THE BELIZE CITRUS CERTIFICATION PROGRAMME (BCCP) GUIDELINES

1. Introduction:

The BCCP has been established to ensure that citrus growers in Belize have healthy, horticulturally desirable citrus varieties and rootstocks available in order to maintain the production of citrus. These guidelines are intended to serve as a practical guide containing instructions for growers, nurserymen, and other interested parties to follow the requirements of the mandatory citrus certification programme.

This mandatory citrus certification programme will be administered by the Minister of Agriculture through its agent, the Belize Citrus Certification Programme (BCCP). Most of the day-to-day operations will be administered by the Citrus Research and Education Institute (CREI), the research arm of the Belize Citrus Growers Association. Therefore, if specific questions arise, these questions may be directed to the BCCP located at 9 Miles Stann Creek Valley Road, P.O. Box 72, Dangriga (Telephone 05-23535, Fax 05-23511), Email : crei@btl.net

II. Details on how to comply with the Belize Citrus Certification Programme (BCCP)

A. Registration of nurseries:

a. Requirements for nursery sites and facilities:

i. Who must register?

Each owner of a nursery in Belize intending to grow and propagate citrus trees which will be planted in the ground will have to be registered.

ii. What are the requirements?

Before a nursery can be registered to produce certified citrus trees, several conditions must be met in order to assure persons who are buying and planting citrus trees that these trees were grown under conditions to ensure that the trees are healthy. Most of the following requirements are intended to ensure that plants are grown or held for retail sale without problems due to root pathogens such as *Phytophthora* and nematodes, while some requirements are meant to ensure plant identity.

Specific requirements include:

- ★ run-off irrigation water is led out of the nursery area by means of a suitable drainage system, and the area cannot be contaminated with run-off water from other citrus plantings in the vicinity.
- ★ seed boxes and seedling trays are raised completely off the ground.
- ★ an effective nematode and *Phytophthora* control programme is in place.
- ★ all rootstock plants are grown in polyethylene bags or other suitable containers.
- ★ the soil or potting mix used in boxes, trays, bags, or containers:
  -permits effective drainage
  -is free from harmful pathogens
  -is not mixed or stored on a surface which is or may become infested with soil pathogens.
- ★ roots of plants in containers are not permitted to come into contact with soil outside of the container.
- ★ budded seedlings are kept in rows with gaps between different budwood sources, with the first and last bag or container of seedlings budded from each budwood bundle clearly labeled with the bud source, rootstock, and date of budding.

Nurseries already in operation when the BCCP is implemented will have two years to meet the requirements for registration, although they must begin the registration process at least 18 months after the implementation date of the BCCP. They may not continue operation after that time period has expired unless they have

modified their nursery to meet the requirements as listed above.

New nurseries must be located on sites which have not been planted with citrus before. However, if the sites have been previously planted with citrus, treatment of the soil with pesticides or cultural practices which would eliminate *Phytophthora* and nematodes, would be acceptable. All of the specific requirements for nurseries previously listed must be met.

The nurseries will be inspected by personnel from the BCCP to verify that the requirements are met. Soil samples may be collected to determine the incidence and population levels of soil pathogens.

b. Procedure for nursery registration.

Nursery registration will be issued for one year.

i. New applications

Any person wishing to register a nursery which will either grow or propagate citrus trees or sell certified citrus trees is required to complete and submit an Application Form (Form AF) (Schedule 1) to the BCCP two months before nursery operations are to begin. A non-refundable fee for registration must be included with the completed Form AF. The fees are listed in Schedule 7. The current fee for new applications for nursery registration is $100. Nurseries propagating and growing certified citrus trees or reselling certified citrus trees will be required to renew their registration yearly.

Nurseries which exist and are operating when the BCCP is implemented will have up to 18 months to apply to become a registered nursery. All nursery requirements must be met within two years of the implementation date. This two year requirement is also intended to allow existing nurseries to sell their current stock of trees, because only certified citrus trees are to be sold two years after the implementation date. Nurseries are encouraged to begin propagating using certified budwood as soon as possible, even before the program is officially implemented.

Upon the receipt of an application for nursery registration, the inspector appointed by the BCCP will carry out on-site visits to ensure the compliance of the nursery with the requirements for registration. If the nursery fulfills the requirements, the nursery will be issue a Nursery Registration Certificate (NRC) (Schedule 2) which is valid for one year. The BCCP may carry out unannounced on-site inspections to ensure that nursery operations are being carried out in compliance with the regulations at any time during the year. Nurseries will required to renew their registration yearly. The Nursery Registration Certificate is to be posted prominently at the nursery site.

ii. Renewal applications

Applications for renewal of nursery registration will be made as previously

described for new applications. The application for renewal should be made two months before expiration of the current Nursery Registration Certificate. The non-refundable fee for renewal of nursery registration is currently $100 (refer to Schedule 7), this fee must accompany the completed application form.

c. Cancellation of nursery registration

Nursery registration may be canceled by the BCCP if the owner misrepresents nursery stock as being certified, knowingly sells trees infested with a graft transmissible disease, or otherwise violates the provisions of the Plant Protection Act, Chapter 178 of the Laws of Belize or the BCCP regulations, if such a request is made in writing, or if a nursery inspection reveals that the Guidelines are not being followed.

If nursery registration is canceled by the Board, the owner will be given an appropriate time frame as determined by the board to remedy the problem. The nursery will then be re-inspected and the registration restored if the problem has been remedied or denied registration if the problem has not been remedied. If the registration is denied, the nursery must submit an application as if it were a new nursery applying for registration along with the non-refundable fee.

III. NOMENCLATURE

A. Scheme of the BCCP

The overall scheme of the BCCP is illustrated in Figure 1. The importation of virus free germplasm from a recognized clean stock program, for example the California Clonal Protection Program, will occur when the industry calls for varieties not present in the country as certified varieties. These will undergo post entry quarantine for one year if all tests verify freedom of the diseases that can be diagnosed at the given time. These accessions will then be maintained under protected conditions by the BCCP as certified parent trees or the germplasm bank (mainly five plants of each variety). Selection of proven local varieties will also be carried out, propagated under protected conditions and indexed for diseases that can be diagnosed at the given time, their cleansing process through shoot tip grafting will also be carried out locally or internationally. Once cleansed they will under go post entry quarantine for one year and if verified free of diseases they will become part of the germplasm collection. The parent trees will be the source of budwood for pre-multiplication blocks, varietal blocks and seed source trees. Varieties that are in demand by the industry will be multiplied in the pre-multiplication blocks and the budwood will be for the establishment of multiplication blocks by the BCCP and registered nurseries that require it. The multiplication blocks will be the main sources of budwood for commercial nursery plants.

Each of the classifications of the trees in the scheme will be discussed in this section, followed by a description of the tracking code which will be used in the certification programme.

B. Parent trees

Parent trees are horticulturally true-to-type citrus trees obtained as a pathogen-free accession from a clean stock programme that implements or complies with the FAO/IPGRI Guidelines for the Safe Movement of Citrus Germplasm or other clean stock programme that has been approved by the Minister upon recommendation of the BCCP. Parent trees must test negative for all graft-transmissible diseases and be free of recognizable signs of disease symptoms, bud mutation, and plant pests. All parent trees will be maintained under protected conditions by the BCCP. Reserve parent trees will be retained in the screenhouse at the Central Farm, Ministry of Agriculture Quarantine Facilities, in the Cayo District, and possibly at other locations.

Parent trees will be tested for freedom from CTV once a year, for the freedom from the psorosis complex of viruses and citrus viroids every four years, and for freedom from greening, stubborn, CVC and other testable graft-transmissible agents every five years. The interval between testing may be changed upon recommendation of the Board which advises the BCCP.

Parent trees may be established by importation as pathogen-free accession as previously described, or selected from local clones whose selection will be based on documented evidence of desirable traits, such as yield and/or fruit quality data. Local clones selected to become parent trees must be forwarded as propagating material to a recognized clean stock programme where the clonal selection will undergo shoot tip grafting or other methods of obtaining virus-free germplasm, following by virus indexing to verify freedom of graft-transmissible diseases, and returned as a pathogen-free accession.

All accessions of prospective parent trees will be indexed for CTV, citrus viroids, the psorosis complex of viruses and tatterleaf virus using the methods briefly explained in Box 1. Budwood from the prospective parent trees may be conditionally released upon negative indexing results for the short term indexes, but the subsequent budwood propagations will be subject to recall if cachexia, citrus tatterleaf, or other virus is indicated by the long term indexes. If propagations are recalled because of the findings of a virus, the BCCP will replace the budwood free of charge once clean budwood is available.

The cost of establishing parent trees and related expenses will be borne by the BCCP and the budwood from the accession is to be distributed throughout Belize for the benefit of the citrus industry.

Parent trees may be used as a source of buds to establish varietal block trees, pre- multiplication block trees, multiplication block trees, seed source trees, and certified citrus trees. All budwood cutting must be witnessed by an authorised representative of the BCCP.

