considered a primary site. A primary site is the property on which an initial detection of a disease or pathogen occurs *or* a potentially infected site within 1½ miles of an infected property (i.e., those host areas within the infected area).

A satellite site is a potentially infected property more than 1½ miles from any infected property. A satellite site, by definition, can be anywhere except within the 1½-mile area around any infected property.

Delimiting surveys will be carried out on all primary sites. They will also be conducted on satellite sites when there is evidence of the possible spread of the pathogen to or from the infected property. The following conditions define those properties that will be surveyed as satellite sites:

- Any property that has received propagative material or transplants of field crops from an infected property within 2 years. For woody plants, 3–4 years will be the minimum, as determined on a case-by-case basis.
- Any property that has been the source of propagative material or transplants of field crops planted on the infected property within 2 years. For woody plants, 3–4 years will be the minimum, as determined on a case-by-case basis.

The frequency of the delimiting survey will depend on the time it takes to cover the area, the resources available for repeat surveys, and whether a decision is made to suppress or eradicate the WBGV. A maximum interval should be determined by program managers or based on the results of a review by a technical committee. In lieu of any decision, a suggested maximum interval would be 1 month between surveys.

Monitoring/ Evaluation Survey

A decision to suppress or eradicate the WBGV evaluation will require a monitoring/ evaluation survey to check on the WBGV population. A cross transit survey would generally be used.

Orientation of Survey Personnel

New personnel will be trained on the job by experienced personnel. A period of up to 3 or more working days may be needed to do this.

Survey Records

Records noting the areas surveyed, sites trapped, dates, locations, and hosts in which detections were made will be maintained.

REGULATORY PROCEDURES

Instructions to Officers

Regulatory actions should be required until the pest is eradicated or declared established with no further suppression or control actions. Officers must follow instructions for regulatory treatments or other procedures when authorizing the movement of regulated articles.

Understanding the instructions and procedures will serve as a basis for explaining such procedures to persons interested in moving articles affected by the quarantine and regulations. Only authorized treatment procedures may be used.

General instructions to be followed in regulatory treatments may be found in State regulatory manuals or in the Plant Protection Quarantine, Animal and Plant Health Inspection Service (PPQ, APHIS) Treatment Manual.

Regulated Articles

A variety of articles may present direct or indirect risks of spreading whitefly borne geminivirus (WBGV). The movement of these articles will be regulated to prevent the infection from spreading. Regulated articles include

- Leaves and stems of hosts listed in Addendum 3 that exist in the regulated area (Bell, 1988; Reeves, 1992).
- Nursery plants or other plant material with or without leaves and stems, including propagative material intended for planting.
- Any other product, article, or means of conveyance of any character whatsoever when it is determined by an inspector that it presents a hazard of spread of the WBGV and the person in possession thereof has been so notified.

Quarantine Actions

Regulatory action will be required if an infection is detected. When an infection of a WBGV is detected and formally confirmed, the following steps should be taken:

1. State notifications are issued by field personnel to the property owners or managers of all establishments within 4½ mi of the epicenter that handles, moves, or processes host material that may include material or conveyances capable of spreading the WBGV or the vector(s). Notifications will be issued pending authoritative confirmation or further instructions from the Head of the State Plant Protection Service or the Deputy Administrator of APHIS, PPQ.

2. If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Federal Plant Pest Act (7 U.S.C. 150 dd) until an interim rule can be published in the Federal Register. For information on other legal authorities, see Section II, Parts A and B, of the APHIS Emergency Programs Manual (for plant pests).

3. The Head of the State Plant Protection Service and/or the Deputy Administrator of APHIS will notify other State cooperators of the WBGV detections, actions taken, and actions contemplated.

Quarantine Actions (continued)

4. A narrative description of the regulated area with supporting documents should be developed by State personnel. The regulated area will normally be within an approximate 4½-mi radius around the find and may contain a 1-mi^2 or greater core area where premises may be treated.

5. The State may need to publish an interim rule covering the emergency regulations. The interim rule will announce a date for submitting written comments.

6. After receipt of written comments, a final determination specifying the action decided on will be published.

Regulated Establishments

Efforts to detect the pest within the regulated area will be made at establishments where host material is sold, handled, processed, stored, or moved. Establishments that might be involved include airports, storage or store areas, landfill sites, fruit stands, farmer's markets, produce markets, flea markets, nurseries, and any other establishments that handle host material.

Use of Chemicals

The appropriate State manual and these New Pest Response Guidelines identify chemicals authorized for vector control, methods and rates of application, and any special application instructions. Concurrence by the appropriate State regulatory agency is necessary for the use of any other chemical or procedure for regulatory purposes. If treatments selected or proposed, including those listed in these New Pest Response Guidelines, are not in compliance with current pesticide labels, emergency exemptions will need to be obtained under Section 18, or 24C, special local need (SLN) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended. Regulated articles may be certified for movement after treatment.

Approved Regulatory Treatments

Sanitation:

The removal and destruction of hosts and other material that may be associated with the regulated items is important for geminiviruses.

Host-Free Period:

Imposition of a host-free period of 2–3 months on an otherwise susceptible property, premises, or area.

Steam Sterilization:

The use of steam, as a treatment alone, to conveyances, storage, or other holding areas to destroy any vectors present.

Approved Regulatory Treatments (continued)

Cleaning:

When appropriate, the use of hot soapy water or quaternary ammonium compound, as a treatment, to conveyances, storage or other holding areas, tools or boots, or to host material to effectively destroy any life stages of a vector that may be present.

Fumigation:

The application of an approved fumigant (methyl bromide), as a treatment alone, to hosts to destroy any vectors.

Hot Water:

The application of hot water at a specified temperature, as a treatment alone, to hosts to destroy any vectors present.

Ground Spray:

An approved insecticide or biological insecticide applied to the above-ground parts of nursery stock, including applications under the leaves or as appropriate, to destroy any vectors present.

Soil Treatment:

An approved systemic insecticide applied to the soil of nursery stock to destroy any vectors present.

Polymer Webs:

Sheets of polypropylene fleece covering hosts, especially crops and nursery plants, to prevent feeding by aerial vectors (Harrewijn et al., 1991).

Certified Virus-Free Propagative Material:

The planting of certified virus-free propagative material in the regulated area, away from infected localities.

Principal Activities

The following identifies principal activities necessary for conducting a regulatory program to prevent the spread of WBGV. The extent of regulatory activity required depends on the degree of infection. For example, to safeguard fruit stands throughout the entire regulated area when these stands are only engaged in local retail activity may not be necessary during a localized and light infection. On the other hand, mandatory checks of passenger baggage at airports and the judicious use of road patrols and roadblocks may be necessary where general or heavy infections occur.

Principal Activities (continued)

Principal regulatory activities include the following:

1. Contacting and advising the regulated industry of regulations and required treatment procedures.

2. Issuing compliance agreements, certificates, and permits.

3. Supervising, monitoring, and certifying treatments of host material.

This may (or may not), if determined by program managers or by a technical committee to be practical, include the sampling of commercial shipments from the regulated area for zero tolerance for plant diseases, such as that given by this equation:

$$PEA = e^{-np}$$

PEA is the probability of erroneous acceptance of a field, e is the base of natural logarithms, n is the sample size, and p is the probability that a plant is diseased (Clayton & Slack, 1988).

4. Conducting compliance inspections at regulated establishments such as
   a. Nurseries (Transplant houses)
   b. Fruit stands
   c. Local growers, gardeners, and packers
   d. Farmer's, produce, and flea markets
   e. Farm and garden supply dealers
   f. Commercial haulers of regulated articles
   g. Public transportation officials
   h. Post office contacts
   i. Storage locations (if plants are stored).

5. Monitoring the movement of host material to approved landfills to ensure adequate disposal of regulated articles.

6. Monitoring the destruction of regulated articles to ensure adequate destruction of any life forms of the vector, and thus the WBGV, that may be present.

7. Monitoring the movement of regulated articles through airports and other transportation centers.

8. Observing major highway and quarantine boundaries for movement of regulated articles.

9. Notifying homeowners near detection sites of applicable regulations.

10. If applicable, monitoring to ensure that only resistant host varieties are planted within the regulated area.

Principal Activities (continued)

11. Visiting processing establishments, if present, in regulated areas.

12. Monitoring sale and transfer of infected property to ensure that property users are aware of restrictions on land use.

Removing Areas From Quarantine

Areas placed under regulation may be removed from quarantine requirements after the WBGV has been declared eradicated. Program management will identify areas to be removed when the equivalent of 2, 3, or 4 years, based on characteristics of the host, has passed since the last pathogen recovery. At least 1 year must have elapsed since the cessation of control activities. A Notice of Quarantine Revocation will need to be published when areas are removed from quarantine requirements.

Orientation of Regulatory Personnel

Only trained or experienced personnel will be used initially. Replacement personnel will be trained by the individual being replaced.

Regulatory Records

Records will be maintained as necessary to carry out an effective, efficient, and responsible regulatory program.

Records may include

- Maps
- Chronology of events and actions
- Personnel movement
- Treatment records
- Regulatory activities
- Meeting notes.

CONTROL PROCEDURES

As control procedures are developed, they will be made available to involved States. There will be no Federal involvement in direct control programs. If treatments selected or proposed are not in compliance with current pesticide labels, an emergency exemption will need to be obtained under Section 18, or 24C, special local need (SLN), of FIFRA, as amended.

Complete eradication or suppression of a whitefly borne geminivirus (WBGV) infection in the continental United States may not be possible (Schoulties et al., 1987) except, perhaps, under certain restricted conditions. Eradication was possible for the unrelated potato Y virus (PVY^n) on Prince Edward Island, which is also insect vectored. In this case, the extent of the infection was severely limited.

The following provides approved procedures available for use in most situations. These procedures include biological, mechanical, and chemical controls. Local conditions will determine the most acceptable procedure or combination of procedures to achieve suppression, control, or eradication.

Recommended Pesticides

The treatments prescribed are predicated on an adequate survey. At the initiation of a program, an evaluation of available insecticides for use on program operations will be made.

Selection of Options

Program options may be selected through a decision-making process such as embodied in Table 5-1.

If the infections are:	And the viral population appears to be:	And the hosts are:	Then the option is:
Established in a large contiguous area	→	→	NO ACTION
Present in a number of widely separate and discrete areas	Well established, as measured by: • population, • estimates, • competition, • environment, or • climatological considerations	→	
Established in a small contiguous area	Not well established and population estimates felt to be a result of recent establishment (within 1 year)	Large number of hosts over an extensive area	Biological and cultural controls
		Moderate number of hosts over a well-defined area	Suppression, cultural, and biological controls
Present in only one or a few closely separate and discrete areas		Confined to a limited number of hosts	Control, suppression, and eradication

Table 5-1: Geminiviridae decision-making process

This decision table follows certain limited basic statements and can be considered generally true in a biological sense, provided no other factors intervene. There are some underlying assumptions. For example, it is assumed that the WBGV in question will be able to survive in the same ecological and environmental circumstances as its host(s).

No Action

Factors involved in arriving at a decision of "No cooperative program action" include the following:

Given that the WBGV in question has firmly established itself in the infested area and that

1. It is determined no reasonable effort will be successful in eradicating it (versus a reasonable effort may be successful);

No Action (continued)

or

2. Regulatory or suppressive measures will not be economically sound, based on the area involved or the rate of spread (versus affordable measures);

or

3. On the basis of measurable ecological factors, the WBGV will not be present in sufficient amounts in the environment to warrant control or suppression efforts (versus a serious threat);

or

4. Control of the WBGV is best left to standard cultural practices of virus control (such as host destruction) and other regulatory resources that control the spread and effects of the disease (versus an urgent need to augment natural controls).

If any of these statements are not true, and the contrary is true instead, then a decision to take "No Action" should be carefully evaluated.

Approved Eradication/ Suppression/ Control Options

Various combinations of treatments exist to arrive at a predetermined goal for a specific program that may be either eradication, suppression, or control. This goal, and the strategies useful for eradication, containment, or control, will be determined by State and local personnel or their Technical Advisory Committees or equivalent advisory boards.

Approved Treatments

The following is a list of suggested treatments that may be applicable under certain conditions. The treatments selected will be determined by State and local personnel concerned with a given program and their Technical Advisory Committees or equivalent advisory boards. Addendum 5 lists certain additional treatments that may be available.

NOTE: Ongoing and intensive studies by various agencies of USDA and concerned States on sweetpotato whitefly (SPWF) and silverleaf whitefly (SLW) may result in improved management and control of these pests (Faust, 1992). The probability exists that the goals and objectives of a WBGV program may differ in intent from controls or management schemes developed by this effort. Accordingly, such controls may need specific alterations to suit the objectives of the WBGV program.

NOTE: In those areas where cooler weather prevails for part of the year, the most vulnerable time for the SPWF (SLW) is in late winter, when populations are at their lowest level. During the cool weather period, a combination of several practices could achieve a break in the host cycle of the whitefly (Watson et al., 1992).

Approved Treatments (continued)

1. Biological, Chemical, and Cultural Control of Whitefly Vectors

a. Biological Insecticides

(1). Bacteria
(2). Viruses
(3). Nematodes

Items (1) to (3). Currently, there are no commercial products listed for the SPWF (SLW). It will be necessary to determine whether such products are available at the time of program action for SPWF (SLW) or for any additional suspect vectors identified by the program.

(4). Fungi

(a). Vertalec

Agent: *Verticillium lecanii*

This agent has been discontinued by Novo Biokontrol in the United States (Farm Chemicals, 1995).

Apply as per directions at the highest possible rate given for that host. An exemption may be needed for outside applications. Extremely toxic to aphids and whiteflies (Rondon et al., 1980).

(b). *Cladosporium* sp. (Samways & Grech, 1986)

Not available in the United States.

Apply 4×10^8 conidia per milliliter as a spray in water with 0.1 percent Tween added as a wetting agent. Use as a cover spray, taking particular care to spray the shoots and the area immediately surrounding them. Repeat every 2 weeks as necessary.

(c). Naturalis-L (Wright, 1992)

Agent: *Beauveria bassiana* strain ATCC 74040

Apply 2.3×10^7 conidia per milliliter as a spray in an emulsifiable oil formulation. Use as a cover spray at the highest possible rate given for that host. Repeat every week as necessary. Excellent activity against aphids and whiteflies.

Approved Treatments (continued)

Another product with this agent is is Mycotrol™- WP by Mycotach Corp., which is registered for use on vegetables, ornamentals, and cotton (Carruthers, 1995; Ferguson, 1995).

(d). *Neozygites fresenii*

Not yet available. Research in Arkansas, however, may provide a viable product (Ferguson, 1995).

(e). *Paecilomyces fumosoroseus*

Under testing in Weslaco, Texas. It caused a natural epidemic in whiteflies in Texas broccoli fields (Carruthers, 1995).

(5). Juvenile Hormones

The following juvenile hormone mimics or insect growth regulators have been found to be useful.

(a). Kinoprene (ZR - 777; Anon., 1976)

Discontinued 1985 by Zoecon Corp. (Farm Chemicals, 1995)

Apply at a rate of 0.1 to 0.13 percent to hosts. Extremely effective against homopterans.

(b). Margosan O

Has antifungal, antibacterial, and antiviral activity as well. This is a neem extract.

Apply as above (Farm Chemicals Handbook, 1995).

b. Augmentation of Predators or Parasites in Infected Area(s)

This technique is applied by mass rearing of the most highly efficient parasites or predators for mass release in infected areas. The use of beneficial insect planes (BIP), a type of model airplane controlled by radio, may be used to release parasites over a 50-acre field in 6–7 minutes with less mortality than with conventional airplanes (Anon., 1993b).