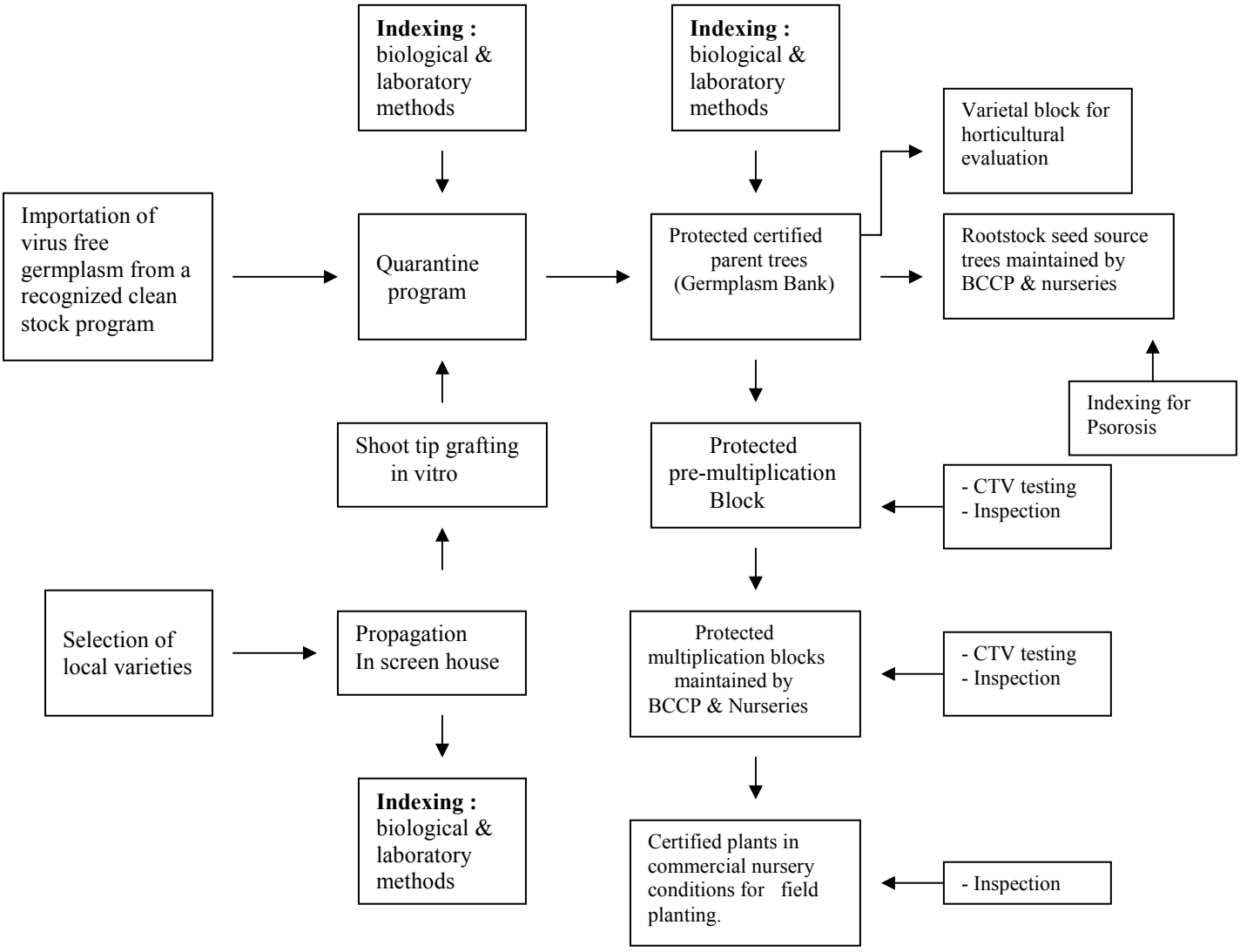

Figure 1. Flow Diagram of the Belize Citrus Certification Program

Box 1.
Protocol for indexing of new accessions into the BCCP

★ Short term indexes for CTV (conducted by ELISA), citrus viroids (except cachexia), and psorosis complex of viruses.

Freedom from viroids will be verified by biological indexing using citron scions maintained under warm temperature, followed by extraction and analysis on polyacrylamide gels if no symptoms are expressed in citron.

Freedom from cachexia may be verified from testing the inoculated citrons used for viroid index by the sequential polyacrylamide gel electrophoresis method and/or reverse transcriptase polymerase chain reaction assays with the use of proper positive and negative controls in either test.

Freedom from the psorosis complex of viruses may be verified by biological indexing using sweet orange seedlings or budlings maintained under cool temperature conditions as indicator plants with positive and negative controls included in the tests.

Freedom from citrus tatterleaf virus may be biologically tested by graft inoculating a nurse plant, such as rough lemon or sweet orange, and top-working with a live bud of Rusk citrange as the indicator plant under cool temperature conditions.

★ Long term indexes for freedom from citrus cachexia viroid and citrus tatterleaf virus will be performed by planting some of the varietal block trees on Orlando tangelo or Clemelin rootstock (susceptible to cachexia) and on Carrizo or other citrange or citrumelo rootstock (susceptible to citrus tatterleaf). These trees should be observed for two years for virus symptoms. These long term indexes should be completed by the time the trueness of type of the fruit has been verified.

C. Seed source trees

Seed source trees are trees that supply seed which is intended to be used as rootstocks for citrus propagations. Several requirements have been established to ensure that all seed source trees appear healthy, are free of the psorosis complex of viruses (the only virus shown to be seed transmitted in citrus), and are horticulturally true-to-type. True-to-type rootstocks are desirable because often the "off-type" rootstocks (heterozygotic) are more susceptible to soil pathogens and/or virus diseases, such as blight, viroids, citrus tatterleaf, and woody gall.

Seed source trees, as well as the immediate surrounding trees, are required to be free from bark and leaf symptoms of the psorosis complex of viruses, citrus blight, citrus viroids, decline, gummosis, or any other recognisable symptoms of disease. Additionally, the prospective seed source tree must be free of bud mutation.

Applications on behalf of trees which exist and are bearing fruit at the time of implementation of the BCCP must be made for prospective seed source trees within three months after the implementation date of the BCCP. After this time, only trees which have been propagated from parent trees may be considered as prospective seed source trees.

a. Procedure to certify a seed source tree

i. Procedure

A procedure has been established for the certification of seed source trees:

- ★ Before submitting a Seed Source Tree Application (Form STA), attached as Schedule 4, to request evaluation and testing of a candidate tree for certification as a seed source tree, the applicant should make a visual inspection of the tree and the immediate surrounding trees. This inspection by the applicant should verify freedom of recognizable disease symptoms, and to make sure the candidate tree is bearing fruit typical of the variety. If there is an obvious problem with the tree and the tree is likely will not be certified as a seed source tree, then there is no reason to pay a non-refundable deposit for the evaluation and testing.

- ★ To apply for a tree to be certified as a seed source tree, a Seed Source Tree Application (Form STA) must be completed. This form requires information as to the variety of the tree, location within a nursery or grove, and a sketched map to enable BCCP authorised personnel to locate the tree. The map should have enough detail so that a person who is unfamiliar with the nursery or farm can find the tree location. Upon completion of Form STA, the original copy and a non-refundable fee (currently set $40, see Schedule 7) should be forwarded to the BCCP, c/o

CREI, P.O. Box 72, Dangriga, 9 Miles Stann Creek Valley Road.

★ When the Form STA is received, BCCP authorised personnel will visually evaluate the candidate tree, and the immediate surrounding trees, to determine whether they meet the requirements outlined in the Seed Source Tree Certificate (STC). Additionally, budwood will be collected from the candidate tree for biological indexing for the psorosis complex of viruses.

★ If all the responses are positive from the list on Form STC, and upon completion and verification of freedom of the psorosis complex of viruses, the Seed Source Tree Certificate (Form STC), attached as Schedule 5, will be completed by the BCCP authorised representative, and returned by registered mail to the applicant. The biological indexing will require several months for completion, and the STC cannot be returned until the indexing has been completed. Because of this, application for certification of seed source tree must be made at least eight months in advance of the intended use of the seed from the tree.

★ A seed source tree is certified for four year time period.

★ All certified seed source trees and surrounding trees will be visually inspected annually by an authorised representative of the BCCP.

★ Certification of seed source trees may be cancelled by the BCCP for the following conditions:

- symptoms of virus or other bud-transmissible disease appear on the seed source tree or any of the trees immediately surrounding the tree
- careless handling of seeds collected from the seed source tree or of the records
- evidence of mutation on fruit and/or foliage of the seed source tree
- other violations of the provisions of the Plant Protection Act or of the BCCP
- biological indexing indicates the presence of the psorosis complex of viruses in the seed source tree
- the certificate holder voluntarily requests cancellation

★ If the certification of a seed source tree is canceled because of a problem with one of the trees immediately surrounding the certified tree, the owner will have 30 days to remedy the problem. If the problem has been remedied by removal of the affected tree upon inspection at the end of the 30 days, the certification may be reinstated.

b. Re-certification of a seed source tree

A seed source tree may be re-certified by making an application for re-certification. The non-refundable fee for re-certification is currently $40. The application and testing requirements for re-certification are the same as described for certification. Re-certification is for a period of four years.

D. Pre-multiplication block (P-MB) trees

Pre-multiplication block (P-MB) trees are specially designated nursery propagations intended to multiply supplies of budwood rapidly for use for the propagation of multiplication blocks trees or certified citrus trees and maintained by the board. Budwood used to establish P-MB trees must come from parent trees maintained by the BCCP. All budwood cutting must be witnessed. P-MB trees shall be planted in the field or container grown and maintained under protected conditions such as in a screenhouse or greenhouse. Experience from other citrus producing areas indicate that P-MB trees under protected conditions are not likely to become infected with CTV and usually have more buds for use in the early spring when demand for buds is highest.

a. Numbering and identification of P-MBs

P-MB trees must be separated from other plantings and properly and adequately labeled. Each block or group of trees which comprise a P-MB will be assigned a P-MB number by the Board. The P-MB number is recorded on the right-hand portion of the Bud Cutting Form (BCF, Schedule 3)(refer to Figure 2 for an example). The P-MB numbers will be assigned by the board in sequential order beginning with P-MB1. No P-MB number will ever be repeated. The P-MB number is important for tracking and locating progeny of bud source material.