Approved Treatments (continued)

c. Introduction of Exotic Natural Enemies

This technique is carried out by USDA, Agricultural Research Service (ARS), and other agencies and institutions. It is assumed that the WBGV will be vectored by local SPWF, and therefore a need exists to identify natural enemies to help suppress the local population of SPWF, SLW, and any other local vector(s) discovered as a result of program efforts. Available natural enemies in production in the United States (whose efficacy may need to be tested) are the following:

1. *Catana parcesetosa*
   (Podumbu, India)

2. *Chrysoperla carneal/comanche/oculata/rufilabris*
   (Farm Chemicals Handbook, 1995)

3. *Delphastus pusillus*
   (Heinz & Parrella, 1994)

4. *Encarsia formosa*
   (Angelohori, Greece)
   (Nile Delta, Egypt)
   (Kampang Saen, Thailand)

5. *Encarsia* sp. nr. *hispida*
   (Sete Lagoas, Brazil)

6. *Encarsia lutea*
   (Frascati, Italy)
   (Mazotos, Cyprus)
   (Givat Haim, Israel)
   (Ein Gedi, Israel)
   (Golan, Israel)
   (Mazarron, Spain)

7. *Encarsia luteola*
   (see Heinz & Parrella, 1994)

8. *Encarsia* sp. nr. *pergandiella*
   (Sete Lagoas, Brazil)
   (Tucuman, Argentina)

9. *Encarsia transvena*
   (Taparbhani, India)
   (Matapur, India)
   (Murcia, Spain)

   Encarsia sp. nr. *transvena*
   (Shan-Hua, Taiwan)

Approved Treatments (continued)

10. *Encarsia* sp.
(Murcia, Spain)
(Shan-Hua, Taiwan)
(Chiang Mai, Thailand)
(Kuala Lumpur, Malaysia)
(Benguet, Phillippines)
(Azua, Dominican Republic)

Encarsia sp. A ?*strenua*
(Taparbhani, India)

11. *Eretmocerus* sp.nr. *californicus*
(Simmons & Minkenberg, 1994)
(Brawley, California)

E. californicus
(Farm Chemicals Handbook, 1995)

12. *Eretmocerus mundus*
(Murcia, Spain)

13. *Eretmocerus* sp. nr. *mundus*
(Cairo, Egypt)
(Murcia, Spain)

14. *Eretmocerus* sp. A
(Padappai, India)

15. *Eretmocerus* sp.
(Thirmala, India)
(Shan-hua, Taiwan)
(Tainan, Taiwan)
(Wufeng, Taiwan)
(Mission, Texas)
(College Station, Texas)
(Murcia, Spain)
(Mazarron, Spain)
(Anzio, Italy)
(Castel Gondolfo, Italy)
(Testa Di Lespe, Italy)
(Frascati, Italy)
(Cairo, Egypt)
(Gat, Israel)
(Golan, Israel)
(Negev Desert, Israel)
(Shan-Hua, Thailand)
(Kampang Saen, Thailand)
(Sai Noi, Thailand)

16. *Serangium parcesetosum*
(Podumbu, India)
(Already under test in Parker, Arizona - Carruthers, 1995)

Approved Treatments (continued)

17. *Serangium* sp.
(Kuala Lumpur, Malaysia)

Other natural enemies currently in quarantine:

18. *Encarsia lutea*
(Mazotos, Cyprus)

19. *Eretmocerus* sp.
(Thailand)

20. *Eretmocerus* sp.
(Thailand-Poinsettia)

21. *Eretmocerus* sp. 2
(Thailand)

22. *Eretmocerus* sp. 3
(Thailand)

23. *Encarsia* sp.
(Philippines)

24. *Encarsia* sp.
(Thailand-Poinsettia)

25. *Encarsia* sp.
(Thailand)

Encarsia sp. 2
(Thailand)

Encarsia sp.
(Taiwan)

Eretmocerus sp.
(Taiwan)

Syrphidae
(Philippines)

Coccinellidae
(Taiwan)

Coccinellidae sp. 2
(Taiwan)

Coccinellidae
(Malaysia)

(See Anon., 1994; Goolsby, 1995)

NOTE: The predator(s) must be carefully selected to decrease the chances of scattering the vector population and thus spreading the virus.

d. Conservation of Predators and Parasites

This treatment refers to the conservation of natural enemies, native or introduced, through integrated procedures, highly selective predator- or parasite-friendly insecticides or techniques, biological insecticides, and cultural practices favoring predators and parasites.

e. Enablement of Predators and Parasites

This treatment refers to augmenting the ability of predators and parasites to attack the host with greater efficiency or to be more tolerant of insecticides or other practices through selective breeding of the most efficient predators and parasites. Gene manipulation may also be

Approved Treatments (continued)

involved (Caprio et al., 1991; Hoy, 1990). The work of Marjorie Hoy (University of Florida, Gainesville) on genetic improvement of natural arthropod enemies is instrumental to this concept, and her expertise should be consulted in designing an enablement program.

f. Insecticides

The following insecticides are effective against whitefly adults and nymphs. They are not effective or have very little effect against the egg stage. Specific information is mentioned, where possible, under each insecticide. The pyrethroids are also efficient in controlling the spread of viruses, apparently because the vectors are intoxicated particularly fast. Intoxication results in feeding inhibition and flight induction, two obvious features in the prevention of vector inoculation.

Aerial application methods are being reduced in favor of ground rigs that use high-pressure sprays with nozzles angled for underleaf coverage (Leidner, 1994). This an obvious adaptation to the SPWF habit of laying most eggs on the underside of leaves and to its habit of congregating on young, upper leaves of the host (see Addendum 7, Vector Life History).

(1). Dimethoate

Apply only when host is in flush growth stage to the entire canopy, specifically flush growth. Used as a water-based full-cover spray.

NOTE: Broad spectrum insecticide.

(2). Insecticidal Soap

Apply this "safe" natural insecticide whenever whiteflies are found as a foliar spray to hosts at the highest rate given for that host. Repeat every 2 weeks.

(3). Malathion

Apply whenever whiteflies are found as a foliar spray to hosts at the highest rate given for that host. Repeat every 2 weeks (Ware, 1980).

(4). Nicotine Sulfate

Apply whenever whiteflies are found as a foliar spray to hosts at the highest rate given for that host (Ware, 1980).

Approved Treatments (continued)

(5). Disulfoton

Apply in granular form (i.e., DiSyston 15G) as a broadcast application at the highest rate given for that host or at the rate of 4.48 kg ai/ha immediately before transplanting (Pirone et al., 1988).

NOTE: This is a broad-spectrum systemic insecticide effective against both mites and insects. To be used in conjunction with (6) below (Pirone et al., 1988).

(6). Acephate

Apply as a foliar spray (i.e., Orthene 75 percent EC) at the highest rate given for that host or at the rate of 0.84 kg ai/ha at approximately 2-week intervals, or more often if whitefly colonies are evident (Pirone et al., 1988).

NOTE: This is a broad-spectrum insecticide effective against aphids, whiteflies, and other insects.

(7). Imidacloprid

Apply in liquid or granular form to the soil or as a spray for foliar applications. Imidacloprid is highly systematic and should be applied at the rate given for that host. A section 18 exemption may be needed. This is available for tomatoes, cole crops, lettuce, and cucurbits (Leidner, 1994).

(8). Fulfill

A new foliar treatment for which a section 18 exemption may be available in 1996 (Leidner, 1994). It presumably may be used in conjunction with soil treatments.

g. Mineral Oils

Mineral oils may interfere with the transmission of nonpersistent plant viruses by whiteflies. Although the mechanism of action is not completely understood, the oil seems to interfere with the attachment or removal of virus particles from the mouthparts (Lowery et al., 1990; Qiu & Pirone, 1989). In combination with an insecticide, especially a pyrethroid, and a whitewash, mineral oils are very effective against whiteflies (Lowery et al., 1990).

Approved Treatments (continued)

The oils are quite effective against the egg stage, or rather, the first instar crawlers, which die while attempting to eclose from the egg (Liu & Stansly, 1995).

The following are suggested oils:

Sunoco Sunspray U-F	(Liu & Stansly, 1995)
Sunoco Sunspray 6 E	(Lowery et al., 1990)
Sunoco Sunspray 7 E6V	(Makkouk & Menassa,1985)
Bayol 52	(Gibson & Rice, 1986)
SC811	(Gibson & Rice, 1986)
Luxan Oil H	(Asjes, 1991)
Duphar-7E Oil	(Asjes, 1991)
JMS Stylet Oil	(Qiu & Pirone, 1989)

Suggested application times: weekly

h. Sugar Esters

Certain esters (currently under development) produced by leaf hairs on the surface of a noncommercial species of tobacco leaves are toxic to whiteflies and environmentally safe for use as insecticides. Contact:

Horicultural Crops Quality Laboratory, Beltsville, MD; J. George Buta, (301) 504-5598; or

i. Polymer Webs

The use of polymer webs may result in fewer whiteflies. See 3.b. below. This technique is especially effective in greenhouses (Berlinger et al., 1988).

j. Mulches

Yellow mulch can be used to attract whiteflies, which are then killed by the heat generated in the mulch (Cohen, 1984).

In Florida, a modification of this technique uses aluminum mulch for spring crops and yellow (or orange) mulch for fall crops. This rotation appears to delay SPWF infestations (Leidner, 1994). However, J. Polston, University of Florida (personal communication, 1995), states that aluminum works best in Florida and that yellow mulch works best in Israel.

Approved Treatments (continued)

k. Cultural Control

(1). Yellow Sticky Strips/Traps

Plastic yellow sheets are coated with an insect trapping compound for control of whiteflies (Farm Chemicals Handbook, 1995). These are larger sheets used for mass trapping to affect the pest population, such as Chroma-line Bright Yellow No. 611-L or Reuter Laboratory Sticky Bars, item no. 142.

(2). Bug Vacuum

The use of an industrial vacuum to remove insect pests from crops would seem to work best with low-canopy herbaceous hosts such as vegetables.

A commercial vacuum such as the Beetle Eater (Thomas Equipment Ltd., Centreville, New Brunswick) or the Bug Beater (Sukup Manufacturing Co., Sheffield, Iowa) may be used. All whitefly hosts in the area around an infection of the virus, both commercially grown and wild, should be vacuumed several times during the season. Use of this equipment apparently will not spread viral diseases in the area through mechanical means (Anon., 1990; Boiteau et al., 1992).

(3). Host Destruction

See 4. below. Direct destruction, including shredding, tillage, and residue disposal of all virus hosts in the area around an infection of the virus, including wild and domesticated hosts that support vector populations, in an attempt to reduce whitefly populations in the area. Rigorous weed control or destruction should be practiced only where alternate wild virus hosts are involved (Jones, 1991; Watson et al., 1992). Timing may be important in temperate areas, such as defoliation of hosts in early autumn to prevent a rapid SPWF buildup the following year (Meyerdirk et al., 1986).

2. Vector Avoidance

If the vector(s) is present only at certain times of the year, it may be possible to alter scheduled commercial plantings for a time (the host-free period) when the vector is not present (Klein & Wyatt, 1989). Other practices include geographic separation and upwind planting of melons and cotton from winter vegetable fields and delayed planting of cotton to coincide with optimum heat unit accumulation (Watson et al., 1992), which is unfavorable to the vector life cycle.

Approved Treatments (continued)

The early spread of the vector (and consequently the virus) can be partly controlled by extending the time between fall crop harvest and spring crop planting (the crop-free period) by just a few weeks (Leidner, 1994). Likewise, spring fields should be destroyed as early as possible, and fall crops should be planted as late as possible to reduce losses (Leidner, 1994).

The destruction of host plants around an area as given in 4. below could also be considered a form of vector avoidance.

3. Biochemical, Physical, and Cultural Integrated Pest Management for Control of Geminiviruses

a. Host Resistance

(1). Genetic Engineering: Pathogen-Derived Resistance

Aside from the direct destruction of hosts, antisense technology offers an alternative for growers of commercial hosts during the regulatory period. Presently, this technology is available for only a few host–virus interactions, such as tobacco and barley yellow mosaic virus, a potyvirus (Becker, 1993).

(2). Conventional

If a gene is known that provides resistance to the effects of a virus, it may be transferred by classical plant breeding, recombinant technology, or a combination of both to a susceptible host. For example, watermelon mosaic virus (WMV) can be inhibited by a resistant cantaloupe gene that permits melon plants to recover from the initial symptoms, causing the yield and quality of the fruit to be significantly better than that of unprotected WMV-infected plants (Clark, 1993).

Note that the virus is still present because the host is not rendered immune by these treatments. However, because virus replication is inhibited, the virus loses its infectivity as a result of the normal process of viral degradation.

Selection of the appropriate treatment and procedures will need to be made at the time a program is under consideration.

b. Passive Protection

Polymer Webs (and Plastic Screens)

Approved Treatments (continued)

Sheets of polypropylene fleece or plastic screens may be used to cover low-lying herbaceous hosts, especially crop or garden plants. Aphids and whiteflies cannot penetrate the web of synthetic fibers with their stylets. Provided the sheets are regularly inspected for damage, the host will be fully protected against virus transmission.

In a passive way, this method will also cut down on aphid and whitefly numbers, especially numbers of apterous aphids, by reducing the available food supply (Berlinger et al., 1988; Harrewijn et al., 1991). Weeds under the cover should be eliminated because they compete for available resources and may shade the hosts.

Consideration should also be given, for those hosts that require it, to uncovering crops or garden hosts at the 50 percent flowering stage to provide for pollination by bees (or other insects) or good growth. This removal cannot take place if it will occur during vector pressure, because the plants will be very rapidly infected (Perring et al., 1989). Timing of any removal must also take into account the objectives of the program and whether eradication or suppression is the goal.

Pyrethroids

The pyrethroids are also efficient in controlling the spread of viruses, apparently because the vectors are intoxicated particularly fast. Intoxication results in feeding inhibition and flight induction, two obvious features in the prevention of vector inoculation. See also 1.g. above (Perrin & Gibson, 1985).

Mineral Oils

In combination with an insecticide, especially a pyrethroid, and a whitewash, mineral oils are very effective in the control of viral spread (Lowery et al., 1990). See also 1.g. above.

The following are suggested oils:

Sunoco Sunspray 6 E	(Lowery et al., 1990)
Sunoco Sunspray 7 E6V	(Makkouk & Menassa, 1985)
Bayol 52	(Gibson & Rice, 1986)
SC811	(Gibson & Rice, 1986)
Luxan Oil H	(Asjes, 1991)
Duphar-7E Oil	(Asjes, 1991)
JMS Stylet Oil	(Qiu & Pirone, 1989)

Suggested application times: weekly or biweekly, depending on local conditions.

Approved Treatments (continued)

Clean Culture Option—Virus-Free Propagative Material

Planting only certified virus-free propagative material in the regulated area, away from infected localities, and in conjunction with other measures such as the use of resistant or tolerant cultivars, is an important means of exclusion. This option is the keystone to the Canada/U.S.A. PVY^{n} Management Plan (Anon., 1993c).

4. Direct Destruction

The only other way to eradicate or suppress the disease is to destroy the contaminated host.

Once the host or hosts have been destroyed, no new hosts will be planted for a minimum of 2–3 months or longer, depending on the host, in both commercial and residential areas.

On residential properties, no new hosts will be planted for 5 years within 50 feet of each find.

a. Nurseries

In nurseries where an infected host is found, all host plants in the nursery will be removed and burned or disposed of in an approved landfill. No host material will be planted in the nursery for a period of 2–3 months or longer, depending on host.

b. Adjacent Woody Hosts

In commercial and residential plantings, host trees or shrubs, including wild hosts, adjacent to and surrounding any infected host will be selectively removed. Rigorous control of alternate weed hosts should be practiced where possible, especially where wild hosts are involved (Jones, 1991).

Leaves removed by defoliation will be burned or buried in an approved landfill. At residential locations, the leaves and fruit will be collected and removed to an approved site where they may be safely burned or buried.

c. Adjacent Herbaceous Hosts

Herbaceous hosts, including wild hosts of the virus, will be destroyed by cultivation or herbicides. Round-up® or a similar herbicide will be used to eliminate all herbaceous hosts in a 50-foot radius from any find. This area will be kept free of herbaceous or other hosts and weeds for a period of 2–3 months or longer.

Orientation of Control/ Eradication Personnel

Only trained and experienced personnel will be used initially. Replacement personnel will be trained by the individual being replaced.

Eradication/ Control Records

Records noting the locations, dates, number and type of treatments, and materials and formulations used will be maintained for all areas treated.

Monitoring

An effective monitoring program will be implemented to aid in the evaluation of program efforts and environmental impact. The application of pesticides will be assessed through the use of appropriate monitoring program criteria. The evaluation must effectively address Agency, cooperator, and public concerns.

The program plan should include at least the following elements:

1. Determine the efficacy of any pesticide used against the target pest.

2. Evaluate dye needs to monitor aerial applications, especially

   a. Droplet size
   b. Droplet distribution
   c. Identification of drift components
   d. Verification of spray block boundaries
   e. Identification of skips

3. Sampling to evaluate the effect of a WBGV program on the environment will be conducted in accordance with an environmental monitoring plan. These plans include pre- and postapplication sampling and observations to determine the impact on soil, water, vegetation, and nontarget species. Carcass searches are a part of this monitoring.

CONTACTS

When a whitefly borne geminivirus (WBGV) program is implemented, its success will depend on the cooperation, assistance, and understanding of many involved groups. The following groups should be continually informed of all operational phases of an emergency program.

1. Federal, State, county, and municipal agricultural officials;
2. Grower groups;
3. Commercial interests;
4. Universities;
5. State and local law enforcement officials;
6. Public health;
7. Foreign agricultural interests;
8. National, State, and local news media; and
9. The general public.

PATHWAY EVALUATION

Natural Means

Whitefly borne geminiviruses (WBGV) are spread by whiteflies. WBGV can be carried by wind currents in the upper atmosphere. These vectors have the potential to carry WBGV up to 20 miles. The whitefly alates have the ability to efficiently locate suitable hosts in the area where they find themselves.

Travel and Commerce

Fresh leaves appear to present a risk. Leaves or plants may be transported illegally for consumption or for medical or propagative reasons by individuals.

Inroduction of any WBGV through plants brought in for planting and associated materials is said to be more probable than through natural means.

ADDENDUM 1

Definitions

Aerial Treatment—Applying an insecticide or pesticide by aircraft over a treatment area.

Array—The vector trapping pattern in the delimiting survey area located around a detection.

Array Sequence—The intensity of vector traps within an array, beginning with the core area and continuing outward through each buffer area, ending with the outer buffer area.

Buffer Area—The area extending a prescribed distance beyond the boundary of the core, the 1-, 2-, 3-, and 4-mile buffers.

Commercial Production Area—An area where host material is grown for wholesale or retail markets.

Confirmed Detection—A positive laboratory identification of a submitted pathogen as a whitefly borne geminivirus (WBGV).

Containment—The effective confinement of the targeted WBGV population(s) to a specified geographical locality through effective survey, regulatory, and control measures.

Control—The effective stabilization or containment of the targeted WBGV population(s) in a specified geographical locality through effective survey, regulatory, and control measures.

Core Area—The 1-mi^2 area surrounding any confirmed WBGV detection.

Day Degrees—An accumulation of heat units above a developmental threshold.

Delimiting Survey—Determining whether infected host(s) exists and, if so, the extent of the geographical area the infected host(s) occupies.

Detection—The collection and identification of any WBGV from a host.

Detection Survey—An activity to determine the presence of WBGV, conducted on susceptible hosts in an area not known to be infected.

Developmental Threshold—The minimum (or maximum) temperature below (or above) which physiological development stops (peaks).

Epicenter/Focal Point—The initial site of an infection.