For P-MBs grown in the field, each clone shall be separated in the row by a gap no less than 2 feet, well separated from other nursery propagations (certified citrus trees, varietal block trees and seed source trees). First and last trees of a single clone in the P-MB must be properly labeled with the clone, date budded and the P-MB number using durable markers. For P-MB trees grown in containers, each clone should be separated by 12 inch gaps on the benches, with the first and last container properly labeled with the clone, date budded, and the P-MB number.

Vigourous rootstocks, such a Volkamerian lemon, or rough lemon should be used for P-MB trees. A 2x3 ft or 2x2 ft spacing is recommended for P-MB trees grown in the field.

Regular and strict pest and disease control measures should be applied at all times to P-MB trees. Fertilize on a regular basis to ensure good growth of trees.

b. Age of P-MB trees

P-MB trees may be used as sources of certified budwood for use in propagation of multiplication block trees or certified citrus trees for up to 24 months without a requirement of testing for CTV, or other viruses. The age of P-MB trees is determined by the date of propagation of the P-MB tree. As an example, if a P-MB tree was propagated on 30 Sep 95, the 24 month anniversary would be 29 Sep 97. If the P-MB trees are tested for CTV between the 18^{th} - 22^{nd} month and found free of CTV, or if CTV is present in low incidence (less than 5 percent) and the CTV-infected trees are removed immediately, the P-MB trees may be used as a source of budwood for multiplication block trees or certified citrus trees for up to the end of 30 months. The testing requirements and time for which buds may be cut from P-MB trees are subject to periodic review by the BCCP and are subject to change.

P-MB trees may be used as varietal block trees or certified citrus trees after they no longer qualify as P-MB trees because of time limits.

E. Multiplication block (MB) trees

Multiplication block (MB) trees are specially designated nursery propagations intended to multiply supplies of budwood rapidly for use for the propagation of certified citrus trees. Budwood used to establish MB trees must come from parent trees or from pre-quick multiplication block trees maintained by the BCCP. All budwood cutting must be witnessed. MB trees may be planted in the field and maintained under protected conditions such as in a screenhouse or greenhouse. Experience from other citrus producing areas indicate that MB trees under protected conditions are not likely to become infected with CTV and usually have more buds for use in the early spring when demand for buds is highest.

a. Numbering and identification of MBs

MB trees must be separated from other plantings and properly and adequately labeled. Each block or group of trees which comprise a MB will be assigned an MB number by the nursery. This MB number is recorded on the right-hand portion of the Bud Cutting Form (BCF, Schedule 3)(refer to Figure 2 for an example). The MB numbers will be assigned by the nursery in sequential order beginning with MB1. No MB number will ever be repeated. The MB number is important for tracking and locating progeny of bud source material.

For MBs grown in the field, each clone shall be separated in the row by a gap no less than 2 feet, well separated from other nursery propagations (certified citrus trees, varietal block trees and seed source trees). First and last trees of a single clone in the MB must be properly labeled with the clone, date budded and the MB number using durable markers. For MB trees grown in containers, each clone should be separated by 12 inch gaps on the benches, with the first and last container properly labeled with

clone, date budded, and the MB number. Budwood cut from MB trees may be used only for propagation of certified citrus trees.

Vigourous rootstocks, such a Volkamerian lemon, or rough lemon should be used for MB trees. A 2x3 ft or 2x2 ft spacing is recommended for MB trees grown in the field.

Regular and strict pest and disease control measures should be applied at all times to MB trees. Fertilise on a regular basis to ensure good growth of trees.

b. Age of MB trees

MB trees may be used as sources of certified budwood for use in propagation of certified citrus trees for up to 24 months without a requirement of testing for CTV, or other viruses. The age of MB trees is determined by the date of propagation of the MB tree. As an example, if a MB tree was propagated on 30 Sep 95, the 24 month anniversary would be 29 Sep 97. If the MB trees are tested for CTV between the 18th - 22nd month and found free of CTV, or if CTV is present in low incidence (less than 5 percent) and the CTV-infected trees are removed immediately, the MB trees may be used as a source of budwood for certified citrus trees for up to the end of 30 months. The testing requirements and time for which buds may be cut from MB trees are subject to periodic review by the BCCP and are subject to change.

MB trees may be used a varietal block trees or certified citrus trees after they no longer qualify as MB trees because of time limits.

F. Varietal block trees

The purpose of the varietal block is to enable quick verification of trueness of type of the fruit. In the longer term, it enables production records to be obtained. Such records are valuable when making decisions as to varieties and/or clones to use for future groves. A varietal block must have at least five trees which are established from trees propagated from some of the first buds cut from new introduced prospective parent trees or from bud sources directly imported to establish parent trees.

The trees in the varietal block will be planted in the field on rootstocks which have been recommended or proven for the industry and using a spacing commonly accepted for the particular variety. By using suitable rootstocks for some of the varietal block trees, the long term indexing for cachexia and citrus tatterleaf virus may be accomplished. The trees comprising the varietal block will be plainly identified using durable markers.

Varietal blocks may be maintained by the BCCP or by any grower or nurseryman. As the purpose of varietal block trees are to provide a more rapid

evaluation of horticultural characteristics, the trees in varietal blocks which belong to a grower or nursery must be made available to the BCCP for evaluation.

Varietal block trees which are maintained by the BCCP may be used to cut budwood for the establishment of quick multiplication block trees and/or certified citrus trees for a period of up to 24 months beginning with the date of the propagation of the varietal block tree, provided the testing requirements for quick multiplication block trees are followed. All budwood cut from varietal block trees must be witnessed. Budwood cutting will not be permitted from varietal block trees maintained by individuals or nurseries.

G. Certified Citrus Trees

Certified citrus trees are the trees which are used for planting in the field and can be propagated only from budwood collected from multiplication block trees maintained by the BCCP or an authorized representative, or pre-multiplication block trees and parent trees maintained by the BCCP. Each certified citrus tree will be identified with a tag which contains the nursery registration number, the scion variety, the rootstock variety, and the date of budding. The use of certified citrus trees ensures the recipient grower that he is receiving a plant capable of its full genetic potential without hindrance due to virus and virus-like pathogens.

IV. Tracking budwood and certified citrus trees

A. Variety identification

Varieties are identified by a brief abbreviation or letter code. Scions have a two letter code while rootstocks have a three letter code. Additional codes will be added as needed by the BCCP.

Oranges :

VA - Valencia:

CM - Campbell	MD - Midknight
CT - Cutter	OL - Olinda
DL - Delta	RH - Rhode Red

NV - Navel:

CC - Cara Cara	PW - Parent Washington
LL - Lane Late	TZ - Thompson Zimmerman
NH - Newhall	

BO - Blood Orange:
- MO - Moro Blood
- SB - Sanguinelli Blood

OG - Oranges:

AB - Ambersweet	PB - Parson Brown
HM - Hamlin	PI - Pineapple
MS - Mid Sweet	RB - Roble
MV - Madam Vinous	RP - Ridge Pineapple

Grapefruit :

GF - Grapefruit:

DC - Duncan	SR - Star Ruby
FG - Flame	RR - Rio Red
MB - Marsh Brown	RY - Ray Ruby
MG - Marsh	TP - Thompson Pink
MR - Marsh Reed	

Mandarins and Tangerines:

MN - Mandarin:

DY - Dancy	NM - Nova
FG - Fallglo	PG - Page
MC - Murcott	PK - Ponkan
SB - Sunburst	RN- Robinson

Tangelos:

TG - Tangelo:
- MT - Minneola
- OT - Orlando
- Ug - Ugli

Tangore:

TR - Tangore:
- DW - Dweet
- OR - Ortanique

Limes :

LM - Limes:
- MX - Mexican Lime
- TH - Tahiti Lime

Lemons :

LE - Lemon:
- BR - Bearss
- ER - Eureka
- LS - Lisbon
- ML - Meyer

Citrons:

CT - Citron:
- AZ - Arizona 861
- EC - Etrog Citron
- S1 - S-1Citron

Others :

CA - Calamondin

KQ - Kumquat
PM - Pummelo

RS - Rootstock: (note 3 character codes)
639 - Hybrid between Cleo X *P. trifoliata*
Ben - Benton Citrange
C35 - C-35 Citrange
Car - Carrizo Citrange
Clo - Cleopatra Mandarin
Ctu - Citrumelo
CiO - *Citrus obovoidea*
FyD - Flying dragon *P. trifoliata*
GoT - Gou Tou
Mil - Milam
PSL - Palestine sweet lime
RpL - Rangpur lime
Rsk - Rusk citrange
Rub - Rubidoux *P. trifoliata*
SCS - Sun chu sha mandarin
SFS - Smooth flat seville
SHD - Hog shaddock
Ski - Sunki mandarin
SOr - Sour orange
SRL - Schaub rough lemon
Swg - Swingle Citrumelo
Tri - *Poncirus trifoliata*
Tyr - Troyer citrange
Vlk - Volameriana lemon
ZhL - Zhu Luan

B. The BCCP Germplasm Code

There is only one way which budwood may be cut in the BCCP to establish P-MB , two ways for MB trees and three way for certified citrus trees. The parent trees (PT) maintained under protected conditions by the BCCP at the CREI facilities will serve to establish P-MB's, parent trees and P-MB's for MB's and budwood for certified citrus trees may be cut from multiplication blocks (MB) maintained by the BCCP or authorized representative, or pre-multiplication blocks (P-MB) and parent trees (PT) maintained by the BCCP. These are coded as:

PT :Parent Tree

P-MB: Pre-multiplication block trees

MB: Multiplication block trees

The BCCP germplasm code indicates in the following order:

- variety and cultivar
- source tree where budwood originated
- year the cultivar was imported
- individual plant number

Example : VARH-PT-94-105

• variety and cultivar VARH	VA: Valencia	RH: Rhode Red
• block where budwood originated	PT : Parent Tree	
• year the cultivar was imported	94: 1994	
• individual plant number.	l05: plant number	

When budwood is cut from parent trees this is the type of code which would be entered on the left hand portion of the Bud Cutting Form (BCF) as the Clone/Variety Identification number when budwood cutting is witnessed.