Definitions (continued)

Eradication—The confirmed removal of a population(s) of the targeted WBGV in a specified geographical locality, as determined by a negative survey for 2 years. Also, the effective reduction in numbers per unit area of a population(s) of the targeted WBGV to the zero point as determined by a negative survey for 3 years.

Fumigation—The application of an approved fumigant to hosts.

Generation (Life Cycle)—The period of time required for the vector to complete all stages of development.

Ground Spray—Using ground spray equipment to apply an insecticide or pesticide to the above-ground parts of host vegetation in a WBGV-infected area.

High-Risk Area—Any area or location that could harbor a WBGV as a result of the presence of hosts, the natural effect of wind on the vector, or through transport, storage, or presence of at-risk businesses or activity.

Host—A plant species capable of supporting WBGV replication.

Infection—The collection of one or more WBGV-infected hosts or the detection of a single infected host determined to be associated with a current infection.

Infected Area—A distance of 1½ miles from all detection sites unless biological factors indicate the need for more or less area.

Monitoring/Evaluation Survey—Using interdependent visual and perhaps vector trapping surveys in an area where a control, suppression, or eradication treatment is in progress to evaluate the effectiveness of the application.

PPQ-APHIS-USDA—Plant Protection and Quarantine, Animal and Plant Health Inspection Service, U.S. Department of Agriculture.

Primary Site—A property on which an initial detection of a disease or viable pathogen occurs or a potentially infected site within 1½ miles of an infected property.

Regulated Area—An area that extends at least 4½ miles in all directions from an infected property.

Regulated Articles—All known or suspected hosts of a WBGV or any other suspected product or article.

Regulatory Inspection—Visual examination of host material and containers at establishments where regulated articles are grown, handled, processed, or moved. Under some circumstances this examination can include discretionary trapping of vectors around selected establishments.

Definitions (continued)

Satellite Site—A potentially infected property that is beyond 1½ miles from an infected property.

Suppression—The effective reduction in numbers per unit area of a population(s) of the targeted WBGV in a specified geographical locality through effective survey, regulatory, and control measures.

Trap Survey—Determining the presence or absence of a vector by the use of traps placed in a predetermined pattern and serviced on a given schedule.

Urban/Residential Area—An area containing multiple or single family dwellings or commercial and industrial facilities.

Visual Survey—Examining hosts for visual signs of infection, either in the field or in regulated establishments, or monitoring the movement of regulated articles.

ADDENDUM 2

Safety

Personal and public safety must be a prime consideration at all times. Safety practices should be stressed in preprogram planning and through the duration of actual program operations. Supervisors must enforce on-the-job safety procedures.

ADDENDUM 3

Hosts

Disclaimer:

This host list is only intended as a guide to known hosts. It is as complete as a reasonable search of the literature could be within a given timetable. Changes in what are presumed to be known hosts and the discovery of previously unknown whitefly borne geminiviruses (WBGV) and hosts make it necessary to use this list with care. The latest information on any WBGV under consideration for a program must be used at the time of that program.

	Host	
Virus	Scientific Name	Common Name
Abutilon Mosaic Virus	*Abelmoschus esculentus*	Okra (MacIntosh et al., 1992)
	Abutilon spp.	
	Abutilon pictum	
	Arachis hypogaea	Peanut
	Cucumis melo	Melon
	Cucumis sativus	Cucumber
	Cyamopsis tetragonoloba	Guar
	Datura stramonium	Jimson weed
	Datura tatula	Blue-flowered thorn-apple
	Glycine max	Soybean
	Gossypium hirsutum	Cotton
	Hibiscus spp.	
	Lycopersicon lycopersicum	Tomato
	Malva spp.	
	Malva parviflora	Little mallow
	Malva silvestris	High mallow
	Momordica balsamina	Balsam apple
	Nicotiana glutinosa	Clammy-leaved tobacco
	Nicotiana tabacum	Common tobacco
	Phaseolus lunatus	Lima bean

	Host	
Virus	Scientific Name	Common Name
Abutilon Mosaic Virus (continued)	*Phaseolus vulgaris*	Garden bean
	Sida micrantha	
	Sida rhombifolia	Arrowroot sida
	Solanum tuberosum	Potato
NOTE: Host List from Brunt et al., 1990.		
Acalypha Yellow Mosaic Virus	*Acalypha* spp.	Copperleaf (Briddon & Markham, 1995)
African Cassava Mosaic Virus	*Datura ferox*	Jimson weed
	Datura stramonium	
	Hewittia sublobata	
	Jatropha multifida	
	Laportea aestuans	
	Manihot esculenta	Cassava
	Manihot glaziovii	Ceara rubber tree
	Nicandra physalodes	Apple of Peru
	Nicotiana benthamiana	
	Nicotiana clevelandii	
	Nicotiana debneyi	
	Nicotiana glutinosa	Clammy-leaved tobacco
	Nicotiana rustica	Aztec tobacco
	Nicotiana tabacum	Tobacco
	Solanum nigrum	Black nightshade
NOTE: Host List from Brunt et al., 1990.		
Ageratum Yellow Vein Virus	*Ageratum conyzoides*	Whiteweed (Wong et al., 1993)

	Host	
Virus	Scientific Name	Common Name
Asystasia Golden Mosaic Virus	*Asystasia* spp.	— (Briddon & Markham, 1995)
Bean Calico Mosaic Virus	*Glycine max*	Soybean
	Malva parviflora	Little mallow
	Nicotiana benthamiana	
	Phaseolus acutifolius	Tepary bean
	Phaseolus lunatus	Lima bean
	Phaseolus vulgaris	Garden bean
	Vigna radiata	Mung bean
	Vigna unguiculata	Cowpea
NOTE: Host List from Brunt et al., 1990.		
Bean Dwarf Mosaic Virus	*Phaseolus vulgaris*	Garden bean (Morales et al., 1990)
	Sida spp.	
NOTE: This virus may infect other plant genera, but existing detection techniques do not clearly distinguish between closely related geminiviruses. Also, it seems to have an extraordinary capacity to adapt to previously unknown hosts.		
Bean Golden Mosaic Virus	*Cajanus cajan*	Pigeon pea
	Glycine max	Soybean
	Macroptilium lathyroides	One-leaf clover
	Malvastrum coromandelianum	
	Pachyrhizus erosus	Jicama
	Phaseolus acutifolius	Tepary bean
	Phaseolus coccineus	Scarlet runner bean
	Phaseolus longepedunculatus	

	Host	
Virus	Scientific Name	Common Name
Bean Golden Mosaic Virus	*Phaseolus vulgaris*	Garden bean
	Vigna radiata	Mung bean
	Vigna angularis	Adzuki bean
NOTE: Host List from Brunt et al., 1990.		
Bhendi (Okra) Yellow Vein Mosaic Virus	*Abelmoschus esculentus*	Bhendi (Okra) (Harrison et al., 1991)
Chinese Squash Leaf Curl Virus	*Cucurbita* sp.	Squash (Hong et al., 1995)
Chino del Tomate Virus	*Capsicum annuum*	Bell pepper
	Capsicum frutescens	Tabasco pepper
	Datura stramonium	Jimson weed
	Datura tatula	
	Lens culinaris	Lentil
	Lens esculenta	Lentil
	Lycopersicon esculentum	Common tomato (Brown & Nelson, 1988)
	Lycopersicon pimpinellifolium	Currant tomato
	Malva parviflora	Little mallow
	Nicotiana benthamiana	
	Nicotiana clevelandii	
	Nicotiana glutinosa	
	Nicotiana rustica	Aztec tobacco
	Nicotiana tabacum	Common tobacco
	Phaseolus vulgaris	Garden bean

	Host	
Virus	Scientific Name	Common Name
Chino del Tomate Virus	*Vigna radiata*	Mungbean (Polston et al., 1993)
NOTE: Host List from Brunt et al., 1990.		
Cotton Leaf Crumple Virus	*Abutilon* spp.	
	Althaea spp.	
	Castanospermum australe	Delgado bean
	Glycine max	Soybean
	Gossypium hirsutum	Cotton
	Hibiscus cannabinus	Kenaf
	Malva parviflora	Little mallow
	Phaseolus acutifolius var. *latifolius*	Tepary bean
	Phaseolus angularis	Adzuki bean
	Phaseolus vulgaris	Garden bean
	Vicia spp.	
NOTE: Host List from Brunt et al., 1990.		
Cotton Leaf Curl Virus	*Abelmoschus esculentus*	Okra
	Alcea rosea	Hollyhock
	Corchorus spp.	
	Corchorus fasicularis	
	Gossypium barbadense	Sea island cotton
	Hibiscus cannabinus	Kenaf
	Hibiscus sabdariffa	Roselle
	Lycopersicon esculentum	Tomato (Yassin, 1978)
	Sida alba	

	Host	
Virus	Scientific Name	Common Name
NOTE: Host List from Brunt et al., 1990.		
Cowpea Golden Mosaic Virus	*Vigna unguiculata*	Cowpea
	Vigna unguiculata ssp. *sesquipedalis*	Yard long bean
	Vigna unguiculata ssp. *dekindtiana* var. *dekindtiana*	Wild cowpea
NOTE: Host List from Brunt et al., 1990.		
Croton Yellow Vein Mosaic Virus	*Ageratum conyzoides*	Whiteweed
	Croton bonplandianum	A weed
	Lycopersicon lycopersicum	Tomato
	Phaseolus vulgaris	Garden bean
	Sonchus brachyotis	A sowthistle
NOTE: Host List from Harrison et al., 1991.		
Dolichos Yellow Mosaic Virus	*Lablab purpureus*	Hyacinth bean (Harrison et al., 1991)
Eclipta Yellow Vein Virus	*Eclipta* spp.	— (Briddon & Markham, 1995)
Euphorbia Mosaic Virus	*Datura metel*	Hindu datura
	Datura stramonium	Jimson weed
	Euphorbia heterophylla	Painted spurge
	Euphorbia prunifolia	Painted euphorbia
	Fagopyrum esculentum	Buckwheat
	Nicandra physalodes	Apple of Peru
	Nicotiana benthamiana	

	Host	
Virus	Scientific Name	Common Name
Euphorbia Mosaic Virus	Nicotiana glutinosa	
NOTE: Host List from Brunt et al., 1990.		
Horsegram Yellow Mosaic Virus	*Arachis hypogaea*	Peanut (Harrison et al., 1991)
	Cajanus cajan	Pigeon pea
	Clitoria ternatea	Butterfly pea
	Glycine max	Soybean
	Indigofera hirsuta	Hairy indigo
	Macroptilium lathyroides	One-leaf clover
	Macrotyloma uniflorum	Horsegram
	Phas Mosaic Virus *eolus lunatus*	Lima bean
	Phaseolus vulgaris	Garden bean
	Vigna angularis	Adzuki bean
	Vigna mungo	Black gram
	Vigna radiata	Mung bean
NOTE: Host List from Brunt et al., 1990.		
Indian Cassava Mosaic Virus	*Manihot esculenta*	Cassava (Brunt et al., 1990)
Jatropha Mosaic Virus	*Croton lobatus*	
	Jacquemontia tamnifolia	Small-flowered morning-glory
	Jatropha gossypifolia	Belly-ache weed
	Jatropha multifida	Coral plant
	Jatropha podagrica	
	Phaseolus vulgaris	Garden bean
NOTE: Host List from Brunt et al., 1990.		

	Host	
Virus	Scientific Name	Common Name
Lima Bean Golden Mosaic Virus	*Phaseolus lunatus*	Lima bean (Brunt et al., 1990)
Macrotyloma Mosaic Virus	*Macrotyloma* spp.	Horsegram (Briddon & Markham, 1995)
Malvaceous Chlorosis Virus	*Sida carpinifolia*	(Christie et al., 1986)
Malvastrum Yellow Vein Mosaic Virus	*Malvastrum coromandelianum*	(Harrison et al., 1991)
Melon Leaf Curl Virus	*Citrullus lanatus*	Watermelon
	Cucumis melo	Melon
	Cucumis sativus	Cucumber
	Cucurbita maxima	Pumpkin
	Cucurbita moschata	Pumpkin
	Cucurbita pepo	Pumpkin
	Phaseolus vulgaris	Garden bean
NOTE: Host List from Brunt et al., 1990.		
Merremia Mosaic Virus	*Datura stramonium*	Jimson weed
	Phaseolus acutifolius	Tepary bean
	Phaseolus coccineus	Scarlet runner bean
	Phaseolus limensis	Lima bean
NOTE: Host List from Polston et al., 1993.		
Mung Bean Yellow Mosaic Virus	*Macroptilium atropurpureum*	Purple bean (R. N. Singh, 1983).
	Vigna mungo	Black gram (D. P. Singh, 1982)
	Vigna radiata	Mung bean (D. P. Singh, 1982)

	Host	
Virus	Scientific Name	Common Name
Mung Bean Yellow Mosaic Virus	*Glycine max*	Soybean (Dhingra & Chenulu, 1985)
Experimental Host Range	*Brachiaria remosa*	A grass
	Cajanus cajan	Pigeon pea
	Eclipta prostrata	Eclipta
	Macroptilium lathyroides	One-leaf clover
	Macrotyloma uniflorum	Horsegram
	Phaseolus vulgaris	Garden bean
	Vigna aconitifolia	Moth bean
	Vigna radiata	Mung bean
	Xanthium strumarium	Broad-leaf cocklebur
NOTE: Experimental Host Range from Brunt et al., 1990.		
Okra Leaf Curl Virus	*Abelmoschus* spp.	Okra (N'Guessan et al., 1992)
Pepper Huasteco Virus	*Capsicum annuum*	Pepper (Garzon-Tiznado et al., 1993)
	Lycopersicon lycopersicum	Tomato (Garzon-Tiznado et al., 1993)
Pepper Mild Tigre Virus	*Capsicum annuum*	Bell pepper (Brown et al., 1989)
	Capsicum frutescens	Tabasco pepper (Brunt et al., 1990)
	Datura stramonium	Jimson weed (Brown et al., 1989)
	Lycopersicon esculentum	Tomato (Brown et al., 1989)
	Nicotiana tabacum	Tobacco (Brown et al., 1989)
Philippine Tomato Leaf Curl Virus	*Lycopersicon esculentum*	Tomato (Retuerma et al., 1972)

	Host	
Virus	Scientific Name	Common Name
Potato Yellow Mosaic Virus	*Lycopersicon lycopersicum*	Tomato
	Nicotiana tabacum	Tobacco
	Petunia x *hybrida*	Garden petunia
	Solanum tuberosum	Potato
NOTE: Host List from Roberts et al., 1988.		
Pseuderanthemum Yellow Vein Virus	*Pseuderanthemum* spp.	False eranthemum (Briddon & Markham, 1995)
Rhyncosia Mosaic Virus	*Rhyncosia minima*	(Christie et al., 1986)
Experimental Host Range	*Abelmoschus esculentus*	Okra
	Cajanus cajan	Pigeon pea
	Canavalia ensiformis	Jackbean
	Glycine max	Soybean
	Gossypium hirsutum	Cotton
	Nicotiana glutinosa	
	Nicotiana tabacum	Tobacco
	Macroptilium lathyroides	
	Phaseolus vulgaris	Garden bean
NOTE: The Experimental Host Range is from Brunt et al., 1990.		
Serrano Golden Mosaic Virus	*Capsicum annuum*	Serrano chili (Acosta-Leal & Quintero-Montelongo, 1989)
	Capsicum frutescens	Tabasco pepper (Brown & Poulos, 1990)
	Datura stramonium	Jimson weed (Acosta-Leal & Quintero-Montelongo, 1989)
	Lycopersicon esculentum	Tomato (Rosset et al., 1990)

	Host	
Virus	Scientific Name	Common Name
Seranno Golden Mosaic Virus	*Nicotiana tabacum*	Tobacco (Acosta-Leal & Quintero-Montelongo, 1989)
	Phaseolus vulgaris	Garden bean (Acosta-Leal & Quintero-Montelongo, 1989
Sida Golden Mosaic Virus	*Sida* spp.	Jute (Briddon & Markham, 1995)
Squash Leaf Curl Virus	*Cucurbita ecuadorensis*	
	Cucurbita foetidissima	Buffalo gourd
	Cucurbita lundelliana	
	Cucurbita martinezii	
	Cucurbita maxima	Pumpkin
	Cucurbita mixta	Pumpkin
	Cucurbita moschata	Pumpkin
	Cucurbita pepo	Pumpkin
	Cucurbita texana	Wild marrow
	Phaseolus vulgaris	Garden bean (Cohen et al., 1983)
NOTE: Hosts from McCreight & Kishaba, 1991.		
Texas Pepper Virus	*Capsicum annuum*	Pepper
	Datura stramonium	Jimson weed
	Lycopersicon lycopersicum	Tomato
	Lycopersicon peruvianum	A tomato
	Nicotiana benthamiana	
	Nicotiana clevelandii	
	Nicotiana rustica	Aztec tobacco
	Nicotiana tabacum	Tobacco

	Host	
Virus	Scientific Name	Common Name
Texas Pepper Virus	*Physalis wrightii*	
NOTE: An Experimental Host List from Stenger et al., 1990.		
Tobacco Leaf Curl Virus	*Acanthospermum hispidum*	Bristly star-bur (Marriappan & Narayanasamy, 1972)
	Ageratum conyzoides	Whiteweed (Brunt et al., 1990)
	Blainvillea rhomboidea	(Marriappan & Narayanasamy, 1977)
	Capsicum annuum	Bell pepper (Brunt et al., 1990)
	Datura stramonium	Jimson weed (Yassin & Abu-Salih, 1972)
	Eupatorium chinense	(Kobatake et al., 1981)
	Flaveria australasica	Marriappan & Narayanasamy, 1977)
	Lonicera japonica	Japanese honeysuckle (MacIntosh et al., 1992)
	Lycopersicon esculentum	Tomato (Kobatake et al., 1981)
	Nicotiana clevelandii	(Brunt et al., 1990)
	Nicotiana debneyi	(Brunt et al., 1990)
	Nicotiana glutinosa	(Brunt et al., 1990)
	Nicotiana tabacum	Tobacco (Marriappan & Narayanasamy, 1977)
	Petunia x *hybrida*	Petunia (Brunt et al., 1990)
	Physalis floridana	(Brunt et al., 1990)
	Solanum melongena	Eggplant (Brunt et al., 1990)
	Zinnia elegans	Zinnia (Padmanabhan & Pathmanabhan, 1978)
Tomato Golden Mosaic Virus	*Datura stramonium*	Jimson weed (Polston et al., 1993)
	Lycopersicon lycopersicum	Tomato (Polston et al., 1993)

	Host	
Virus	Scientific Name	Common Name
Tomato Golden Mosiac Virus	*Nicotiana benthamiana*	
	Nicotiana clevelandii	
	Nicotiana debneyi	
	Nicotiana glutinosa	
	Nicotiana tabacum	Tobacco
	Petunia x *hybridia*	Petunia
	Physalis floridana	
	Solanum pennelli	
NOTE: Host List from Brunt et al., 1990.		
Tomato Leaf Crumple Virus	*Lycopersicum* spp.	Tomato (Nakhla et al., 1994)
Tomato Leaf Curl Virus	*Acanthospermum hispidum*	Bristly star-bur (Mariappan & Narayanasamy, 1972)
	Abelmoschus esculentus	Okra (Yassin, 1978)
	Ageratum conyzoides	Whiteweed (Harrison et al., 1991)
	Asystasia coromandeliana	(Olivares et al., 1972)
	Brassica pekinensis	Chinese cabbage (Olivares et al., 1972)
	Brassica juncea	Indian mustard (Olivares et al., 1972)
	Capsicum annuum	Bell pepper (Olivares et al., 1972)
	Croton bonplandianum	(Harrison et al., 1991)
	Cucurbita maxima	Squash (Olivares et al., 1972)
	Datura stramonium	Jimson weed (Green et al., 1987)

	Host	
Virus	Scientific Name	Common Name
Tomato Leaf Curl Virus	*Lonicera japonica*	Japanese honeysuckle (Green et al., 1987)
	Lycopersicon esculentum	Tomato (Dhanju & Varma, 1986)
	Nicotiana benthamiana	A tobacco (Green et al., 1987)
	Petunia x *hybrida*	Petunia (Green et al., 1987)
	Physalis floridana	(Green et al., 1987)
	Raphanus sativus	Radish (Olivares et al., 1972)
	Solanum melongena	Eggplant (Olivares et al., 1972)
	Sonchus brachyotis	A sowthistle (Harrison et al., 1991)
	Vigna unguiculata subsp. *sesquipedalis*	Yard-long bean (Olivares et al., 1972)
Tomato Mottle Virus		
Known hosts from Tomato mottle virus (TMoV) are given below. This list, from Abouzid, et al., 1992, and Polston et al., 1993, may not be complete, and new hosts may be expected as research on the virus continues.		
	Lycopersicon esculentum	Tomato
	Lycopersicon cheesmanii	
	Lycopersicon chilense	
	Lycopersicon hirsutum	
	Lycopersicon pennellii	
	Lycopersicon peruvianum	
	Lycopersicon pimpinellifolium	Currant tomato
	Nicotiana benthamiana	
	Nicotiana edwardsonii	
	Nicotiana tabacum	Tobacco
	Phaseolus vulgaris	Common bean

	Host	
Virus	Scientific Name	Common Name
Tomato Mottle Virus	*Physalis wrightii*	Wright groundcherry
	Physalis alkekengi	Chinese lantern
	Physalis ixocarpa	Tomatillo
	Solanum viarum	Tropical soda apple (McGovern et al., 1994)
Tomato Yellow Leaf Curl Virus		
With clear symptoms:		
	Datura stramonium	Jimson weed
	Euphorbia sp.	(Maxwell et al., 1994)
	Hyoscyamus desertorum	A henbane
	Jatropha sp.	(Maxwell et al., 1994)
	Lycopersicon esculentum	Tomato
	Lycopersicon hirsutum	(Berlinger & Dahan, 1989)
	Lycopersicon hirsutum form *glabratum*	(Berlinger & Dahan, 1989)
	Lycopersicon pennellii	(Berlinger & Dahan, 1989)
	Lycopersicon peruvianum	Wild tomato (Zakay et al., 1991)
	Lycopersicon pimpinellifolium	Currant tomato (Zakay et al., 1991)
	Nicotiana benthamiana	A tobacco
	Nicotiana glutinosa	A tobacco
	Sida sp.	(Maxwell et al., 1994)
Symptomless hosts:		
	Chaerophyllum sp.	A chervil
	Cynanchum acutum	A swallow-wort
	Lens esculenta	Lentil

	Host	
Virus	Scientific Name	Common Name
Tomato Yellow Leaf Curl Virus	*Malva nicaensis*	A mallow
	Malva parviflora	Little mallow
	Nicotiana tabacum	Tobacco
	Phaseolus vulgaris	Garden bean
	Sonchus oleraceus	Annual sowthistle
NOTE: Only the swallow-wort and the little mallow were found to be naturally infected. Host List from Cohen & Antignus, 1994.		
Tomato Yellow Mosaic Virus	*Datura stramonium*	Jimson weed (Polston et al., 1993)
	Lycopersicon hirsutum	(Brunt et al., 1990)
	Lycopersicon lycopersicum	Tomato
	Lycopersicon lycopersicum var. *cerasiforme*	Cherry tomato
	Lycopersicon pimpinellifolium	Currant tomato
	Petunia x *hybrida*	Petunia (Polston et al., 1993)
	Solanum tuberosum	Potato
NOTE: Cherry and currant tomato are natural hosts. Host list from Debrot & Centeno, 1985.		
Experiemental Host Range	*Nicandra physalodes*	Apple of Peru
	Nicotiana benthamiana	
	Nicotiana glutinosa	

	Host	
Virus	Scientific Name	Common Name
Experimental Host Range	Nicotiana tabacum	Tobacco
	Petunia x *hybrida*	Petunia
	Physalis floridana	
	Physalis peruviana	Cape gooseberry
	Solanum melongena	Eggplant
	Solanum tuberosum	Potato
NOTE: Experimental Host Range from Brunt et al., 1990.		
Watermelon Chlorotic Stunt Virus	*Citrullus colocynthis*	Colocynth (Brunt et al., 1990)
	Citrullus lanatus	Watermelon (Walkey et al., 1990)
Watermelon Curly Mottle Virus	*Citrullus lanatus*	Watermelon
	Cucumis melo	Melon
	Cucumis sativus	Cucumber
	Cucurbita maxima	Pumpkin
	Cucurbita moschata	Pumpkin
	Cucurbita pepo	Pumpkin
	Phaseolus vulgaris	Garden bean
NOTE: Host list from Brown & Nelson, 1986.		

ADDENDUM 4

Technical Survey Information

Cross Transit Survey:

Draw two straight lines on a map that will intersect each other and run through high-risk areas:

- Host production (agricultural) areas
- Areas where hosts are in abundance (wild hosts, etc.)
- Downwind high-risk areas up to 20 miles away
- Suburban/urban areas whose residents are likely to travel to and from whitefly borne geminivirus (WBGV)-infected areas
- Coastal and port of entry areas where hosts are available.

Both lines should bisect the area under survey. They do not need to be perpendicular to each other but should both run through the most suitable local sites that have been identified.

Survey Procedures:

1. If host(s) are in new flush:

   a. Examine all hosts along the transit. If there are many hosts along the transit (as in a field or grove), select one from every 10 most likely localities. A minimum sample along any one transit should be 10 host localities. The selection of sampling sites may follow criteria given in a manual recently developed by the Animal and Plant Health Inspection Service (APHIS) Plant Protection Laboratories for sweetpotato whitefly (SPWF) (Anon., 1995). In any event, unless otherwise recommended, the selection should be biased toward upwind and downwind borders of a given field or grove where vectors are likely to congregate, especially if a strongly predominant wind direction is present (Fargette et al., 1993; N'Guessan et al., 1992).

   Each host locality may be sampled, depending on the type of host.

   Hosts are best sampled if aggregated in wild stands or in cultivated fields. These fields or stands should be sampled at a minimum of five different sites, following a predetermined pattern agreed to beforehand by program staff or a technical advisory committee.

   b. Restrict examination to host(s), especially a host with new growth. In particular, pay attention to new growth that appears stunted; has chlorotic mottling or mosaic in leaves; or in some hosts with a bright yellow

Technical Survey Information (continued)

mosaic, or with upward (or downward) leaf curling, is distorted, or is otherwise abnormal in appearance and seems to show any one of the visual symptoms of WBGV (Polston et al., 1993) or of an attack of its vector. In general, check host(s) that appear to be unhealthy.

Samples of plant tissue, especially leaves, should be taken immediately from different parts of the plant.

Leaf sampling may be modeled along the lines of the leaf sample collection procedure for Canada/U.S.A. potato Y virus (PVY^n) under the Canada/ U.S.A. PVY^n Management Plan (see D.5). Leaf sampling procedures given in the APHIS SPWF Biological Control Manual may also be followed (Anon., 1995). Modifications may be necessary owing to the specific pathogen (of WBGV) and vector (SPWF biotype/other species) involved.

c. Knock whitefly vectors, if any, into an oiled pan or aspirate into a collecting jar.

Precautions should be taken to ensure that no whiteflies are accidentally spread through collection methods or procedures.

d. Label each sample with the collector's name, the date, and the exact location in enough detail so that someone else can find the spot.

e. Send vials (bottles) to a designated center for identification and processing. Insect samples may be sent in live if suitable, or otherwise in 50 percent ethanol, if they are to be frozen for blot tissue analysis. Otherwise they can be sent in jars of alcohol if the intent is identification of the insect only.

2. When host(s) are not in new flush

a. Examine the undersides of mature foliage for dead, parasitized insect vectors or mummies as well as living specimens. Because dead insect vectors adhere to the leaves, they can be used for identification in the absence of living specimens (EPPO, 1992).

b. Examine all suspect secondary or reservoir hosts, such as herbaceous weeds and shrubs, that show typical visual symptoms of WBGV and are found in or near infected properties along the transit. This includes backyard and field locations that are relatively easy to examine (Vicchi & Bellardi, 1988).

c. Follow the procedures as given above.

Technical Survey Information (continued)

The survey should be run weekly or biweekly until it is determined, through negative finds, that WBGV is not present in a given area. Transit lines may be moved in the judgment of the survey officer responsible for that area in an attempt to cover more favorable hosts or new locations.

Inspection Procedures:

Visual surveys and aids are more effective during periods of low insect vector populations and mobility. Traps may be deployed when vector populations are high or flight times (of whitefly vectors) are estimated to peak at a given time. Butler et al., 1989, discusses various collection techniques for SPWF.

1. Survey of new flush on hosts. Look for symptoms of WBGV, evidence of the vector's presence, and colonies (especially) of the vector. This technique is best in the spring.

2. Generally, look for certain signs, such as plant stunting, mottling, leaf curling, interveinal chlorosis, distorted leaves, and other year-round evidence characteristic for the WBGV. These signs may be checked throughout the year.

3. Traps may be used for the purpose of determining the populational numbers of the vector, for identifying the whiteflies present, or for determining flight times or flight periods or releases of the vector. They are not suitable for collecting the live insects needed to determine whether a virus is being carried by them.

Horizontal and vertical traps are used, but which is better is controversial. Traps on the ground are said to catch more SPWF than those above ground level, but this claim is also in dispute. Finally, traps above canopy level may also catch predators of SPWF, which may be an undesirable result. In this case, it would be better to place the traps within the canopy of the host or on the ground, if possible. Traps on the ground run the risk of dust, damage, or other unfavorable conditions unless short-time interval sampling is practiced. This, however, may require personnel in excess of program funding (Butler et al., 1989).

a. Green or yellow pan traps (Halbert et al., 1986) are for use among hosts with a low canopy such as soybean or vegetable fields. They may be used for the purpose of determining the populations of the whitefly vector or identifying the whiteflies present.

Traps are made of clear plastic sandwich boxes, each with an 11x11-cm green or yellow tile ceramic within, and filled with water containing ethylene glycol (Irwin & Goodman, 1981; Raccah et al., 1985). The actual color depends on the preferences of the whitefly. For SPWF this is yellow, in the 520- to 760-nm range (Butler et al., 1989). Traps are mounted at canopy level with double-ended clamps and support stands. Traps are to be serviced every day, and water is to be changed at least once a week (every day in the tropics).

Technical Survey Information (continued)

An alternate pan trap is the mosaic green pan trap. This trap consists of a 12x12-cm mosaic green ceramic tile in a plastic sandwich box. The box is filled with a 50 percent aqueous solution of ethylene glycol. This box is mounted at canopy level by means of a metal pole and a double-chemistry clamp (Irwin & Kampmeier, 1989).

Another alternate pan trap is one of aluminum, 23 cm diameter, and painted yellow inside. These traps may be filled with water and placed on the ground under low-canopy hosts (Adlerz, 1987).

b. Suction traps (Carver, 1978) may or may not be recommended for a given

arthropod vector owing to their comparatively great cost per trap. A trap design described as an "inexpensive suction trap" cost $300.00 in 1987 and required 20 staff-hours of labor for construction and erection (Allison & Pike, 1988). It is possible that a suction trap design incorporating color as part of its attraction could be developed and deployed in such a way that a few traps could cover a large area, but this is currently not available. However, if a given vector is flying at that time of year and suction traps have collected adequate numbers of that vector in the past, then this method may prove well worthwhile.

NOTE: If color is incorporated into the design, it may greatly affect both the size of the sample and its species composition. Deciding which color to use may be complicated, because manufacturers use different bases and pigments to produce colors that appear the same to humans, but not to insects. For that reason, different species of insects may react differently to two different preparations. This may be of concern in a survey for one specific insect, i.e., SPWF (Taylor & Palmer, 1972).

c. The whitefly trap (Berlinger, 1980) is best suited for low-canopy hosts or inside greenhouses.

The trap is constructed of 9.0-cm-diameter plastic petri dishes. The cover of a dish is glued horizontally, upside down, onto a rod (or placed directly on the ground). This inverted cover is painted yellow or a yellow plastic sheet is placed into it. Because whiteflies respond to yellow in the 520- to 620-nm range (520–760 nm for SPWF), a color in that range should be used. On the Munsell color file, a color with 5Y 8.5/12 (500–700 nm) would be appropriate.

The inverted cover with color is the base and stationary part of the trap. The portable part also consists of a plastic petri dish. The bottom is smeared with a thin layer of tanglefoot or similar sticky material. Benzene may be used to dilute the sticky material, if necessary. This bottom is placed on the yellow trap base and exposed by removing the top of the dish. When it is time to

Technical Survey Information (continued)

service the trap, the top is placed over this bottom and the whole trap is removed. A fresh bottom may be put in place and the catch transported to the labatory with appropriate collection data written on the top with a grease pen.

Generally, the trap is placed at a level that depends primarily on the height of the flight activities of the whitefly species in question. This level can range from ground level to above host height.

No figures have been given for trap spacing. From 5 to 20 traps were deployed in a greenhouse of unknown size for populational studies (Berlinger, 1980). Other types of sticky traps are discussed in Butler et al., 1989.

Backtracking:

It may be necessary to trace the source(s) of an infection. Because SPWF is assumed to have a low flight speed capability, in this case it is possible to measure long distance movement. The potential exists for good resolution of source regions under all meteorological conditions. An objective trajectory model has been developed to accomplish this (Scott & Achtemeier, 1987).

The next step would be the development of a predictive model to determine where the vector(s), and the related pathogen, may travel next. Preliminary data indicate that aphids, at least, prefer prefrontal conditions of moderate to strong southwesterly air flows.

A model for local-level or short-range migration is still under development. This type of migration occurs near the ground level during morning and midday hours, with one peak. Because they are poor flyers, SPWF tumble quickly to the ground boundary level and are driven by the wind as they drift about, landing on particular plants mostly by chance, staying on suitable hosts, and moving away again from those that are not (Byrne et al., 1994).

Leaf Sample Collection Procedures:

The leaf sample collection procedures for PVY^n are given here as a general guide. As stated in Survey Procedures above, modifications may be necessary for a specific program on a WBGV.

Leaf Sample Collection
PVY^n Management Plan
(in hectares & meters)

1. The objective of sampling is to provide the laboratory with high-quality leaf samples that are representative of the crop being examined.

Technical Survey Information (continued)

appropriate HL and R intervals in a manner similar to the instructions in 5. on page 11.6.

b. **Example 2:**
Suppose that an irregularly shaped 6-ha field is to be sampled. It must be sampled at rate of 400/6=67 plants/ha. Find the number nearest to 67 in the body of Table 11-1 (example ** = 63). This number indicates that a head-land interval of approximately 22.5 m, with sampling every 7 m along the rows, will provide the appropriate total sample (377 approx. = 400). Pick a random number between 1 and 22 and a number between 1 and 7 to select the randomized starting point (in from the corner of the field). Proceed using the appropriate "along the row" interval of 7 m between samples and head-land interval of 22.5 m, in manner similar to the instructions in 5. on page 11-6. This will result in a sampling rate of approximately 67/ha for a 6-ha field (in this case 63/ha for a total of 377 samples, if all paced measurements are exact). Obviously, paced measurements will rarely all be exact. Therefore, keep track of the total number of samples collected for the field, and "top-up" randomly (in this case with approximately 23 samples) or randomly skip samples as required, to meet the desired sample size of 400 for the field.