C. The Certified Nursery Plant Code

The nursery code plus the information on the multiplication blocks will be used after the germplasm repository code to make the certified nursery plant code in this manner:

VARH-PT-94-105 #05-5-96

Nurseries are assigned a Nursery Registration Number upon receiving their Nursery Registration Certificate (Form NRC for propagating nurseries). The Nursery Registration Number begins with # followed by two digits; for example: #05

#05: John Doe Nursery

This will be followed by a MB (multiplication block) number followed by the year the certified citrus tree was budded.

5: Multiplication Block number 5
96: The certified citrus tree was budded in 1996

Another example:

GFMB-PT-94-2-#05-3-96

GFMB:	Grapefruit Variety Marsh Brown
P-MB2/96:	Pre-Multiplication Block 2 and year established

94:	Year 1994 when variety was imported
2:	Parent Plant number
#05:	Identifies John Doe nursery
3:	Quick Multiplication Block 3
96:	The certified nursery tree was budded in 1996

In all instances BCCP will maintain records of the equivalent codes.

D. Witnessing Budwood Cutting

All budwood cutting required to produce certified citrus trees requires witnessing, this includes budwood cut for propagation of P-MB trees, MB trees, varietal block trees, seed source trees as well a certified citrus trees. The person from the BCCP who witnesses the budwood cutting is to ensure that budwood is cut from the appropriate tree, that the number of buds cut is correct, and that the labeling of the budwood bundle(s) is correct. Witnesses will only observe the budwood cutting, the actual budwood cutting will be carried out by nursery staff. All witnessing of budwood cutting activity is to be recorded on the left-hand portion of the Bud Cutting Form (BCF), attached as Schedule 3. An example follows in Figure 2.

The buds which originate from one parent tree, or from a group of P-MB or MB trees which have originated from one parent tree, should be wrapped together to form a budwood bundle which will be properly marked as the source, clone, and time of cutting.

As far as possible, a budstick should contain approximately 11 buds, but counted as 10 buds. This is to allow for damage to some buds which may occur during budcutting and transport.

Orders for buds should be placed as far in advance as possible. For example orders for MB establishment should be made at least 7 months in advance since they have to be multiplied in the pre-multiplication blocks. For buds distributed through the BCCP, the allocation will be on a first come, first served basis.

After the left-hand portion of the BCF has been completed, a copy is forwarded to the BCCP, c/o CREI, P.O. Box 72, Dangriga, 9 Miles Stann Creek Valley Road (Telephone 05-23535, Fax 05-23511), and the original Form BCF accompanies the budwood bundle.

The nursery receiving the budwood bundle completes the right-hand portion of Form BC. After bud take (number of buds which are alive at about four weeks after grafting) has been determined, the completed report is to be returned to BCCP, and a copy is retained by the nursery. An example of how to complete the right-hand portion of Form BC is shown at the bottom of Figure 2.

When budwood cutting is witnessed from MB trees, the left-hand portion of Form BC is completed in a manner as shown in the top portion of Figure 3. The person receiving the buds, which only can be cut from MB trees for the establishment of certified citrus trees, completes the right-hand portion of Form BC. See the example at the bottom of Figure 3.

E. Movement of Certified Citrus Trees

When certified citrus trees are moved from the nursery, a Tree Movement Form (TM Form), attached as Schedule 6, must be completed by the nursery selling the trees. Refer to the example in Figure 4 for the completion of the TM Form.

F. Roster of Registered Nurseries

A roster of registered nurseries will be maintained by BCCP. The roster will be published at regular intervals by the BCCP, and will be provided to anyone requesting the roster.

V. Fees for Services

Registration and certification will be carried out by qualified inspectors and budwood cutting witnesses which have been trained by CREI. The BCCP will organize registration and certification visits; ensure the collection of fees; and maintain appropriate records and accounts.

Fees not paid within 30 days of billing will be considered past due. A penalty of five percent (5%) of the unpaid balance will be charged

The schedule for registration and certification fees has been determined on the basis of time and testing costs required for each service. The fees will also be adjusted at least yearly to take into account the Consumer Price Index and in light of prices in neighboring countries.

A. Nursery Registration

A nursery must be registered to produce certified citrus trees as described in Section II B above.

The non-refundable fee for nursery registration or re-registration will be $100.

B. Seed Source Tree Certification

The fee for seed source tree certification or re-certification will be $40. The fee covers the cost of one visual inspection annually and the indexing costs for the psorosis complex of viruses.

Figure 2

- Example of the left hand portion of the Bud Cutting Form (BCF) filled out when buds were cut to establish a MB.

The purpose of this form is to record all bud cutting of certified budwood which is to be used to establish certified scion trees, varietal block trees, pre-multiplication block trees, multiplication block trees and certified citrus trees.

Check as appropriate: ____ Buds are being cut to establish varietal block trees
____ Buds are being cut to establish pre-multiplication block trees
_✓__ Buds are being cut to establish multiplication block trees
____ Buds are being cut to establish seed source trees

Left hand portion to be filled out by witness.

Clone/variety identification Number	No. of buds cut	Rebud *	Date cut
VARH-PT-94-105	500		May 30, 1998

Bud supplied by : BCCP/CREI
(Nursery Name)

Siganture: WoGrovere
Address: CREI, 9mls Stann Creek Valley Rd.
Nursery Registration No.: #01
Witness: Jack Doings JDoings
(Name and Signature of BCCP Authorized representative witnessing the bud cutting)

*Rebud = check this column if the buds are being used to rebud previously budded rootstock. The same clone/variety identification number must be used as was used initially.
Retain a copy and submit the original to : Belize Citrus Certification Programme c/o CREI, PO. Box 72, Dangriga Town, or 9 mls Stann Creek Valley Rd./Tel: 05-23535, Fax: 05-23511.

After this portion of the bud cutting form is complete, the nursery supplying the buds keeps a copy, and the original copy accompanies the budwood bundle

- In this Example, the right-hand portion of the Bud Cutting Form (BCF) is completed by the nursery receiving the buds:

Right-hand portion, to be filled out by the nursery receiving the buds. Submit the completed form within 30 days of receipt of buds.

After the bud take has been verified, the original Bud Cutting Form is completed and signed by the nursery receiving the buds. The original form is sent to BCCP c/o CREI, and the nusery keeps a copy for their records.

Rootstock	Nursery block/s.house	Row/bench	No. of trees budded	MB No.**
Volka	Block 5	Rows 1-5	250	MB3
Swingle	Block 5	Rows 6-10	250	MB3

I hereby certify that this information is true and correct to the best of my knowledge.

Buds received by: Milton Briggs M&B Nursery
(person receiving buds) (nursery name receiving buds)

Nursery Registration No. #20 Address: SteadFast Community

Milton Briggs M. Briggs
(Name and title for receiving nursery) (Signature)

**MB= Multiplication block number assigned if a multiplication block is being established

Figure 3

Example of the Bud Cutting Form (BCF) when buds are cut to establish certified citrus trees. In this example, we will assume the buds were cut from the MB which was established in the previous example (figure 2).

The purpose of this form is to record all bud cutting of certified budwood which is to be used to establish certified scion trees, varietal block trees, pre-multiplication block trees, multiplication block trees and certified citrus trees.

Check as appropriate: ____ Buds are being cut to establish varietal block trees
____ Buds are being cut to establish pre-multiplication block trees
____ Buds are being cut to establish multiplication block trees
_✓__ Buds are being cut to establish certified citrus trees
____ Buds are being cut to establish seed source trees

Left hand portion to be filled out by witness.

Clone/variety identification Number	No. of buds cut	Rebud *	Date cut
VARH-PT-94-105-#20-3- 98	5000		January 8, 1999

Bud supplied by : M&B Nursery
(Nursery Name)

Siganture: M Briggs
Address: Steadfast Community, Mile 19 Stann Creek Valley Rd.
Nursery Registration No.: #20
Witness: Jack Deings JDeings
(Name and Signature of BCCP Authorized representative witnessing the bud cutting)

*Rebud = check this column if the buds are being used to rebud previously budded rootstock. The same clone/variety identification number must be used as was used initially.
Retain a copy and submit the original to : Belize Citrus Certification Programme c/o CREI, PO. Box 72, Dangriga Town, or 9 mls Stann Creek Valley Rd./Tel: 05-23535, Fax: 05-23511.

The buds cut from the MB trees is witnessed, and the witness fills out the left-hand portion of the Bud Cutting Form. A copy is retained by the nursery, and the original is forwarded to the BCCP.

In this Example, the same nursery used the buds to establish certified citrus trees. The right-hand portion of the Bud Cutting Form (BCF) is completed after bud take as been determined and the original copy returned to the BCCP. The BCCP will issue tags for the identification of the certified citrus plants.

Right-hand portion, to be filled out by the nursery receiving the buds. Submit the completed form within 30 days of receipt of buds.

Rootstock	Nursery block/ s. house	Row/bench	No. of trees budded	MB No.**
Cleopatra	Block 10	Rows 1-10	2500	
Swingle	Block 10	Rows 11-20	2500	

I hereby certify that this information is true and correct to the best of my knowledge.

Buds received by: Milton Briggs (person receiving buds) M&B Nursery (nursery name receiving buds)

Nursery Registration No. #20 Address: Steadfast. Community

Milton Briggs (Name and title for receiving nursery) M.Briggs (Signature)

**MB= Multiplication block number assigned if a multiplication block is being established

The right-hand portion of the Bud Cutting Form is completed by the nursery doing the budding. The original copy is sent to BCCP c/o CREI, and the nursery keeps a copy for their records.