9. Always remember that the objective of sampling is to provide the laboratory with high-quality leaf samples that are truly representative of the crop being examined. It is the responsibility of the person collecting the samples to ensure that this objective is achieved.

<u>Nylon Membrane Preparation and Squashing Procedures</u> (Gilbertson et al., 1991):

1. **Preparation of Nylon Membranes**

a. Any positively charged nylon membrane used for nucleic acid hybridization should suffice. Do not touch membrane with hands at any time.

b. Cut rectangular-shaped pieces of nylon membrane (the size will depend on the number of samples and an appropriate container for storing or sending membranes, but the size should not exceed 10x10 cm).

c. Cut off the lower right corner of the membrane to be used; this step is essential for subsequent orientation of the membrane and identification of the appropriate sample at a later date.

Technical Survey Information (continued)

d. Do not allow the nylon membranes to become wet; do not touch them with bare hands or allow them to contact dirt, soil, etc.

e. Label nylon membranes with collector's initials and geographical location in lower left corner with a pencil or pen and the date the sample was squashed in the lower right corner.

2. **Preparation of Leaf Disks for Squashing**

a. Place leaves on a clean surface (i.e., a paper towel or piece of writing paper).

b. Use a flame-sterilized cork burner (a 4- to 8-mm diameter is recommended) to excise an appropriate number of leaf disks from each leaf sample. At least two disks from each leaf sample should be excised for each membrane.

c. Flame-sterilize cork borer between each sample.

3. **Leaf Disk Membrane Arrangement**

a. Make a key as to the location of samples in relation to the notched lower right corner.

b. Include controls on all membranes; controls would be uninfected leaves as well as known geminivirus-infected leaves.

c. Duplicate samples on each membrane, if possible, and replicate membranes for all samples. If probes are to be used, it is advisable to do at least five membranes for each group of samples to be probed. It is more efficient to arrange the disks from one sample on whatever number of membranes are to be used and to squash all these disks on each membrane and then go on to the disks from the next sample.

4. **Squashing Samples on Membranes**

a. Use a sterile round-bottom glass rod, the base of a sterile tube, or some other sterile or clean instrument with a solid round bottom to squash leaf disks on nylon membranes. It is best to place a membrane on a clean sheet of filter or writing paper or a paper towel for squashing. Use a gentle but firm rolling motion to squash the leaf disk (being careful not to bend the membrane too far or it will break). This motion should result in a green to

Technical Survey Information (continued)

brown spot on the membrane, depending on the age and condition of the leaf and the plant species.

b. Fibrous leaf residue can be removed with a sterile pair of forceps.

c. The rod(s) and forceps should be flame sterilized between each different sample.

5. **Drying**

Allow membranes to air dry (there will be water from the squashed disk).

6. **Labeling**

Membranes can be labeled by writing directly on them with pencil.

7. **Storage**

Membranes can be stored at room temperature at all times, once disks have been squashed and membranes dried. The membranes should be placed in sealed or regular plastic bags, after which they can be stored, sent, or from which samples can be immediately lysed.

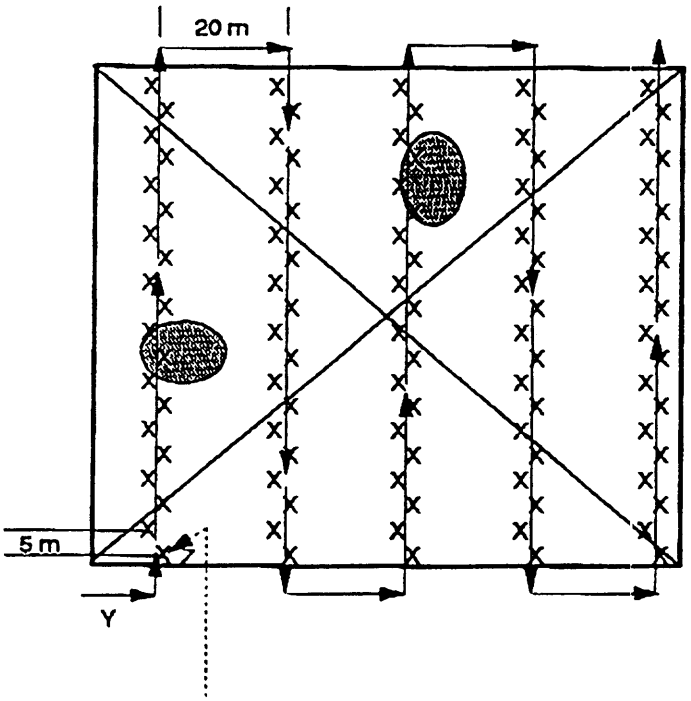


Figure 11-1: Example of 20mHL x 5mR "grid" sampling pattern. Arrows illustrate path walked through field. Random start of grid in from corner Y m across head-land, Z m into field. Shaded areas are schematic representations of pockets of infection. Note lines of sampling if "X" pattern had been used.

Sample numbers per ha that are provided by various head-land by row (HL X row) grid patterns.

meters along rows	meters across headland rows																				
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0
1.0	10000	6667	5000	4000	3333	2857	2500															
1.5		4444	3333	2667	2222	1905	1667	1481	1333	1111												
2.0				2000	1667	1429	1250	1111	1000	833	714	625										
2.5						1143	1000	889	800	667	571	500	444	400								
3.0									667	556	476	417	370	333	267							
3.5											408	357	317	286	229	190						
4.0												313	278	250	200	167						
4.5													247	222	178	148	127					
5.0														200	160	133	114	100[#]				
6.0														167	133	111	95	83	74			
7.0																95	82	71	63[#$]	57	52	
8.0																83	71	63	56	50	45	42
9.0																	63	56	49	44	40	37
10.0																			44	40	36	33
12.5																				32	29	27
15.0																					24	22
17.5																						19
20.0																						17

ADDENDUM 5

Technical Control Information

Polymer Webs:

On the declaration of a regulatory zone or when domesticated or commercial plantings are visible above the soil surface:

1. Completely cover susceptible herbaceous hosts with polypropylene fleece as soon as possible. The fleece of choice is Lutrasil LS10® or equivalent for aphids and Agril® or equivalent for whiteflies.

2. Sheets should be inspected for damage on a regular basis to ensure that the fleece remains intact over the host.

3. Control of other pests may be achieved by spraying the appropriate pesticide over the sheets.

Glyphosate (Round-up®):

Use Round-up® to kill infected or infested hosts when a whitefly borne geminivirus (WBGV) is detected during the growing season and to destroy susceptible hosts after field bioassays. The herbicide may be boom-applied at the rate of 4 quarts (3 lb a.i.) per acre (3.4 kilos a.i. per hectare) overall. For spot treatment, use a hand or knapsack sprayer and apply a solution containing 2 ounces of Round-up® per gallon of water.

ADDENDUM 6

Special Considerations for Home Gardens

Factors in Regulatory Decisions:

Home gardens and similar situations may present a lower risk of viral spread because their produce may not be commercially distributed and they may (or may not) be well tended and treated for pests, including possible vectors. Because viruses occur in diverse situations, survey techniques; regulatory actions; and control, suppressive, or eradicative procedures will be decided on a case-by-case basis. Procedures will be mutually approved by cooperating State and local regulatory officials. Factors in regulatory decisions include

- Proximity of site to areas of commercial production
- Size of garden
- Movement of hosts and vector(s)
- Changes in size or location of garden on a property over the years
- Proximity of site to dwellings
- Suitability of the whitefly borne geminivirus (WBGV) to such regulatory measures (e.g., Abutilon mosaic virus may not be suitable owing to risks of its spread on numerous hosts with serious economic consequences; but watermelon chlorotic stunt virus may be so regulated owing to a singular host that may not be prevalent in the area).

Some of these factors may also apply to the choice of survey, control, suppressive, or eradicative techniques at commercial sites.

Regulatory Options:

These include

- Control, suppression, or eradication measures
- Prohibition of host crops at the infected site.

Alternative options may be developed if deemed necessary. A quarantine or compliance agreement may or may not be required.

ADDENDUM 7

Life History

Systematic Position:

Geminiviruses

Superkingdom:	Prokaryotae
Kingdom:	Virus
*Class:	Plant Viruses
Family:	Geminiviridae
Genus:	Geminivirus, subgroup III

*Class is not a recognized term for plant viruses at this time. It is used here to give a continuity framework to the WBGV, as covered by this document.

Subgroup III is the largest and most important of the three subgroups in this genus. It is distinguished from the other subgroups by the possession of two molecules of circular single-stranded DNA, a generally narrow host range for each virus but with a wide spectrum of hosts in the higher plant families for the subgroup, and its transmission by whiteflies (Francki et al., 1991). There are 47 viruses in this group and 10 probable members. Seven were added since Francki et al., 1991, from which this list was taken, and eight more were found in the literature. Briddon & Markham, 1995, added another eight without explanation and did not list the following: Chinese squash leaf curl, cowpea golden mosaic, malvastrum yellow vein mosaic, merremia mosaic, and Philippine tomato leaf curl viruses. Rapid developments in this field may render the current list invalid within a few years.

Acronym	Virus	Geographical Distribution
AbMV	Abutilon mosaic virus (MacIntosh et al., 1992)	Europe (MacIntosh et al., 1992); South America, West Indies (Brunt et al., 1990); USA (Polston, personal communication, 1995)
ACMV	African cassava mosaic virus (= Cassava latent virus)	Africa, Malagasy, Seychelles, Zanzibar, Pemba (Brunt et al., 1990)
AGMV	Asystasia golden mosaic virus (Briddon & Markham, 1995)	(Not available)
AYMV	Acalypha yellow mosaic virus (Briddon & Markham, 1995)	(Not available)
AYVV	Ageratum yellow vein virus (Wong et al., 1993)	Singapore (Malaysia) (Wong et al., 1993)
BCMV	Bean calico mosaic virus	Mexico (Brunt et al., 1990)

Acronym	Virus	Geographical Distribution
BDMV	Bean dwarf mosaic virus (= Bean chlorotic mottle virus) (Brunt et al., 1990; Morales et al., 1990)	Colombia (Morales et al., 1990); Brazil, Argentina, Latin America (Brunt et al., 1990)
BGMV	Bean golden mosaic virus	Brazil, Guatemala, Puerto Rico, Jamaica, Venezuela, Dominican Republic (Brunt et al., 1990); Several strains exist, Dominican Republic, Georgia, Florida (Polston, personal communication, 1995)
BYVMV	Bhendi (Okra) yellow vein mosaic virus (Harrison et al., 1991)	India (Harrison et al., 1991)
CdTV	Chino del tomate virus (Tigre Disease) (Brown & Nelson, 1988)	Mexico (Brown & Nelson, 1988); Texas (Brunt et al., 1990)
CGMV	Cowpea golden mosaic virus	Nigeria, Mozambique, possibly in Tanzania, Kenya, and Niger; Pakistan and India (?) (Brunt et al., 1990)
CLCrV	Cotton leaf crumple virus	California, Arizona, Mexico, India (?), Middle East (?) (Brunt et al., 1990)
CLCV	Cotton leaf curl virus	Sudan (Yassin, 1978); Nigeria (Brunt et al., 1990)
CSLCV	Chinese squash leaf curl virus	China (Hong et al., 1995)
CYVMV	Croton yellow vein mosaic virus (Harrison et al., 1991)	India (Harrison et al., 1991)
DYMV	Dolichos yellow mosaic virus (Harrison et al., 1991)	India (Harrison et al., 1991)
EuMV	Euphorbia mosaic virus	Brazil, Venezuela, Puerto Rico, Florida (Brunt et al., 1990)
EYVV	Eclipta yellow vein virus (Briddon & Markham, 1995)	(Not available)

Acronym	Virus	Geographical Distribution
HYMV	Horsegram yellow mosaic virus	India (Harrison et al., 1991)
ICMV	Indian cassava mosaic virus	India (Harrison et al., 1991); Sri Lanka (Brunt et al., 1990)
JMV	Jatropha mosaic virus	Puerto Rico, Kenya (Brunt et al., 1990)
LGMV	Lima bean golden mosaic virus	Niger (Brunt et al., 1990)
MaMV	Macrotyloma mosaic virus (Briddon & Markham, 1995)	(Not available)
MCV	Malvaceous chlorosis virus	Puerto Rico (Christie et al., 1986)
MLCV	Melon leaf curl virus	California (Brunt et al., 1990)
MMV	Merremia mosaic virus	Puerto Rico (Bird et al., 1975)
MYMV	Mungbean yellow mosaic virus	Japan (Ikegami, 1988); India, Bangladesh, Pakistan, Philippines (Brunt et al., 1990); Thailand (Honda et al., 1986)
MYVMV	Malvastrum yellow vein mosaic virus (Harrison et al., 1991)	India (Harrison et al., 1991)
OLCV	Okra leaf curl virus (Briddon & Markham, 1995)	West Africa (Swanson & Harrison, 1993)
PHV	Pepper huasteco virus (Tigre Disease; Rizado Amarillo Disease) (Garzon-Tiznado et al., 1993)	Mexico (Garzon-Tiznado et al., 1993)
PMTV	Pepper mild tigre virus (Tigre Disease) (Brown et al., 1989)	Mexico (Brown et al., 1989); Texas (Brunt et al., 1990)
PTLC	Philippine tomato leaf curl virus	Philippines (Retuerma et al., 1972)
PYMV	Potato yellow mosaic virus	Venezuela (Roberts et al., 1988)

Acronym	Virus	Geographical Distribution
PYVV	Pseuderanthemum yellow vein virus (Briddon & Markham, 1995)	(Not available)
RMV	Rhynchosia mosaic virus	Puerto Rico (Brunt et al., 1990)
SGMV	Serrano golden mosaic virus (Brown & Poulos, 1990)	Mexico (Acosta-Leal and Quintero-Montelongo, 1989); Arizona (Brown & Poulos, 1990)
SiGMV	Sida golden mosaic virus (Briddon & Markham, 1995)	(Not available)
SLCV	Squash leaf curl virus	California (Cohen et al., 1989); Philippines (Dolores et al., 1988)
TGMV	Tomato golden mosaic virus	Costa Rica (Rosset et al., 1990); Brazil (Chatchawankanphanich et al., 1993)
TLCV	Tobacco leaf curl virus (= Honeysuckle yellow vein virus) (= Tomato yellow dwarf virus) (MacIntosh et al., 1992)	Europe (MacIntosh et al., 1992); Japan (Kobatake et al., 1981); Sudan (Yassin, 1978); India (Marriappan & Narayanasamy, 1977); Philippines (Olivares et al., 1972); Taiwan (Green et al., 1987)
TLCV	Tomato leaf curl virus	India (Harrison et al., 1991); Egypt (Shaheen, 1977); Sudan (Yassin, 1978); Taiwan, Australia (Chatchawankanphanich et al., 1993)
TLCrV	Tomato leaf crumple virus (Briddon & Markham, 1995)	Central America, Mexico (Nakhla et al., 1994)
TMoV	Tomato mottle virus (Gilbertson et al., 1993)	Florida (Polston et al., 1993) (Abouzid, et al., 1992)
TPGV	Texas pepper virus (Stenger et al., 1990)	Texas (Stenger et al., 1990)

Acronym	Virus	Geographical Distribution
TYLCV	Tomato yellow leaf curl virus	Israel (Zakay et al., 1991); Sicily (Rapisarda, 1990); Italy, Turkey (Czosnek et al., 1990); Cyprus (Anon., 1980); Europe (MacIntosh et al., 1992); Jordan (Suwwan et al., 1988); Lebanon (Makkouk et al., 1979); Egypt (Shaheen, 1977); Saudi Arabia (Mazyad, 1979); India (Verma et al., 1975); Senegal (D'Hondt & Russo, 1984); Sudan (Makkouk & Laterrot, 1983); Cape Verde, Mali, Nigeria (Czosnek et al., 1990); Taiwan, Thailand (Czosnek et al., 1990); Dominican Republic, Jamaica (Polston et al., 1994); Singapore (?) (Wong et al., 1993)
Different forms of this virus exist—(Polston, personal communication, 1995):		
AUTYLCV	Australian tomato yellow leaf curl virus	Australia
STYLCV	Sandinian tomato yellow leaf curl virus	Sardinia
ThTYCCV	Thailand tomato yellow crumple curl virus	Thailand

Acronym	Virus	Geographical Distribution
TYMV	Tomato yellow mosaic virus	Brazil (Brunt et al., 1990); Venezuela (Debrot & Centeno, 1985); India (Verma et al., 1975)
WCMoV	Watermelon curly mottle virus	Arizona (Brown & Nelson, 1986)
WCSV	Watermelon chlorotic stunt virus	Yemen (Walkey et al., 1990); Saudi Arabia (?) (Brunt et al., 1990)

Probable members (Francki et al., 1991):
Eggplant yellow mosaic virus
Eupatorium yellow vein virus
Lupin leaf curl virus
Papaya leaf curl virus
Sida yellow vein virus
Solanum apical leaf curl virus
Soybean crinkle leaf virus
Wissadula mosaic virus (Briddon & Markham, 1995)

Epidemiology

The dynamics of most plant disease epidemics in the ecosystem are strongly governed by meteorological parameters.

The whitefly-transmitted geminiviruses are usually acquired by adult whitefly vectors. Almost without exception, they are transmitted in a persistent manner by *Bemisia tabaci* biotypes of the sweetpotato whitefly (SPWF). If acquired by an immature form, the virus is retained when the vector moults but is not passed on to vector progeny.