Figure 4

When certified citrus trees from this current example are removed from the nursery, a Tree Movement Form (TMF) must be completed by the nursery. The person receiving the trees must indicate to the nursery the intended planting location.

Schedule 6

BELIZE CITRUS CERTIFICATION PROGRAMME (BCCP)
TREE MOVEMENT FORM (TMF)

The purpose of this Tree Movement Form (TMF) is to record the movement of certified trees from the nursery where they were propagated or sold to their planting location. This form must be completed by the person selling the trees.

Date: *May 10th 1999*

In compliance with the BCCP, I hereby state that the following certified citrus trees are going to be moved:

VARH-PT-99-105-#20-3-99 (cleo-Blck 10-rows 1-10 - Jan. 99)
Variety/Clone Identification Number/ Nursery Number/ MB No. and year established (taken from the Bud Cutting Form which was completed when the buds were cut for these trees)/Rootstock used/Block or House/Row or Bench/Date buds were cut.

2400
Number of certified citrus trees being removed

Maya Mountain Citrus Ltd. *Mile 55 Southern Highway, Stann Creek Dist.*
Name of person or farm receiving the trees Address

Grove/block location(s) where the certified citrus trees are to be planted (contact the Belize Citrus Certification Programme for the codes of the grove/block locations if this information is unknown):

Blocks F3 (1500 trees) Block F4 (900 trees)

I hereby certify that this information is true and correct to the best of knowledge.

Trees supplied by: *Milton Briggs* (Print name and Title) *M.Briggs* (Signature)

Nursery name and Registration No. *M&B Nursery #20*

Address: *Steadfast Community, Mile 19 Stann Creek Valley Rd.*

Retain a copy and submit the original to: BCCP c/o Citrus Research and Education Institute, PO. Box 72, Dangriga Town, or 9 miles Stann Creek Valley Road (Telephone 05 –23535, Fax 05-23511)

If the person receiving the trees does not have a block designation code, he/she should contact CREI for this information.

Upon completion of the Tree Movement Form (TMF), a copy should be retained by the nursery, and the original forwarded to the BCCP c/o CREI.

C. Parent trees

The cost of indexing and/or testing of parent trees will be borne by the BCCP as long as the germplasm is to be released for the benefit of the whole citrus industry. When the tests results have been judged satisfactory to the BCCP, the tree will be declared as a certified parent tree.

D. Witnessing Budwood Cutting

Budwood cutting will be witnessed by certified inspectors to ensure that the buds are cut from the appropriate tree and that the correct number of buds are cut. The witnesses will observe the budwood cutting only and not be required to cut buds which will be carried out by nursery staff. Nurseries should request to the BCCP at least ten days in advance that one inspector be available on the designated date for witnessing budwood cutting. The fee currently charged for budwood cutting witnessing would be $5.00/1000 budeyes cut, with a minimum charge of $5.00.

E. Pre-multiplication block trees

The cost of indexing and/or testing of pre-multiplication blocks trees will be borne by the BCCP as long as the budwood is to be used for the propagation of multiplication block trees. P-MB trees do not require testing unless they are to be used for cutting budwood for an addition six months past the 24 month time limit, then ELISA testing for freedom of CTV is required.

F. Multiplication block trees

MB trees do not require testing unless they are to be used for cutting budwood for an addition six months past the 24 month time limit, then ELISA testing for freedom of CTV is required. The fee for collection and processing the material from each composite sample of five MB trees for ELISA is currently established at $3.00.

G. Certified citrus trees

All certified citrus trees will carry a tag attesting to their certification status. The tag will indicate that tree was propagated according to the requirements of the BCCP and list the clone (variety), rootstock, nursery number and date propagated, and include a bar stripe code which will enable tracking of the plant back to its original source. These tags are required for all certified citrus trees, and are provided by the BCCP for a cost of $0.10 to nurseries based on bud cutting reports (Form BC).

H. Fee guidelines for virus testing

The fees schedule for the required pathogen tests are:

	$

CTV (ELISA) quick multiplication block trees (5 tree composite)	3.00
CTV (ELISA) tree over 2 yrs old	10.00
Citrus viroids (Citron/Page)	10.00
Citrus viroids (PCR)	10.00
psorosis complex of viruses	10.00
Citrus tatterleaf virus	10.00
Cachexia	10.00
Woody gall	10.00
Citrus Variegated Chlorosis	10.00
Greening	10.00
Stubborn	10.00

VI. Considerations for citrus growers

A. Rehabilitation considerations for Belize citrus

Citrus growers in Belize can expect that existing trees on sour orange rootstock will die from tristeza decline over the period of the next few years as has happened in Panama, Puerto Rico, Haiti, and Jamaica following the introduction of the brown citrus aphid (BrCA). This raises the question as to how citrus growers, especially small growers, may maintain a profitable citrus production in spite of this threat of tristeza decline on sour orange. Tristeza decline often slowly reduces the vigour and productivity of trees on sour orange rootstock so that it is almost unnoticed if the trees are seen daily or weekly. Usually only stress conditions will result in quick decline and rapid death of trees. Only when trees on CTV-tolerant rootstocks are present for comparison does it become obvious that CTV decline has begun. It is hard to predict when CTV decline will strike a specific group or block of trees, it may be weeks or it may be years. However, preparations should begin now to prevent the loss of productivity. Factors which must be considered in order to address this question are briefly reviewed here.

a. Use replacement citrus trees which are on CTV-tolerant rootstocks only.

Tristeza decline will cause poor growth and kill trees planted on sour orange rootstock. To continue to plant trees on sour orange rootstock will ensure that no productivity will be realised from these trees. Trees should be planted only on CTV-tolerant rootstocks. All rootstocks except sour orange and *Macrophylla* are considered to be CTV-tolerant. Rootstocks selected for use based on consideration of the variety being grown, soil type, and desired horticultural characteristics.

b. Plant only certified citrus trees as replacements.

Why is this necessary? Sour orange has been grown as the rootstock of choice in Belize because it tolerates the presence of many other viruses in the budwood. When alternative CTV tolerant rootstocks are used for propagation, these other viruses (which do not affect sour orange rootstock) will result in very poor, non-productive citrus trees. For example, citrus exocortis and other citrus viroids will severe limit growth on citrange, citrumelo, *Poncirus trifoliata*, and sweet lime rootstocks. Cachexia will affect growth on trees on mandarin and *Macrophylla* rootstocks. Citrus tatterleaf (also called citrange stunt virus) will affect trees on citrange, citrumelo, *P. trifoliata*, and lemon rootstocks as well as limit growth and productivity of lemons and limes grown on other rootstocks. Woody gall will limit trees grown on lemon-type rootstocks. Citrus blight will be most severe on most citrange, lime, and lemon rootstocks but less of a problem on mandarin and selected citrumelo rootstocks. **By planting only certified citrus trees, any CTV tolerant rootstock may be considered without concern because these other viruses will not be present. Additionally, it insures that severe strains of CTV are not further distributed through planting materials.**

When buying citrus plants, growers should insist on the seeing the Nursery Registration Certificate (Form NRC) to ensure the nursery is registered. All certified citrus trees have a tag which attests to their certification status. This tag will also indicate the registration number of the nursery, variety (clone), rootstock, and date propagated.

c. Selection of nursery plants

The BCCP provides testing to ensure that you, as a grower, will receive certified citrus trees having the highest productive potential that is available. Testing of the propagation sources for the various graft-transmissible pathogens and viruses ensure the freedom from these pests. Nurseries are inspected and are expected to achieve control of fungal pathogens and nematodes of the soil, especially *Phytophthora*. Therefore, knowing the symptoms of infestation with the soil pathogens and avoiding these plants is a useful strategy which can result in ensuring that you plant the best possible citrus trees.

Observe the nursery where the certified citrus trees are being propagated before buying trees. The plants should not be standing in pools of water for prolonged periods of time. Look for yellow midribs on the certified citrus plants. Yellow midribs indicate a serious problem with the root system, usually a *Phytophthora* infestation. Do not purchase citrus trees from nurseries having plants with yellow midribs. Ask the nurseryman to unpot some plants which you are considering to purchase. There should be a vigorous system of fibrous roots readily evident. If you see only a tap root and a few fibrous roots, shop around with other nurseries. Plants not having an ample fibrous root system will perform well and will grow poorly for you.

B. Strategies for dealing with CTV decline on sour orange rootstock and the BrCA:

a. Consider planting new trees on CTV-tolerant rootstock between presently planted trees which are on sour orange rootstock (interplanting).

Concern is often expressed that in order to re-establish productive trees on CTV-tolerant rootstocks, trees on sour orange rootstock have to be removed. Because newly planted trees will take 3-4 years to yield well, removal of existing producing trees represents a loss of income for a few years. Strong consideration should be given to planting immediately replacement trees in between the existing trees. Because newly planted trees which are too shaded will not grow well, some selective pruning might be required to ensure that the new tree(s) receive sunlight, yet the productivity of existing trees can be maintained until such time as CTV renders them totally non-productive.

Growers having larger blocks of trees may want to remove whole blocks of trees as tristeza decline renders them non-productive, and to replant the blocks using new certified citrus trees on CTV-tolerant rootstocks. Growers need to compare present yields with past yields to determine when the block has reached the point where grove care and maintenance costs are greater than the return from the fruit. Over a period of several years, all of the citrus could be replaced by productive certified citrus trees. Growers with few trees would be better suited to plant between existing trees to maintain their production.

b. Should insecticides be applied to keep the aphid from spreading tristeza?