Another closely related aleyrodid, *Bemisia lonicerae,* has been implicated in the transmission of TLCV from wild honeysuckle to tomato (Osaki et al., 1979). However, Mound & Halsey, 1978, synonymized this species under *B. tabaci,* saying only that there were four pupal cases (for their examination) in the British Museum. They later stated that the large number of names for SPWF results from the structure of the pupal case, which depends on the form of the host plant leaf, and added that pupal cases on glabrous leaves have no elongate setae on the dorsum, whereas those on hairy leaves may have up to seven pairs.

A newly named species, *Bemisia argentifolii* (Bellows et al., 1994; Perring et al., 1993) is the biotype B of recent literature. A number of features, including slight morphological differences, DNA sequences, host plant range, physiological differences, and a plant disorder called squash silverleaf, seem to offer substantial evidence that this form is indeed a separate species. However, the same authors state that the genus *Bemisia* is in need of revision and that we may be dealing with a a species complex. Because such a task has not been carried out, this document, in effect, is treating biotype B as part of the *Bemisia tabaci* species complex, with notes where different under description and biology.

WBGV may have an effect on the vector. TLCV was shown to significantly reduce the fecundity and fertility of SPWF, although the lifespan was not affected (Butter & Rataul, 1977).

Epidemics of plant viruses transmitted by arthropods result from recurring movement of infective vectors through the ecosystem, with subsequent infections of plants through the introduction of the pathogen by vectors. In these systems, an epidemic requires the interaction of four components: the host plant, the arthropod vector, the virus, and their interactions with the environment.

Epidemiology (continued)

In SPWF, geminiviruses are transmitted in a persistent manner. The virus may be retained when the vector moults, depending on the geminivirus involved. Optimum-acquisition feeding is generally greatest for 1-day-old SPWF, and inoculation-access feeding is generally greatest for 3-day-old SPWF. The virus can be lost or it may be transmitted in a persistent manner up to the life of the adult insect. The virus does not multiply in the vector and is not transmitted directly to progeny (Brunt et al., 1990). A helper virus is not required for transmission. It is not transmitted by touching of plants, by seed, or by pollen. It can be transmitted by grafting or mechanical inoculation.

Transmission over long distances of geminiviruses is not known. This transmission depends on the retention time and the transmission rate. A third factor is the number of hosts a given infected vector can inoculate.

Environmental factors influencing transmission are relative humidity and temperature. A high relative humidity of about 80–90 percent and temperatures around 25–30 °C, when combined, increase virus transmission in aphids by 30-35 percent. However, trans-mission rates are reduced by nearly 50 percent if the relative humidity drops to 50 percent while the temperature stays the same (M. N. Singh et al., 1988). How or whether whitefly transmission of a geminivirus is similarly influenced has not been studied.

A long-term epidemiological goal is to provide a set of principles or guidelines on which to base virus epidemiological models and control strategies that take into account the total ecology of a virus and its vector(s) in agricultural systems (Irwin & Kampmeier, 1989). As more information becomes available about the biology and spatial components of plant disease epidemics, it will be possible to forecast their origins and spread more accurately, resulting in enough information to take the steps needed to control, suppress, or even eradicate a virus from a given area. The following is a short summary of key factors.

Clonal Transmission (Vegetative Propagation)

This is the deliberate collection, transport, storage, and planting of the whole or part of an infected host by humans through clonal propagation.

Vector Movement

Because plant viruses cannot exist outside the host, vectors must be present to carry them during the growing season. Geminivirus vectors are whiteflies. These are small to minute life forms greatly influenced by climatological factors in their movement.

Vector Movement (continued)

1. **Vertical Displacement**—Vertical displacement may be illustrated by dividing the troposphere into four layers. The lowest is the vector pool population on the hosts. Immediately above the crop canopy are the vectors within the surface boundary layer (lower 10 to 20 m). Above this is the 1-km planetary boundary layer where turbulence and surface effects such as inversions dominate. The uppermost layer is the area where the vectors have become involuntarily uplifted by convection into the free atmosphere.

Vectors in the surface boundary are involved in local or short-duration movement. This movement directly accounts for most local virus spread in a field. Vectors in the planetary boundary are true migrants. In the case of arthropods, movement from this layer to the host canopy is determined by an individual's physiological state, dictated by the depletion of fuel reserves through flight. Changes in the environment also play an important part in vector movement from the upper layers.

For SPWF, this type of migration occurs near the ground level during morning and midday hours, with one peak. Because SPWF are poor flyers, they tumble quickly to the ground boundary level and are driven by the wind as they drift about, landing on particular plants mostly by chance, staying on suitable hosts and moving away again from those that are not (Byrne et al., 1994).

2. **Horizontal Translocation**—Whitefly vectors of geminiviruses are generally assumed to have low flight speed capabilities; thus, it is assumed that they are controlled by air movement. This makes it possible to measure long distance movement (See Scott & Achtemeier, 1987).

3. **Flight Patterns**—Whitefly flight requires the right combination of physiological and meteorological factors. Wind is the most obvious stimulus for the spatial component of arthropod flight. Wind speed controls takeoff thresholds and distance between landings. Wind direction influences direction of flight and thus direction of spread. Different species or biotypes may react in very different ways to the same stimuli. Time of day is also a factor in flight because different species may have different activity cycles.

4. **Barriers**—Barriers, both living and artificial, can alter the pattern or timing of a plant virus epidemic. Barriers work by physically excluding (or including) the vector(s), by altering the flight path, or both. The spread of a virus within a field may be characteristic of its vector, but in the case of WBGV, it seems that there are strong border effects as a result of the accumulation of the vector (*Bemisia tabaci*) on the borders of exposed fields under the influence of prevailing winds. This accumulation results in a larger number of infected host plants along the upwind and downwind borders, with gradually diminished numbers toward the center, thus forming an open V-shaped gradient (Fargette et al., 1993; N'Guessan et al., 1992).

Vector Movement (continued)

5. **Environmental Stimuli**—Environmental stimuli that alter arthropod vector activity and the subsequent inoculation potential of host plants can dramatically change rates of viral epidemics in the ecosystem. These stimuli can be physical or chemical. Some examples include

a. **Canopy Cover**—Canopy cover is the ratio of the amount of ground covered by plants to total ground area. A dense canopy may attract some vectors and deter others, depending on the species.

b. **Foliage Color**—The exact green or green/yellow color perceived by a vector may be or may not be attractive in eliciting alighting responses, depending on the species.

c. **Leaf Pubescence**—Increased leaf trichome density apparently retards virus epidemics by reducing probing frequency and total time spent probing by the vector.

d. **Host Genotypes**—Different cultivars may not alter probing behavior, but depending on the pattern of susceptible hosts to resistant hosts and nonhosts, the dilution of infections may delay an epidemic long enough to reduce the effects of early infection and diminish the threat of seed transmission.

e. **Insecticides**—Many insecticides prevent the spread of a virus because they act quickly on the arthropod vector and may in fact decrease the spread of a virus, as vector activity is increased.

Biology

Once within the host, geminiviruses characteristically induce nuclear changes such as segregated nucleoli, fibrillar bodies, and virus particle aggregates in cells of the vascular region of the cytoplasm of leaves and flowers (Christie et al., 1986).

Inclusion bodies are probably involved in viral replication. The properties and functions of the component proteins and genomic DNAs are active topics of research.

Natural Enemies

There are no direct predators of a plant virus, which, after all, exists within the host or vector. Resistance within the host is the result of the interaction of a multitude of chemical processes initiated by both the host and the pathogen while in contact in a favorable environment. The genes of the host plant that control disease resistance do so by changing or adapting the physiological processes of the plant, so that infection or disease development is neutralized or prevented from operating (Lucas et al., 1985).

Natural Enemies (continued)

However, predators of vectors are another matter. Predators may affect viral spread by vectors in different ways. For example, if the vector population is low, predators may reduce vector numbers enough to lower the rate of infection. If the vector population is high, predators may increase vector activity and thus increase viral spread.

Natural Protecttion

Protection from a geminiviral epidemic is best in Northern regions, where viral and vector activity is limited to absent, depending on the locality, owing to freezing temperatures. Greenhouses are an exception.

Vector Life History

To date, SPWF is the central vector implicated in WBGV transmission. Brief details of its life history are recounted here.

1. Systematic Position
   Class: Insecta
   Order: Homoptera
   Family: Aleyrodidae
   Genus: Bemisia
   Species: tabaci
   Common Name: Sweetpotato whitefly

The genus *Bemisia* (reviewed by Mound and Halsey, 1978) is a moderately large group of 38 species. However, only three species (*B. giffardi, B. hancocki, B tabaci*-SPWF) have wide distributions. *B. argentifolii* (silverleaf whitefly [SLW]) probably also has a wide distribution (as far north as Canada and as far south as the Caribbean by some unofficial reports) but is known only from Florida and California at present (Bellows et al., 1994).

SPWF has by far and away the most hosts in 63 plant families (*B. hancocki* is next, with hosts in 19 plant families). However, if SLW is considered a separate species from SPWF, it may replace SPWF in terms of hosts and very likely in distribution and numbers (Blua & Toscano, 1994).

SPWF is spread throughout many areas of the world, including England, Spain, Jordan, Iran, Nigeria, Chad, Sudan, Rhodesia, Madagascar, Pakistan, Thailand, China, Taiwan, Malaysia, Sumatra, New Guinea, Australia, Fiji, Brazil, Argentina, Jamaica, Puerto Rico, Russia, Japan, and the United States, including California, Arizona, Texas, and Florida.

2. Identification Characters (Butler et al., 1989; Hill, 1983)

a. Adult: Minute; 1–1.2 mm in length. Two pairs of broad, flat, white wings, flat over pale yellow body; body covered with a white waxy bloom. The SLW (biotype B) is, on average, slightly smaller than SPWF (Bellows et al., 1994).

"Pupa": About 0.7 mm in length, integument of last nymphal instar transparent with red eyes of adult visible through it. This is actually the last (inactive fourth) instar.

Vector Life History (continued)

Thoracic trachial fold widths narrower in SLW than in SPWF, submarginal seta ASMS4 absent in SLW, present in SPWF (Bellows et al., 1994).

Nymph: Greenish white, oval in outline, scale-like, and somewhat spiny.

Egg: About 0.2 mm long and pear-shaped. White when laid, turning to brown. Anchored upright to leaf by a tail-like appendage inserted into a stoma (of the leaf).

NOTE: Fixed differences occur in allelic frequencies between SLW and SPWF populations. These are single-primer polymerase chain reaction (PCR)-amplified DNA sequences (Bellows et al., 1994).

3. Biology (Butler et al., 1989):

a. Emergence: Adults emerge dorsally through the "pupae" through a T-shaped fissure in the integument, usually between 8 a.m. and 12 noon. Parthenogenetic reproduction occurs with unfertilized eggs, which only produces males. The same thing happens to eggs of females in interspecific crossing studies between SPWF and SLW, indicating that the eggs were not fertilized, as would be expected if they were separate species (Bellows et al., 1994). The male-to-female ratio is close to 1:1 to 1:6. The adults frequently remain on or near the "pupal case" for 10–20 minutes to spread and dry their wings and began feeding soon thereafter.

b. Mating: Adults can mate a few hours after emergence. Males exhibit aggressive behavior, approaching the female with antennal strokes and wing fluttering that may last up to 20 minutes, although actual mating is no longer than 4 minutes. Attempts failed to force mating between SPWF and SLW, because copulation did not occur between the two biotypes (Bellows et al., 1994).

c. Preoviposition: The preovipositional period lasts from 1 to 8 days at average temperatures of 21.6–26.5 °C. At temperatures of 13.8 °C it takes 22 days.

d. Oviposition: Under field conditions, no eggs are laid below 22.8 °C. Females insert the ovipositor through the epidermal layer into the mesophyll tissue of the leaf to firmly embed the eggs. Most eggs are laid on the underside of the leaves. A female lays 28 to 81 eggs on average, with a high of 300 reported. However, SLW lays more eggs (avg. 51.2) than SPWF (avg. 31.8) (Bellows et al., 1994; Bethke et al., 1991). Starved females do not oviposit, and maximum oviposition occurs within the first week of adult life, declining thereafter. When laying eggs, females prefer younger upper leaves of the host.

Vector Life History (continued)

e. Egg Hatch: Mortality of the eggs is low, partly because they are inserted into the host tissue. However, temperature does affect egg hatch, as follows:

Temperature	Hatch Rate
15 °C	62 percent
20 °C	81 percent
25 °C	92 percent
35 °C	98 percent
40 °C	59 percent

f. Development: There are three larval instars. The first instar may take 3 days, the next 2 days, and the last 1.9 days at 25.4 °C or 4.5, 2.7, and 2.6 days at 31 °C.

The "pupal" stage is actually the last (fourth) larval instar. It may last 4.7 days at 25.4 °C and 6.2 days at 31 °C.

Total development from egg to adult may vary from 60.5 days at 14 °C to 19.3 days at 25 °C. Differences in experimental results are explained on the basis that development time varies significantly depending on the cultivated or wild host inhabited. Development also goes faster during long-day photoperiod as opposed to short days at the same temperature.

In any event, SPWF can develop 11-12 generations per year in many tropical and subtropical areas of the world.

g. Longevity: Females are longer lived than males. Longevity depends on temperature. In the field, males may live for 6.4 to 34 days and females from 14.5 to 55.3 days at temperatures ranging from 12.7 to 26.5 °C.

h. Behavior: Females seem to prefer younger upper leaves for oviposition. In fact, eggs are generally found in the top, nymphal stages in the middle, and "pupae" on the bottom of some hosts. Adults may be distributed throughout. Although it is hard to make generalizations, SPWF seem to distinctly aggregate on and around a main stem leaf, which location varies according to plant growth and kind of host.

i. Feeding: SPWF feed without leaving visible symptoms on the leaves other than secondarily resulting from honeydew deposits (i.e., sooty mold, sticky cotton). SLW, however, seems to cause a visible plant disorder referred to as squash silverleaf (Bellows et al., 1994). It also processes more phloem sap than SPWF, resulting in more severe expressions of secondary results (Blua & Toscano, 1994).

Vector Life History (continued)

j. Population Dynamics: SPWF population dynamics may be characterized by exponential growth. This is not surprising in view of the basic demographic parameters; short generation time, relatively long reproductive period, and high reproductive rate.

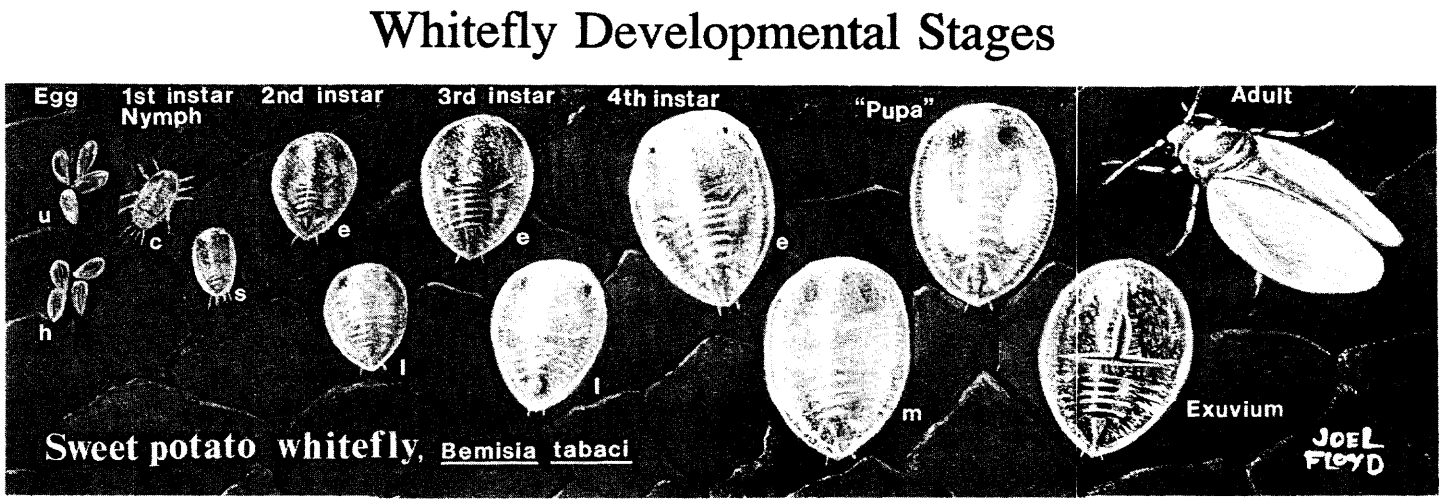
In many areas of the world, SPWF populations are regulated by various natural parasites. When SPWF populations suddenly increase, this well could be a sign of the failure of the parasite or predator complex to control these populations, perhaps as a result of the use of insecticides.

A study of SPWF showed that most of the mortality occurred during the crawler and first-instar nymphal stages. Egg mortality was minimal. Crawler mortality was attributed to climatic factors (low humidity, high temperatures) and to predation. First instar mortality was also caused by host-plant effects. Moderate morality in the two remaining nymphal stages and the "pupal" stage was attributed to weather and parasites.

In general, data gathered from various sources show that SPWF populations decline with cool temperatures or high rainfall, owing to temperature, declining host availability, increase in the availability of leaf substrate in areas where other hosts may be planted or grow during the winter (not possible in very cool temperate zones), a decline in reproduction, or an increase in mortality. The persistence of leaves and rate of aging during winter months depends on the host and whether it dies before completion of SPWF development.

Several models have been developed to characterize SPWF. One, based on cotton plants, used the life-table parameters. This includes temperature-dependent development oviposition and survival of all stages, tied in to emigration rates.

The second model is based on a simple degree-day exponential population growth-rate equation and is the one given on page 1.2 - Life Cycle Information (also, see Figure 14-1). It is based on the assumption that although other factors such as host-plant effects are important, SPWF populations will usually increase in an exponential manner during most of the growing season.



u=uneclosed, h=eclosed, c=crawler, s=stationary, e=early, m=middle, l=late

Figure 14-1: Life cycle of the sweetpotato whitefly (Bemisia tabaci)

ADDENDUM 8

Identification of Specimens

As many specimens as possible of suspect samples are to be collected for screening and identification by the local designated identifier.

Preservation and Shipment of Samples:

The following procedures were developed for the Canadian/U.S.A. potato Y virus (PVY^n) Management Plan. In general, these procedures may be followed for leaf samples. Different procedures may be necessary for other plant parts and for vectors. Field procedures may also differ depending on the identification technique used.

Instructions for the Preservation and Shipment of Leaves for Laboratory Diagnosis of PVY^n

1. The normal sample size per plant is the terminal three leaflets attached to the petiole of a compound leaf (these should remain connected) from the upper portion of the plant. However, if the leaflets are less than 4 cm long, the number of leaflets collected (still attached to a single petiole or stem) should increase in compensation to provide a total tissue mass equivalent to three leaflets 4 cm in length. If this cannot be done, then take the whole compound leaf.

2. The leaf samples should be bagged in composites of 100. Loosely folding over the opening of the bag and stapling it shut is a good way of sealing the bag. The bag should not be sealed airtight, particularly if it is warm or damp; if necessary, make breathing holes.

3. The leaf samples should be cooled (BUT NOT FROZEN) to 5 °C as soon as possible. This should be done within hours of picking (particularly on warm days). If ice packs (-15 to -20 °C) are used, they should be insulated with two or three layers of paper or other packing material and be placed in the middle or top of the cooler. Two 6″×6″ ice packs per cooler are usually sufficient. Avoid packing the leaves too tightly.

4. If the leaves are to be shipped to the laboratory by courier, they should be held overnight in refrigerated storage. For shipment, the bags of leaves should be packed loosely in styrofoam containers and placed in cardboard boxes. An ice pack should be included, but it should be sufficiently insulated with paper so not to freeze any leaves.

5. A complete content list should be placed on the top of the samples or with the bill of lading (if an overnight courier is used) and signed (if possible) by the person collecting the sample.

6. A field log of sampling dates, samples submitted, etc., is recommended to ensure sample continuity from the field to the laboratory.

7. Shipment of samples should be postponed if it is apparent that the package will be held in transit over a holiday or a weekend.

Identification of Specimens (continued)

8. Regular communication (e.g., by phone or fax) between collectors and the destination labatory is recommended to optimize the use of testing resources.

Instructions for the Preservation and Shipment of Leaves for Laboratory Diagnosis of PVY[n] (continued)

9. If confirmatory testing (after screening test) is to be performed at another laboratory, the leaf samples should be placed in good-quality paper bags, then packed and shipped as indicated above.

Identification Techniques:

Before identification can begin, it may be necessary to assess the quality of the sample. The following classification scheme may be used as a guide:

Good—Sample tissue contains no broken down tissue, and entire sample is in good condition.

Fair—Sample tissue is almost completely intact with some breakdown evident.

Poor—Sample tissue contains some breakdown, but intact tissue is present from each sample and can be bioassayed.

Very Poor—Sample tissue is largely broken down with no intact tissue from each sample. Such samples should NOT be bioassayed.

The following are various procedures for identification. The technique selected for WBGV may depend on program needs and goals.

1. Polymerase Chain Reaction—The most sensitive identification uses polymerase chain reaction (PCR). This technique amplifies a virus' unique nucleic acid sequence and makes enough additional copies of it for quick and reliable fingerprinting. Amplification takes only 3–5 hours, and the results are available in 1–2 days.

2. Direct Tissue Blot Immunoassay—The direct tissue blot immunoassay (DTBI) is an immunoassay technique that uses direct blotting of plant or animal tissue onto nylon (preferred; Navot et al., 1989) or nitrocellulose membranes. The assay is specific, sensitive, reliable, and rapid. Large numbers of samples may be assayed in this way. The technique precisely locates any antigens present in plant hosts or animal tissue. The blots can be carried out in the field with just a few instructions and then transported to diagnostic laboratories for processing. Results will still be valid, if blots are stored properly, for at least 1 month after the sample is taken. Blots can be stored permanently after processing (Bravo-Almonacid et al., 1992; Hsu et al., 1993).

Identification of Specimens (continued)

Field Procedures (Navot et al., 1989):

Plants: Leaves, flowers, and other plant parts may be squashed onto a dry nylon membrane, using a hard object such as a glass rod or pen. Stems are cut longitudinally or sliced serially from the apex to the crown and squashed. Fruits are cut open and imprinted on the membrane.

Insects and Mites: Carry live to the laboratory for immediate freezing at -20 °C. When frozen, vector bodies may be squashed on a nylon membrane as above.

3. Enzyme-Linked Immunosorbent Assay—this procedure, called ELISA for short, is currently the easiest laboratory detection method for most geminiviruses and is generally applicable for most virus detection with available virus-specific or group-specific antisera. A sample of the plant part most likely to contain the virus must be collected from hosts, especially from parts showing suspect symptoms, and sent to the laboratory, with full collection data (Klein & Wyatt, 1989).

In general, a small sample of the specimen is ground in a buffer and incubated in a specimen microtiter plate for a few hours before adding an enzyme-labeled monoclonal antibody. This sample is incubated for a few more hours with the final addition of a color-forming substrate subsequently quantitated in a ELISA plate reader.

In some cases, specimens may have to be established or grafted to plant hosts for weeks or months from germplasm to increase the pathogen titer to determine the presence of a given virus in an ELISA test. This procedure is not recommended for a program if samples from suspect host plants can be processed and determined within a reasonable time frame (Bravo-Almonacid et al., 1992).

4. Squash-blot Molecular Hybridization (Bravo-Almonacid et al., 1992)—Use of genomic libraries is essential to this technique. Clones can be obtained from researchers in the field or can be prepared from the samples. Double-stranded DNA is obtained by plasmid purification and restricted with endonucleases to liberate the inserts. These inserts are subjected to electrophoresis in agarose gels, and cDNA viral fragments are obtained. These fragments are in turn purified by electroelution and labeled with radioisotope or nonradioactive labels through a random oligonucleotide priming method. The samples are then subjected to molecular hybridization. Using a specific clone, even a small amount of nucleic acid can be detected. For instance, with PVY^n about 1 ng of virions can be detected after an 8-hour radiographic treatment. This procedure is, therefore, more sensitive than ELISA for virus detection.

5. Immunodiffusion Test—If antisera for virus identification is available, virus isolates taken from vectors may be identified serologically in a sodium dodecyl sulfate solution, in gel diffusion plates (Adlerz, 1987). It should be kept in mind that this test is the least sensitive procedure of those listed.

Identification of Specimens (continued)

Field Procedures (R. P. Singh et al., 1988):

Collect 500 leaves from each site or 10 woody hosts at each locality outlined in the survey, in batches of 100. If not immediately used, store at about 5-10 °C until time to extract the nucleic acids.

ADDENDUM 9

Forms Forms, as developed by the State, may be listed in this section.

ADDENDUM 10

Contributors

Thanks to the following individuals for their assistance in the development of these Guidelines. Dr. Dale Meyerdirk is responsible for seeing that the color drawings of the sweetpotato whitefly (SPWF) by Joel Floyd were available and Dr. Jane Polston is credited with the photographs of the viruses.

Animal and Plant Health Inspection Service:

Dale Meyerdirk
Plant Protection Laboratories
PPQ, APHIS, USDA
4700 River Road
Riverdale, MD 20737

Stephen Poe
Domestic & Emergency Operations
PPQ, APHIS, USDA
4700 River Road
Riverdale, MD 20737

Edward Podleckis
Biological Assessment and Taxonomic Support Staff
PPQ, APHIS, USDA
4700 River Road
Riverdale, MD 20737

Laurene Levy
National Plant Germplasm Center
Bldg. 580, BARC-E
Beltsville, MD 20705

University:

Jane Polston
Gulf Coast Research & Education Center
Institute of Food and Agricultural Sciences
University of Florida
5007 60th Street E
Bradenton, FL 34203

ADDENDUM 11

References

Abouzid, A., et al., 1992. The nucleotide sequence of tomato mottle virus, a new geminivirus isolated from tomatoes in Florida. J. Gen. Virol., 73: p. 3225–3229.

Acosta-Leal, R. and Quintero-Montelongo, S., 1989. Characterization of a virosis of chili transmitted by whiteflies in the Husteca Plain. Rev. Mex. de Fitopatologia, 7(2): pp. 147–149.

Adlerz, W.C., 1987. Cucurbit potyvirus transmission by alate aphids trapped alive. J. Econ. Entomol., 80(1): pp. 87–92.

Allison, D. and Pike, K.S., 1988. An inexpensive suction trap and its use in an Aphid Monitoring Network. J. Agric. Entomol. 5(2): pp. 103-107.

Anonymous, 1980. Annual Report for 1979. Cyprus Agricultural Research Institute, p. 95.

Anonymous, 1990. The bug sucker. In: Florida Magazine. Orlando Sentinel, April 15, 1990: p. 4.

Anonymous, 1993a. Natural compounds that kill plant damaging whiteflies. Quarterly Report of Selected Research Projects, USDA, ARS, April/June: p. 2.

Anonymous, 1993b. 85-Beneficial insect planes. In: New Products. Ag Consultants, 49(5): p. 18.

Anonymous, 1993c. Canada/U.S.A. PVY^{n} Management Plan. Agriculture Canada and USDA, APHIS, PPQ: pp. 1–7 + App. AĐE.

Anonymous, 1994. USDA-APHIS-PPQ-Mission Biological Control Laboratories Sweetpotato Whitefly Natural Enemy Cultures Currently in Production. 04-04-1994. APHIS, PPQ, Biological Control: p. 4.

Anonymous, 1995. Biological Control of Sweetpotato Whitefly Project Manual. USDA, APHIS, PPQ, BCO: p. 67.

Asjes, C. J., 1991. Control of air-borne field spread of tulip breaking virus, lily symptomless virus and lily virus X in lilies by mineral oils, synthetic pyrethroids, and a nematicide in the Netherlands. Neth. J. Pl. Path., 97(3): pp. 129–138.

Becker, H., 1993. Keeping Tabs on Plant Viruses and Viroids: New Tools From Genetic Engineering-Agricultural Research, 41(7): pp. 16-19.

Bell, A. C., 1988. The efficiency of the bulb and potato aphid *Rhopalosiphoninus latysiphon* as a vector of potato virus V. Potato Research, 31(4): pp. 691–694.

References (continued)

Bellows, T. S., et al., 1994. Description of a species of *Bemisia*. Ann. Ent. Soc. America, 87(2): pp. 195–206.

Berlinger, M. J., 1980. A yellow sticky trap for whiteflies: *Trialeurodes vaporariorum* and *Bemisia tabaci*. Ent. Exp. & Appl. 27: pp. 98–102.

Berlinger, M.J., et al., 1989. Honeydew excretion as a possible tool to screen tomato resistance to virus transmission by *Bemisia tabaci*. Proc. Working Group "Breeding for Resistance to Insects and Mites, Marcelin, Switzerland, 4-6: pp. 121-131.

Berlinger, M. J., Dahan, R., and Mordechi, S., 1988. Integrated pest management of organically grown greenhouse tomatoes in Israel. Applied Agricultural Research, 3(5): pp. 233–238.

Bethke, J. A., et al., 1991. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* on cotton and poinsettia. Ann. Ent. Soc. America, 84(4): pp. 407–411.

Bird, J., et al., 1975. Rugaceous viruses in Puerto Rico. In: Tropical Disease of Legumes, Academic Press, New York: pp. 3–25.

Blua, M. J. and Toscano, N. C., 1994. *Bemisia argentifolii* development and honeydew production as a function of cotton nitrogen status. Environ. Entomol., 23(2): pp. 316–321.

Boiteau, G., et al., 1992. Evaluation of a vacuum collector for insect pest control in potato. Am Potato J., 69(3): pp. 157–166.

Bravo-Almonacid, F., Haim, L., and Mentaberry, A., 1992. Rapid immunological detection of potato viruses in plant tissue squashes. Plant Disease, 76(6): pp. 574–578.

Briddon, R. W. and Markham, P. G., 1995. Geminiviridae. In: Virus Taxonomy, Sixth Report of the International Committee on Taxonomy of Viruses. Springer-Verlag Wien, New York: pp. 161–165.

Brown, J. K. and Nelson, M. R., 1986. Whitefly-borne viruses of melons and lettuce in Arizona. Phytopathology, 76(2): pp. 236–239.

Brown, J. K. and Nelson, M. R., 1988. Transmission, host range and virus-vector relationships in chino del tomate virus, a whitefly transmitted geminivirus from Sinaloa, Mexico. Plant Disease, 72(10): pp. 866–869.

Brown, J. K. and Poulos, B. T., 1990. Serrano golden mosaic virus, a newly identified whitefly transmitted geminivirus of pepper and tomato in the United States and Mexico. Plant Disease, 74(9): p. 720.

References (continued)

Brown, J. K., et al., 1989. A whitefly transmitted geminivirus from peppers with tigre disease. Plant Disease, 73(7): p. 610.

Brunt, A., et al., 1990. Viruses of Tropical Plants. CAB International Redwood Press, Melksham, England: p. 602.

Butler, G. D., et al., 1989. Biology, sampling and population dynamics of *Bemisia tabaci*. In: Biology and Population Dynamics of Invertebrate Crop Pests. Intercept Limited, Andover, Hampshire, England, 3: pp. 83–111.

Butter, N. S. and Rataul, H. S., 1977. Effect of TLCV infection on *Bemisia tabaci*. Entomon., 2(2): pp. 163–164.

Byrne, D. N. and Bellows, T. S., 1991. Whitefly biology. Annual Review of Entomology, 36: pp. 431–457.

Byrne, D. N. et al., 1994. Migration and dispersal by the sweetpotato whitefly. Phytoparasitica, 22(4): p. 314.

Caprio, M. A., Hoy, M. A., and Tabashnik, B. E., 1991. Model for implementing a genetically improved strain of a parasitoid. American Entomologist, 37(4): pp. 232–239.

Carruthers, R., 1995. Whitefly fungus on its way to growers. Agricultural Research, 43(5): pp. 16–17.

Carver, M., 1978. The black citrus aphids, *Toxoptera citricida* (Kirkaldy) and *T. aurantii* Boyer de Fonscolombe. J. Australian Ent. Soc., 17(3): pp. 263–270.

Chatchawankanphanich, O., et al., 1993. Nucleotide sequence of a geminivirus associated with tomato leaf curl from India. Plant Disease, 77(11): p. 1168.

Christie, R. G. and Edwardson, J. R., 1986. Light microscopic techniques for detection of plant virus inclusions. Plant Disease, 70(4): pp. 273–279.

Christie, R. G., et al., 1986. Light microscopy of geminivirus-induced nuclear inclusion bodies. Phytopathology, 76(1): pp. 124–126.

Clark, B., 1993. Biotech Briefs. Ag. Consultant, 49(9): p. 12.

Clayton, M.K. & Slack, S.A., 1988. Sample Size Determination in zero tolerance circumstances and the implications of stepwise sampling: Bacterial ring rot as a special case. American Potato Journal, 65(12): pp. 711-723.

References (continued)

Cohen, S., 1984. Transmission and control of whitefly-borne viruses. Phytoparasitica, 12(3–4): pp. 209–210.

Cohen, S. and Antignus, Y., 1994. Tomato yellow leaf curl virus, a whitefly-borne geminivirus of tomatoes. In: Advances in Disease Vector Research. Springer-Verlag, Inc., New York, 10: pp. 259–288.

Cohen, S., et al., 1983. Purification, serology, and vector relationships of squash leaf curl virus. Phytopathology, 73(12): pp. 1669–1673.

Cohen, S., et al., 1989. Acquisition, interference, and retention of cucurbit leaf curl viruses in whiteflies. Phytopathology, 79(1): pp. 109–113.

Czosnek, H., et al., 1990. Geographical distribution of tomato yellow leaf curl virus. A first survey using a specific DNA probe. Phytopathologia Mediterranea, 29(1): pp. 1–6.

Debrot, E. A. and Centeno, F., 1985. Natural infection of potato in Venezuela by tomato yellow mosaic, a geminivirus transmitted by whiteflies. Agronomia Tropical, 35(1–3): pp. 125–138.

Dhanju, K. S. and Varma, J. P., 1986. Natural occurrence of mixed infection of tomato leaf curl virus and tobacco mosaic virus in tomato. Indian J. Virology, 2(1): pp. 108–110.

Dhingra, K. L. and Chenulu, V. V., 1985. Effect of yellow mosaic on yield and nodulation of soybean. Indian Phytopathology, 38(2): pp. 248–251.

D'hondt, M.D. and Russo, M., 1984. Tomato yellow leaf curl in Senegal. Phytopathol. Zeitschrift, 122: pp. 153-160.

Dolores, L.M., et al., 1988. Viral diseases of squash in the Philippines and sources of resistance. Philippine Journal of Crop Science, 13 (Supp. 1): p. 530.

EPPO, 1992. Plum pox potyvirus. EPPO quarantine pest. Data Sheets on Quarantine Pests, A2 list: No. 96, Quarantine Pests for Europe. CABI/EPPO, Wallingford, UK: pp. 921–927.

Fargette, D., et al., 1993. Comparative epidemiology of three tropical whitefly-transmitted geminiviruses. Biochimie (Paris), 75(7): pp. 547–554.

Farm Chemicals Handbook, 1995. Meister Pub. Co., Willoughby, Ohio.