There is no evidence that application of insecticides will reduce the rate of spread of tristeza. In fact, some data suggests that application of insect sprays speeds the spread of the virus by its aphid vectors. Because of the costs of insecticide applications and the lack of evidence that there is any benefit, there should be no applications of insecticides for the control of the brown citrus aphid.

c. If the first trees on sour orange rootstock are starting to show symptoms of quick decline due to CTV, what can you do?

Don't panic but try to make an informed decision. Consider the general location in regard to tristeza decline. Are there nearby groves where CTV is already producing noticeable decline? If so, then it can be expected that CTV decline will probably progress more rapidly through the grove. If the grove is located in an isolated location away from other citrus plantings or if nearby groves are not yet showing areas having CTV decline, the decline may progress slower. In groves which are in isolated locations or where nearby groves are not showing CTV decline, it may be worthwhile to quickly remove the declining tree(s) and to survey surrounding trees to look for honeycombing on the sour orange bark flap, and also removing these trees (Box 1). While there is no guarantee, this timely action may prolong the impeding spread of the CTV which will eventually kill the trees on sour orange in the block.

A decision must be made on whether to plant new certified citrus trees on CTV-tolerant rootstock between existing trees, or to continue with the present trees until the CTV decline reduces the fruit yield to the point where grove care and maintenance costs are greater than the income from sale of fruit, and then remove all the trees at once and replant the block.

Box 2. Field diagnosis of decline tristeza strains by examination of bark patches
A heavy-duty pocket knife and 10X or 20X hand lens are required. Cut a rectangle shaped patch across the budunion of the tree (about 1 cm by 2-3 cm) so that the patch includes a small area of scion, the budunion, and a small area of rootstock. When decline strains of tristeza infect a tree on sour orange rootstock, small, microscopic bristles are produced below the budunion in the sour orange rootstock. These small bristles project into the bark. Thus when the bark flap is removed from the infected tree, the small holes (usually referred to as honeycombing) are apparent on the inner side of the bark flap. A hand lens will often be required to see the honeycombing, but in some instances the pinholes are sufficiently large as to enable seeing them with the unaided eye.

Honeycombing on the inner side of bark flap over the sour orange rootstock

VII. The important graft transmissible and soil pathogens

A. CITRUS VIROIDS

Nature of pathogen:

Citrus viroids constitute one group of important citrus graft transmissible pathogens. Viroids are infectious, circular, single-stranded RNA (nucleic acid material) which are very small in size, ranging in size from 371 bases to about 245 bases, they do not have a coat protein. Citrus exocortis and citrus cachexia diseases, also called

xyloporosis, are include in this pathogen group. The viroids are classified according to their size: the largest viroid is exocortis with 371 bases and the rest of the viroids are placed in groups I through V bases on their size. Cachexia viroid is a group II viroid.

How the pathogen is spread:

All citrus viroids are very easily mechanically transmitted, and spread is quite common on contaminated clippers, pruners, saws, and other implements. Viroids can survive for a period of time on contaminated tools. They are also spread by propagation of viroid infected nursery material. There are no insect vectors or other means of spread reported for the citrus viroids.

Field diagnosis:

Citrus exocortis (called scaly butt in Australia) causes severe and dramatic field symptoms which are expressed as bark scaling on susceptible rootstocks, which include *P. trifoliata.* and hybrids which have *P. trifoliata* as one of the parents, such as citranges and citrumelos. Often the bark scaling symptoms are not expressed until the trees are a few years old, the trees become stunted, decline, and nonproductive.

Citrus cachexia causes stem pitting and gumming in most mandarin rootstocks, *Citrus macrophylla*, Orlando tangelo, sweet lime, and Rangpur lime, and a general stunting of the tree. When the pitting is severe, the trees are small, unthrifty, and nonproductive. This disease is present in most old line bud sources.

The expression of the other citrus viroids are varied and mixed. This may be because viroids rarely occur in field trees as single isolates of a specific viroid, rather they occur as mixtures of two or more viroids. Symptoms often associated with various citrus viroids include presence of gum pockets on citrange or citrumelo rootstocks, yellowing and/or creaks in the bark of scions, stunting, and general unthrify appearance. In Florida there have been several decline symptoms occurring in trees on Swingle citrumelo rootstock which may be due to the presence of mixtures of specific viroid groups, but Kock's postulates have not been fulfilled.

Laboratory diagnosis:

Citrus viroids are usually indexed on etrog citron indicator plants. Exocortis gives the strongest symptoms with severe leaf epinasty and bark splitting. are usually also present when indexing exocortis. Most of the citrus viroids, except for the group II viroids, will also produce petiole browning, petiole wrinkle, and leaf tip browning on citron, but the severity of these symptoms is much less than those expressed by exocortis. The group II viriods do not produce symptoms on etrog citron, and are usually indexed by grafting a Parson's special mandarin or Orlando tangelo onto a suspect tree, and observing for gumming and pitting under the bark of the mandarin 6-9 months later. Orlando tangelo may be used as a rootstock for the scion to be tested, and observations are made 18-24 months later for evidence of gumming and pitting on the rootstock near the bud union.

There are two common laboratory methods of diagnosis of viroids: 1) The sequential polyacrylamide gel electrophoresis method (seq-PAGE) is often used for laboratory diagnosis. This method involves the partial purification of viroids from infected plant tissue, and analyze them by size by electrophoresis on a non-denaturing gel first, then by a denaturing gel. 2) More recently, reverse transcriptase polymerase chain reaction (RT-PCR) assays have been developed for detection of citrus viroids, and group specific primers permit selective detection of specific viroid groups.

B. CITRUS BLIGHT

Nature of pathogen:

Citrus blight is caused by an unknown infectious agent. It has been reported in Florida for over 100 years, and commonly occurs in countries throughout the Caribbean when susceptible rootstocks are used. In Florida blight is estimated to remove two percent of the total number of trees from production annually while in Brazil, the disease removes 10 percent of the trees from production. The disease has been repeatedly graft transmitted to healthy trees using roots or root pieces as inoculum which has been collected from blighted trees. To date citrus blight has not been transmitted using inoculum from above ground tree parts.

Citrus blight is rarely expressed when the majority of the citrus industry is on sour orange rootstock. Other rootstocks which have a field tolerance to blight are sweet orange, Cleopatra mandarin, and some of the citrumelos such as Swingle. Rough lemon, *P. trifoliata*, Carrizo, most citranges and some citrumelos are very susceptible to the disease.

How the pathogen is spread:

The means of spread of citrus blight is unknown. The first occurrences of blight in a grove are at random, and then the disease tends to spread from these foci points. In sour orange and Cleopatra mandarin rootstocks, the diseases often spreads down the row suggesting that root to root grafting between trees may be the cause of spread in these rootstocks.

Field diagnosis:

The first symptoms of blight are often the development of zinc deficiency leaf symptoms on one branch or sector of the tree. The tree becomes a bluish green color instead of a normal green, and wilt occurs in periods of drought. A general decline of the canopy occurs over time with twig dieback and few growth flushes. The fruit become small and few. The trees usually do not die of blight. The blossom set is delayed on blight affected trees. Often rootsprouts grow abundantly from the trunk of the tree. Usually trees are 5-7 years of age and in heavy bearing before any trees in the grove develop blight symptoms, but symptoms may occur at an earlier age in some groves.

Water injection test for blight--Blighted trees have vascular plugs in the xylem and do not take up water if a 1/8th inch diameter hole is drilled in the trunk, and a

syringe used to inject water into the tree. Healthy trees take water using this test method.

Laboratory diagnosis:

Several blight specific proteins have been found and characterized from the xylem vessels (wood) of infected trees. Two of these proteins have been purified and used to produce antibodies. The antibody specific to the 12 kD protein is useful for diagnosis of blight from wood or leaf extracts, and the antibody specific to the 35 kD protein is useful for diagnosis when using xylem extracts collected from roots. A western blot or dot immunobinding assay format is usually used.

Zinc analysis may be run on wood collected from the trunk of trees being tested. In a blighted tree, the zinc is present in 2-4 times the level of that present in wood from healthy trees. Cores may be collected, sectioned, stained, and observed using a light microscope to look for the unique amorphous plugs present only in blight affected trees.

C. CITRANGE STUNT (CITRUS TATTERLEAF VIRUS)

Nature of pathogen:

Citrange stunt, caused by citrus tatterleaf virus, was recognized in the early 1960s. It is known to have been imported from China into many areas on Meyer lemon introductions and possibly other introductions. The disease is characterized by stunted trees when the trees are on *P. trifoliata* or *P. trifoliata* hybrid rootstocks. A virus-induced budunion incompatibility develops, usually when the tree is 5-7 years of age.

Citrus tatterleaf virus has been purified and partially characterized. It is an elongated virus about 650 nm in length. Recently a strain of the virus has been cloned and sequenced.

How the pathogen is spread:

The disease is easily mechanically transmitted and readily graft transmitted. No insect vector has been reported. The virus occurs in many herbaceous hosts.

Field diagnosis:

Observe the budunion of a tree by removing a bark patch. If a budunion crease has developed which has a necrotic, dark line right at the budunion and the tree declining is on a susceptible rootstock, citrus tatterleaf should be suspected. This should be confirmed by laboratory diagnostic tests.

Laboratory diagnosis:

The biological indicator plant commonly used is Rusk citrange. Tatterleaf infections in Rusk citrange or Swingle citrumelo result in distorted leaves having chlorotic spots, and the stem grows in a zig-zag fashion. Carefully regulated cool temperature is critical to getting good symptoms expression in the indicator plants. Often better indexing results are obtained by inoculating budchips from the sample to be tested onto a nurse plant such as rough lemon or citron, then topwork the nurse plant with a live Rusk citrange bud.