Faust, R. M., 1992. Conference Report and 5-year National Research and Action Plan for Development of Management and Control Methodology for the Sweetpotato Whitefly. USDA, ARS, 107: p. 165.

References (continued)

Ferguson, J., 1995. Insect controls. AG Consultant, 51(3): p. 8.

Francki, R. I. B., et al., 1991. Classification and Nomenclature of Viruses. Fifth Report of the International Committee on Taxonomy of Viruses. Springer-Verlag, New York: pp. 171–177.

Garzon-Tiznado, J. A., 1993. Inoculation of peppers with infectious clones of a new geminivirus by a biolistic procedure. Phytopathology, 83(5): pp. 514–521.

Gibson, R. W. and Rice, A. D., 1986. The combined use of mineral oils and pyrethroids to control plant viruses transmitted non- and semi-persistently by *Myzus persicae*. Ann. Appl. Biol., 109(3): pp. 465–472.

Gilbertson, R. L., et al., 1991. Differentiation of bean-infecting geminiviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Disease, 75: pp. 336–342.

Gilbertson, R. L., et al., 1993. Pseudorecombination between infectious cloned DNA components of tomato mottle and bean dwarf mosaic geminiviruses. J. General Virology, 74(1): pp. 23–31.

Goolsby, J. A., 1995. Biological control of sweetpotato whitefly. In: FY 1994 Project Highlights. Mission Biological Control Laboratory & Bozeman Biological Control Facility, Mission, TX.

Green, S. K., et al., 1987. Outbreaks and new records. Leaf curl virus on tomato in Taiwan Province. FAO Plant-Protection Bulletin, 35(2): p. 62.

Halbert, S. E., Zhang, G., and Pu, Z., 1986. Comparison of sampling methods for alate aphids and observations on epidemiology of soybean mosaic virus in Nanjing, China. Ann. Appl. Biol., 109: pp. 473–483.

Harrewijn, P., Ouden, H. den, Piron, P. G. M., 1991. Polymer webs to prevent virus transmission by aphids in seed potatoes. Entomol. Exp. Appl., 58: pp. 101–107.

Harrison, B.D., et al., 1991. Recognition and differentiation of seven whitefly-transmitted geminiviruses from India, and their relationships to African cassava mosaic and Thailand mung bean yellow mosaic viruses. Ann. Appl. Biol., 118(2): pp. 299–308.

Heinz, K. M. and Parrella, M. P., 1994. Biological control of *Bemisia argentifolii* infesting *Euphorbia pulcherrima*: evaluations of releases of *Encarsia luteola* and *Delphastus pusillus*. Environ. Ent., 23(5): pp. 1346–1353.

Hill, D. S., 1983. Agricultural insect pests of the tropics and their control. Cambridge University Press, Cambridge, England, p. 198.

References (continued)

Honda, Y., et al., 1986. Mungbean yellow mosaic virus isolated from mungbean in Thailand. Tech. Bull. Tropical Agric. Res. Center, 21: pp. 189–202.

Hong, Y. G., et al., 1995. Chinese squash leaf curl virus: a new whitefly transmitted geminivirus. Science in China, ser. B - Chemistry Life Sciences & Earth Sciences, 38(2): pp. 179–186.

Hoy, M. A., 1990. Genetic improvement of arthropod natural enemies: becoming a conventional tactic? Proceedings of a UCLA Colloquium held at Frisco, Colorado, 1989. Alan R. Liss, New York, 112: pp. 405–417.

Hoy, M. A., 1990. Genetic improvement of parasites and predators. FFTC-NARC International Seminar on the use of parasitoids and predators to control agricultural pests, Tukuha Science City, Japan, p. 15.

Hsu, H.T., et al., 1993. 17.1.43. Improved detection of plant pathogens by direct tissue blot immunoassay. In: Abstracts, 6th International Congress of Plant Pathology, July 28–August 6, p. 17.1.

Ikegami, M., 1988. Comparative studies between amino acid sequences of potential gene products of Mung bean yellow mosaic virus and other whitefly transmitted geminiviruses. J. Agricultural Science, 33(2): pp. 181–189.

Irwin, M. E. and Goodman, R. N., 1981. Ecology and control of soybean mosaic virus. In: Plant Diseases and their Vectors: Ecology and Epidemiology. Academic Press, New York: pp. 181–220.

Irwin, M. E. and Kampmeier, G. E., 1989. Chapter 2, Vector behavior, environmental stimuli, and the dynamics of plant virus epidemics. In: Spatial Components of Plant Disease Epidemics. Prentice Hall, NJ, Englewood Cliffs: pp. 14–39.

Jones, R. A. C., 1991. Reflective mulch decreases the spread of two non-persistently aphid transmitted viruses to narrow-leafed lupin. Ann. Appl. Biol., 118(1): pp. 79–85.

Klein, R. E. and Wyatt, S. D., 1989. Influence of propagation on incidence of seedborne bean common mosaic virus in the USDA *Phaseolus* Germ Plasm Collection. Plant Disease, 73(9): pp. 759–761.

Kobatake, H., et al., 1981. Ecology and control of yellow dwarf disease of tomato caused by tobacco leaf curl virus. Proceedings of the Kansai Plant Protection Soc., 23: pp. 8–14.

Leidner, J., 1994. Keeping whiteflies under control. Progressive Farmer, State North Carolina, 109(9): p. 2.

References (continued)

Liu, Tong-Xian and Stansly, P. A., 1995. Toxicity of Biorational Insecticides to *Bemisia argentifolii* on Tomato Leaves. J. Econ. Entomol., 88(3): pp. 564–568.

Lowery, D. T., et al., 1990. Control of turnip mosaic virus of rutabaga with applications of oil, whitewash, and insecticides. J. Econ. Entomol., 83(6): pp. 2352–2356.

Lucas, G. B., et al., 1985. Introduction to Plant Diseases. AVI Publishing, Westport, CT, 6: p. 52.

MacIntosh, S., et al., 1992. Detection of three whitefly-transmitted geminiviruses occurring in Europe by tests with heterologous monoclonal antibodies. Ann. Appl. Biol., 121(2): pp. 297–303.

Makkouk, K. M. and Laterrot, H., 1983. Epidemiology and control of tomato yellow leaf curl virus. In: Plant Virus Epidemiology. Blackwell, Oxford, England, pp. 315–321.

Makkouk, K. M. and Menassa, R. E., 1985. Effects of a mineral oil spray on aphid transmission of Zucchini yellow mosaic virus to cucumbers. Arab J. Pl. Prot., 3(1): pp. 18–23.

Makkouk, K. M., et al., 1979. Tomato yellow leaf curl: incidence, yield and losses and transmission in Lebanon. Phytopathologische Zeitshcrift. 96(3): pp. 263–267.

Marriappan, V. and Narayanasamy, P., 1972. *Acanthospermum hispidum,* a new host of tomato leaf curl virus. Madras Agricultural J., 59(6): pp. 355–357.

Marriappan, V. and Narayanasamy, P., 1977. Characterization of virus affecting weeds II. Leaf curl disease. Madras Agricultural J., 64(11): pp. 740–744.

Matthews, R. E. F., 1993. Diagnosis of Plant Virus Diseases. CRC Press, Boca Raton, Florida: p. 112.

Maxwell, D. P., et al., 1994. Widespread occurrence of the Eastern Mediterranean strain of tomato yellow leaf curl geminivirus on tomatoes in the Dominican Republic. Plant Disease, 78 (In Press).

Mazyad, H. M., 1979. Observations on the epidemiology of tomato yellow leaf curl disease on tomato plants. Plant Disease Reporter, 63(8): pp. 595–698.

McCreight, J. D. & Kishaba, A. N., 1991. Reaction of cucurbit species to squash leaf curl virus and sweetpotato whitefly. J. Am. Soc. Horticultural Science, 116(1): pp. 137–141.

References (continued)

McGovern, R. J., et al., 1994. Identification of a natural weed host of tomato mottle geminivirus in Florida. Plant Disease, 78(11): pp. 1102–1106.

Meyerdirk, D. E., et al., 1986. Population dynamics and control strategy for *Bemisia tabaci* in the Imperial Valley, California. Agriculture Ecosystems and Environment, 17(1): pp. 61–67.

Milne, D. L., 1977. Control of citrus nursery pests by soil applications of Dimethoate. Citrus & Subtropical Fruit J., 525: pp. 5–7.

Morales, F., et al., 1990. Isolation and partial characterization of a geminivurs causing bean dwarf mosaic. Phytopathology, 80(1): pp. 96–101.

Mound, L. A. and Halsey, S. H., 1978. Whiteflies of the world. A systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data. British Museum (N. H.), London, pp. 111–124.

Nakhla, M.K., et al., 1994. Two Geminiviruses associated with tomatoes in Central America. Phytopathology, 84(10): p. 1155.

Navot, N., Ber, R., and Czosnek, H., 1989. Rapid Detection of Tomato Yellow Leaf Curl Virus in Squashes of Plants and Plant Vectors. Phytopathology, 79(5): pp. 562-568.

N'Guessan, K. P., et al., 1992. Aspects of the epidemiology of okra leaf curl virus in Cote d'Ivoire. Tropical Pest Management, 38(2): pp. 122–126.

Olivares, F. M., et al., 1972. Progress report on whitefly transmission of tobacco leaf curl virus to vegetables and weeds. Philippine Phytopathology, 8(1/2): pp. 21–29.

Osaki, T., et al., 1979. Yellow vein mosaic of honeysuckle *(Lonicera japonica),* a disease caused by tobacco leaf curl virus in Japan. Annuals Phytopathological Society of Japan, 45(1): pp. 62–69.

Padmanabhan, C. and Pathmanabhan, G., 1978. A leaf curl disease of *Zinnia elegans.* Current Research, 6: pp. 100–101.

Perrin, R. M. and Gibson, R. W., 1985. Control of some insect-borne plant viruses with the pyrethroid PP321 (Karate). International Pest Control, 27(6): pp. 142–145.

Perring, T. M., et al., 1989. Floating row covers for the exclusion of virus vectors and the effect on disease incidence and yield of cantaloupe. J. Econ. Entomol., 82(6): pp. 1709–1715.

Perring, T. M., et al., 1993. Identification of a whitefly species by genomic and behavioral studies. Science, 259: pp. 74–77.

References (continued)

Pirone, T. P., Raccah, B., and Madden, L. V., 1988. Suppression of aphid colonization by insecticides: effect on the incidence of potyviruses in tobacco. Plant Disease, 72(4): pp. 350–353.

Polston, J. E. and Bois, D., 1994. First report of a tomato yellow leaf curl-like geminivirus in the Western Hemisphere. Plant Disease, 78(8): p. 831.

Polston, J. E., et al., 1989. Nucleic acid probes for detection and strain discrimination of cucurbit geminiviruses. Phytopathology, 79(10): pp. 1123--1127.

Polston, J. E., et al., 1990. Association of the nucleic acid of squash leaf curl geminivirus with the whitefly *Bemisia tabaci*. Phytopathology, 80(9): pp. 850–856.

Polston, J. E., et al., 1993. Host range of tomato mottle virus, a new geminivirus infecting tomato in Florida. Plant Disease, 77(12): pp. 1181–1184.

Polston, J. E., et al., 1994. Tomato yellow leaf curl-like geminivirus detected in Dominican Republic. Phytopathology, 84(10): p. 1072.

Qiu, J. Y. and Pirone, T. P., 1989. Assessment of the effect of oil on the potyvirus aphid transmission process. J. Phytopathology, 127(3): pp. 221–226.

Raccah, B., Gal-On, A., and Eastop, V. F., 1985. The role of flying aphid vectors in the transmission of cucumber mosaic virus and potato virus Y to peppers in Israel. Ann. Appl. Biol., 106: pp. 451–460.

Rapisarda, C., 1990. *Bemisia tabaci* vector of TYLCV in Sicily. Informatore Fitopatologico, 40(6): pp. 27–31.

Reeves, D., 1992. Discussion of Possible Strategies to Reduce the Impact of BCA/CTV in the U.S.A. Exclusion Efforts at Ports-of-Entry. In: Minutes of the APHIS Brown Citrus Aphid Stakeholder Workshop. Ladner, D. R., APHIS, IX: p. 9.

Retuerma, M. L., et al., 1972. Preliminary study of the transmission of Philippine tomato leaf curl virus by *Bemisia tabaci*. Philippine J. Plant Industry, 37(1/2): pp. 45–49.

Roberts, E. J. F., et al., 1988. Characterization of potato yello mosaic virus as a geminivirus with a bipartite genome. Intervirology, 29(3): pp. 162–169.

Rondon, P., et al., 1980. Chemical control of plant parasitic nematodes on sugarcane. Nematropica, 10(2): pp. 107-111.

Rosset, P., et al., 1990. Estimation of loss and identification of the geminivirus transmitted to tomato by the whitefly *Bemisia tabaci* in Costa Rica. Manejo Integrado de Plagas, 15: pp. 24–35.

References (continued)

Samways, M.J. & Grech, N.M., 1986. Assessment of the fungus *Cladosporium oxysporum* as a potential biocontrol agent against certain Homoptera. Agriculture systems and environment, 15(4): pp. 231-239.

Schoulties, C. L., et al., 1987. Citrus tristeza virus and vectors: regulatory concerns. Proc. Fla. State Hort. Soc., 100: pp. 74–76.

Scott, R.W. and Achtemeier, G.L., 1987. Estimating pathways of migrating insects carried in atmospheric winds. Environ. Entomol., 16(6): pp. 1244–1254.

Shaheen, A.H., 1977. Survey of pests attacking tomato in Egypt with some ecological notes. Agricultural Research Review, 55(1): pp. 49–57.

Simmons, G. S. and Minkenberg, O. P. J. M., 1994. Field-cage evaluation of augmentative biological control of *Bemisia argentifolii* in Southern California cotton with the parasitoid *Eretmocerus nr. californicus*. Environ. Ent., 23(6): pp. 1552–1557.

Singh, D. P., 1982. Breeding for resistance to mungbean yellow mosaic virus in mung bean and urdbean, *Vigna radiata, Vigna mungo*. Indian J. Genetics Plant Breeding, 42(3): pp. 348–355.

Singh, M. N., et al., 1988. Environmental factors influencing aphid transmission of potato virus Y and potato leafroll virus. Potato Research, 31(3): pp. 501–509.

Singh, R. N., 1983. Yellow mosaic of *Phaseolus atropurpureus* caused by mungbean yellow mosaic virus. Legume Research, 6(1): p. 52.

Singh, R. P., et al., 1988. A survey of the incidence of potato spindle tuber viroid in Prince Edward Island using two testing methods. Can. J. Plant Sci., 68: pp. 1229–1236.

Stenger, D. C., et al., 1990. Biological and genomic properties of a geminivirus isolated from pepper. Phytopathology, 80(8): pp. 704–709.

Suwwan, M. A., et al., 1988. Tomato performance and incidence of tomato yellow leaf curl (TYLC) virus as affected by type of mulch. Scientia Horticulturae, 37(1–2): pp. 39–45.

Swanson, M. M. and Harrison, B. D., 1993. Serological relationships and epitope profiles of isolates of okra leaf curl geminivirus from Africa and the Middle East. Biochimie, 75(8): pp. 707–711.

Taylor, L. R. and Palmer, J. M. P., 1972. Aerial sampling. In: van Emden H. F., Aphid Technology. Academic Press, New York, pp. 189–234.

Verma, H. N., et al., 1975. A whitefly transmitted yellow mosaic virus disease of tomato from India. Plant Disease Reporter, 59(6): pp. 494–498.

References (continued)

Vicchi, V. and Bellardi, M. G., 1988. The role of weeds in the epidemiology of gladiolus viruses and MLO. Acta Horticulturae, 234: pp. 371–378.

Walkey, D.G.A., et al., 1990. Plant virus disease in the Yemen Arab Republic. Tropical Pest Management, 36(3): pp. 195–206.

Ware, G. W., 1980. Complete Guide to Pest Control With and Without Chemicals. Thomson Publishers, Fresno, California: Chapt. 3, p. 24; Appendix A, p. 241; Appendix E, p. 257.

Watson, T. F., et al., 1992. Seasonal dynamics of sweetpotato whitefly in Arizona. Southwestern Entomologist, 17(2): pp. 149–167.

Webb, S.E. and Kok-Yokomi, M.L., 1993. Transmission of cucurbit potyviruses by *Uroleucon pseudambrosiae,* an aphid trapped during epidemics of watermelon mosaic virus 2 in Florida. J. Econ. Entomol., 86(6): pp. 1786–1792.

Wong, S. M., et al., 1993. A geminivirus causing vein yellowing of *Ageratum conyzoides* in Singapore. Plant Pathology, 42(1): pp. 137–139.

Wright, J.E., 1992. Whiteflies: Development of Naturalis, a bionational mycoinsecticide for control. Proceedings - Beltwide Cotton Production Research Conferences, 2: pp. 887-888.

Yassin, A. M., 1978. Whitefly-borne virus diseases of important crop plants in the Sudan. Proc. Fourth Conference Pest Control, Sept. 3-Oct. 3, 1978. Part I: pp. 107–114.

Yassin, A. M. and Abu-Salih, H. S., 1972. Leaf curl of tomato. Technical Bull. Agric. Res. Corp., 3: p. 33.

Zakay, Y., et al., 1991. Screening *Lycopersicon* accessions for resistance to tomato yellow leaf curl virus: presence of viral DNA and symptom development. Plant Disease, 75(3): pp. 279–281.

Zalom, F. G., et al., 1985. Temperature regulation of *Bemisia tabaci* populations in Imperial Valley cotton. J. Econ. Entomol., 78(1): pp. 61–64.

INDEX

A

B

D

E

F

G

H

M

Q

R

T

U-V

W

X-Y-Z