Antibodies are available which permit serological detection, these antibodies are not commercially available. With the sequence information, reverse transcriptase polymerase chain reaction assays are possible, but these assays are not in common use at present.

D. CITRUS TRISTEZA VIRUS

Nature of pathogen:

Tristeza is the most economically important citrus virus in the world. It is caused by citrus tristeza virus, a member of the closterovirus group. The long flexuous viroins which are about 11 x 2,000 nm contain a single RNA of about 6.5 million kD which is encapsidated with multiple copies of a single coat protein, and one end of the virion is encapsidated with a coat protein homologue (p27).

CTV causes a great diversity of biological symptoms which can be grouped into one or more of five different categories: **mild**, not causing noticeable symptoms on commercial citrus; **seedling yellows**, a dwarfing and chlorosis on sour orange, lemons, and grapefruit; **decline**, causing decline and death of trees on sour orange rootstock; **stem-pitting on grapefruit**, causing stunting, decline, small fruit of low quality and quantity on grapefruit scions regardless of the rootstock; and **stem-pitting on sweet orange**, causing stunting, decline, small fruit of low quality and quantity on sweet orange scions regardless of the rootstock. Decline strains of CTV may be controlled by replanting citrus on CTV-tolerant rootstocks. The stem pitting strains of CTV are considered the most serious because there is little which may be done to prevent yield losses when they are widespread in a citrus area. The stem pitting strains of CTV do not kill the trees but can reduce yield by up to 45 percent.

How the pathogen is spread:

CTV is readily graft transmitted. Long spread movement of CTV is usually by movement of CTV-infected nursery materials. The virus may be mechanically transmitted only with great difficulty.

CTV is semi-persistently transmitted by several aphid species with the brown citrus aphid (BrCA), *Toxoptera citricida*, being the most efficient. Once an aphid acquires CTV, it is capable of transmitting the virus for up to 24 hours.

Field diagnosis:

In groves where the trees are on sour orange rootstocks, trees will decline and die from infections with decline strains of CTV. Usually a bulge develops above the budunion. If a bark piece is cut and removed from over the budunion, there will be tiny projections from the sour orange wood and corresponding tiny holed (honeycombing) in the innerside of the bark patch over the sour orange. In Florida some strains kill the trees quickly, and the honeycombing symptoms do not develop. Instead a brown line forms right at the budunion after the wood is exposed to the air for 2-3 minutes. When trees decline due to CTV, usually following stress such as drought, freezes, or high water, the leaves take on a golden yellow appearance. The leaves then drop, and the

fruit remain hanging on the tree. The decline on sour may be quick, within a matter of a few days, or slow and over the period of several months or even years.

Laboratory diagnosis:

Isolates of CTV are biologically indexed using a battery of five indicator plants: Mexican lime as a susceptible host; sour orange seedling to monitor seedling yellows; sweet orange on sour orange budling to monitor decline strains of CTV; Duncan grapefruit seedlings to monitor stem pitting on grapefruit; and Madam Vinous seedlings to monitor stem pitting on sweet orange. Some strains have been found which stem pit rough lemon and volkamer lemon, or Tahiti lime or mandarins.

There are several serological based detection methods for CTV. One monoclonal antibody has been selected which reacts selectively with decline isolates of CTV from Florida and most areas of the Caribbean, but not with mild, non-decline isolates. Double sandwich indirect enzyme linked immunosorbent assays (DASI-ELISA) are the most commonly used. By running a polyclonal, broad spectrum antibody assay alongside the decline strain specific monoclonal antibody assay, mild and decline strains of CTV may be differentiated. This methodology is idea for performing large scale assays.

Several molecular based detection methods are being developed which show promise of differentiating among mild, decline, and stem pitting strains of CTV. One method is called single stranded conformational polymorphism (SSCP) and is performed by running a denaturing polyacrylamide gel of products obtained from a RT-PCR assay. Another procedure, called strain specific probes, uses hybridization assays on the products from RT-PCR assays. While these methods are useful, they do not lend themselves to large scale use at the moment.

E. CITRUS VARIEGATED CHLOROSIS

Nature of pathogen:

Citrus variegated chlorosis (CVC) is one of the most destructive graft transmissible diseases of citrus. In 1987 this disease was recognized at an isolated location in the northern part of Sao Paulo State, Brazil. Now this disease is present in all citrus growing areas of Brazil, including the citrus areas geographically isolated from Sao Paulo State. Additionally, it is present in parts of Argentina and in Paraguay, it has been found and the trees destroyed in Uruguay.

CVC most severely affects trees less than 7-8 years of age. The symptoms of CVC usually begin on one branch, then spread to the rest of the tree. The symptoms include chlorosis and small fruit which ripen early and have a hard rind. Necrotic lesions on the leaves which form a gum deposit on the back side of the leaf. The trees become stunted as the symptoms become chronic but the trees do not die from CVC. The trees produce fruit for the first one or two years of infection, but production stops as the disease becomes chronic. In areas of Sao Paulo State where the disease is widespread, it is almost impossible to plant a new grove and to bring it into bearing.

The disease severely affects all sweet orange varieties. Mandarins and some rootstock varieties are tolerant and do not express the disease symptoms.

Citrus variegated chlorosis (CVC) is caused by a pathovar of *Xylella fastidiosa*, a fastidious phloem inhabiting bacterium. Recent research information suggests that the same pathovar of *X. fastidiosa* also infects coffee producing decline symptoms. Different pathovars of this bacteria cause chronic decline diseases in plum, peaches, grapes, and a number of ornamental and shade trees.

How the pathogen is spread:

CVC has been widely spread throughout Brazil by movement of infected nursery material. There are a number of sharpshooter insects (plant hoppers) which have been shown to transmit the disease. The sharpshooters carry the bacteria in their foregut and pump organ, and lose the ability to transmit the bacteria when they undergo molting. If an adult sharpshooter acquires the bacteria, it retains the ability to transmit for the remainder of its life.

Field diagnosis:

CVC affected trees have a bright chlorosis, but this is also a symptom produced by citrus blight and other diseases. The most characteristic symptom is the production of small fruit which ripen early. The fruit is sweet to taste. The fruit tend to be numerous the first year of infection and to hang in clusters, even in cultivars where this is not expected such as Pera sweet orange. The leaves develop a necrotic spot on the under side directly beneath the yellow chlorosis area on the top side, and a gum deposit forms in the necrotic area.

Laboratory diagnosis:

A polymerase chain reaction assay utilizing nested primers has been developed which enables specific detection for the CVC pathovar of *X. fastidiosa*. The outside primers are specific for *X. fastidiosa* and give a PCR product with any pathovar of the bacterium. The inside primers are specific for the CVC pathovar and produce a product only if the CVC pathovar is present. This is the only pathovar specific assay for diagnosis of CVC. There are numerous other PCR assays reported which detect *X. fastidiosa* in a generic sense.

Several serological based assays have been developed for detection of CVC. It is not possible at present to differentiate the CVC pathovar of *X. fastidiosa* from the other pathovars using any of the polyclonal or monoclonal antibodies which have been reported.

F. CITRUS GREENING

Nature of pathogen:

Greening disease, also called huanglongbing, is caused by a fastidious, phloem-limited bacterium, *Liberobacter sp.* Asian greening is one of the most important diseases of citrus in China, Taiwan, Indonesia and many countries in the east. African greening causes similar losses in South Africa and neighbouring countries. When

young trees become infected, they do not reach production. Older trees develop greening symptoms first on one branch or sector, then become systemically infected and are nonproductive in a few years. The Asian strains of greening express strong symptoms under warm conditions (32-37 C) whereas African strains express strongest symptoms under cooler temperatures (20-24 C). Sweet oranges, mandarins, and mandarin hybrids are most severely affected, but all most all citrus cultivars, species, hybrids and many relatives are susceptible to greening.

How the pathogen is spread:

Two species of citrus phyllids are reported as vectors of greening. The vector present in Asia is *Diaphorina citri* and the common vector in Africa in *Trioza erytreae*. Both vectors in present in many citrus areas, but *T. erytreae* does not survive well in hot dry areas. *D. citri* tolerates warm temperatures and is not normally found in the cooler and at higher elevations of tropical citrus areas, and populations are suppressed by high humidity and rainfall. *D. citri* is present in several countries in South America and has been reported to be as far north as Honduras, but this report is nonconfirmed.

The greening bacterium is persistently transmitted by the phyllid vectors. Usually it is the adults that are capable of transmitting, but 4th instars may also transmit the bacterium. The vectors are not efficient, and most epidemics occur during periods of high phyllid populations and a large amount of inoculum in the area.

Field diagnosis:

Field diagnosis may be based on foliar and fruit symptoms. Some trees should have sectors or branches showing disease symptoms while other trees which have been infected longer will have systemic symptoms. The leaves commonly show a vein chlorosis or a chlorotic mottling. Systemic infected trees will have small leaves commonly with zinc deficiency symptoms. If the syringe water uptake test is performed as when diagnosing citrus blight, the greening infected trees will take up water. Fruit are small and usually remain green. The juice is low in soluble solids, high acid, and has a bitter taste.

Laboratory diagnosis:

Biological indexing may be done to detect greening. If indexing for African greening, temperatures in the greenhouse must be maintained below 25 C, and Asian greening indexing must be done under warm (32-37 C) temperatures. Dweet tangor and Duncan grapefruit are probably the best indicator plants for greening indexing under greenhouse conditions. Mandarin indicator plants are good if tristeza is also present, because mandarins are tolerant of tristeza.

A simple procedure to test for presence of gentisic acid has proven to be useful for large surveys. Recently polymerase chain reaction assays have been developed for both Asian and African strains of greening which give quick specific diagnosis.

G. PSOROSIS COMPLEX OF VIRUSES

Nature of pathogen:

The psorosis complex of viruses includes many diseases of which all give either a leaf fleck or oak leaf pattern when indexing on sweet orange or grapefruit indicator plants. This group of viruses include psorosis A, psorosis B, citrus ringspot, citrus concave gum, additionally indexing on sweet orange and grapefruit would also reveal the presence of cristicortis, the citrus ilarviruses, and impietratura. Most of the viruses in the psorosis complex are not characterized. Only recently has the nature of the psorosis A, B and citrus ringspot viruses become known, and the characterization of these viruses is presently underway. The rest of the viruses in the complex, with the exception of the citrus ilarviruses, have not been purified or characterized. Most members of this group are commonly present in old line cultivars.

How the pathogen is spread:

In Argentina, northern Brazil, and Paraguay, psorosis is naturally spread but the nature of this spread is not known. Evidence suggests that insects are involved as vectors, but pollen could also be a cause of spread. Psorosis has been found to be seed transmitted, especially through *P. trifoliata* and *P. trifoliata* hybrids.

The bark scaling symptoms of psorosis often do not appear for 3-12 years. Because of this, many times budwood is cut from field trees lacking bark scaling symptoms, only to find that the resultant young propagated trees are severely infected with psorosis.

Field diagnosis:

The bark scaling symptoms which occur on the scion are indicative of psorosis. The wood is not dead under the scaling, and often gum oozes from the scaling. If a larger branch is cut through which has bark scaling, a staining of the wood beneath the scaling is usually present. Leaf symptoms of vein flecking and/or oak leaf patterns are easy to overlook in field trees. Observe the young tender flushes which emerge directly from the trunk of the tree during spring flush. Leaf symptoms in field trees are not expressed as the weather becomes warmer.

Laboratory diagnosis:

Indexing on sweet orange and grapefruit indicator plants under cool greenhouse conditions will reveal the presence of psorosis complex of viruses by vein flecking and/or oak leaf patterns. A reliable serological assay is not available because of antibodies expressing strain preferences and the lack of characterization of many of the members of this disease complex. Recently a polymerase chain reaction (PCR) assay has been developed which enables specific detection of psorosis A and B and citrus ringspot, but not the other members of the complex.

H. STUBBORN

Nature of pathogen:

Stubborn is caused by *Spiroplasma citri*, a mycoplasma-like organism which may be cultured. The microorganism is very small and spiro-shaped, but detectable only by using a high quality phase contrast light microscope.

How the pathogen is spread:

In addition to being graft transmitted by use of infected budwood, stubborn is vectored by several species of leafhoppers. *Scaphytopius nitridus* and *Circulifer tenellus* are present in western USA, and *Neoaliturus haemoceps* is present in the Mediterranean area. The leafhoppers have a wide range of hosts, and usually migrate to citrus when the other plants have dried up.

Field diagnosis:

Stubborn infected plants are very stunted. The internodes are short, and the tree canopy is dense and upright growing. The leaves are thick and cupped, some mottling or chlorosis may appear. The fruit are often misshapen, lopsided or acorn shaped, and small. The seed are often aborted. The fruit may remain green at the top. Warm weather is needed for symptoms expression.

Laboratory diagnosis:

Culturing of the *Spiroplasma* is often used to diagnosis stubborn. Positive cultures may be maintained in the freezer in culture media for comparison. Once in culture, light microscopy and/or electron microscopy may be used to verify the size and shape of the microorganism. Biological indexing may be done, but the disease often is not well distributed so the budwood needs to be collected with this poor distribution kept in mind. Common indicator plants include Madam Vinous sweet orange and Duncan grapefruit indicators. Recently, polymerase chain reaction assays have been developed for *S. citri.*

I. NEMATODE DISEASES OF CITRUS

There are three main nematodes which cause damage to citrus: **citrus nematode** (*Tylenchulus semipenetrans*) causing slow decline, **burrowing nematode** (*Radopholus citrophilus*) causing spreading decline; and the **lesion nematode** (*Pratylenchus coffeae*) causing citrus slump.

Nature of the pathogens:

The **citrus nematode**, causing slow decline, usually does not noticeable affect trees unless high populations are present. Trees are not killed, but have reduced vigor. Young replant trees planted into old groves having high nematode populations are usually severely affected. Affected trees may have leaf yellowing, sparse foliage, and small fruit, there is no reponse to fertilizer applications. The sedentary females feed on fibrous roots where they lay eggs surrounded by a gelatinous matrix. This nematode will survive as long as citrus roots survive. Soil fumigation has been used to treat infested soils.

The **burrowing nematode**, causing spreading decline, causes trees to lose vigor and results in thinned canopy having dead twigs, small fruit and leaves. The affected trees are very susceptible to drought. As much as 50 percent of the fibrous roots may be lacking. Feeding by the nematode causes the root to stop elongating. The burrowing nematode can survive for up to 6 months without live roots. The burrowing nematode usually affects a group or cluster of trees, hence the name spreading decline.

The **lesion nematode**, which caused citrus slump, causes trees to decline, have a thin canopy with twig dieback, small leaves and fruit. The symptoms often mimic citrus blight, except the trees will take up water when using the syringe test for blight. This nematode has caused tree losses in Brazil.

How the pathogen is spread:

Movement of nematode infested plants from infected nurseries is a sure way to distribute these pathogens throughout the industry. Care should be paid not to move implements containing soil between different locations.

Diagnosis:

Nematode infections may be diagnosed only by collecting soil samples and fibrous root samples which are analyzed using various extraction methods to separate the nematodes from the roots and soil, followed by observation for presence of nematodes using a high quality light microscope. The following is one method for the extraction of nematodes.

Sugar extraction of soil nematodes:

1. Empty contents of soil bag into a dish tub.
2. Mix soil thoroughly breaking up dirt clods
3. Using a 250 ml beaker, take 2 samples (total 500 ml) and place into a 2.5 liter bucket.
4. Fill with tap water to 2500 ml and stir the soil and water vigorously, first in one direction then the other.
5. Allow 10-15 seconds for sand to settle, then pour the water through nested (80 over 500) wet sieves.
6. Tap water out of sieve. Spray over the 80 mesh screen, then remove it and discard its contents.
7. Gently spray the contents of the 500 mesh screen with it tilted to the bottom of the screen, to a very small area.
8. Wash with very low volume. Spray into a small beaker or directly into centrifuge tubes for sugar extraction of the nematodes, or for Baerman extraction.
9. Pour nematode slurry into 50 ml round-bottom centrifuge tubes. Do not fill more than 25% with silt.
10. Spin at 200 rpms for 5 minutes then slowly decant (supernatant can be discarded or collected for sieving at step 6 and added to sugar rinse).
11. Remove silt debris around tube rim with finger.
12. Fill the tube 3/4 full with sugar (sucrose) solution (540 g/L), stopper and shake vigorously to resuspend silt into sugar.

13. Fill remaining 1/4 volume with more sugar solution and centrifuge at 2000 rpms for approximately 30 seconds.

14. Decant supernatant carefully through a 500 mesh screen and rinse gently to remove sugar solution from nematodes. (Collect rinse supernatant from step 2 here).

15. Backwash screen to collect the nematodes.

J. Phytophthora-Induced Diseases

Phytophthora species are the most damaging soilborne fungi that attack citrus, because they affect virtually any part of the tree at any age. These fungi are worldwide in distribution. They cause losses in production in arid areas that are irrigated and in areas receiving high rain fall. Losses due to *Phytophthora* occur in seedbeds from damping-off; in nurseries from foot rot , gummosis, and root rot; in orchards from foot rot, gummosis, feeder root rot, and brown rot; in packing houses from the further development and spread of brown rot.

Nature of pathogen:

The most common and important *Phytophthora* spp. that attack citrus are *P. parasitica* Dast. and *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian. *P. parasitica* is widespread in most citrus areas and causes foot rot, gummosis, and root rot. It seldom causes infection very high up the trunk. *P. citrophthora* causes gummosis and root rot. It attacks aerial plant parts more frequently that *P. parasitica* and is most commonly the cause of brown rot.

How the pathogen is spread:

Phytophthora spp. are endemic in the soil of citrus orchards in most areas. Infection usually occur by means of zoospores, which are released when free moisture is abundant. Severe outbreaks are associated with prolonged periods of wet weather. Zoospores are attracted to wounds or to the zone of elongation of root tips, where they encyst, germinate, and penetrate directly. The pathogen can penetrate young leaves and green stems directly. It requires a wound or natural growth crack for infection of tree trunks.

Phytophthora spp. usually survive unfavorable periods as chlamydospores in soils or as hyphae or sporangia in decayed roots or other organic matter. They do not compete well as saprophytes.

Field diagnosis:

The most serious diseases caused by *Phytophthora* spp. are foot rot and gummosis. Foot rot is an injury of bark on the trunk or roots near ground level. Gummosis is a rotting of bark anywhere on the tree. Infection occurs through wounds or natural cracks in the bark. The fungus grows into the cambium, producing a necrosis, which is commonly accompanied by abundant gum exudation. Citrus gum is water

soluble and disappears following heavy rains. Badly affected trees have pale green leaves with yellow veins, as is typical of a girdling effect. If a lesion has ceased expansion or the fungus has died, the affected area is surrounded by callus tissue. The lesion do not extend below the bud union of trees on a resistant rootstock. On susceptible cultivars under favorable conditions for disease development, lesions may extend downward into the crown roots as well as upward on the trunk. Nursery trees and young orchard trees can be rapidly girdled and killed. Large trees may be killed but are usually only partially girdled, and the injury causes a decline of the canopy, with defoliation, twig dieback, and short growth flushes. On susceptible rootstocks, lesions may occur below the soil line, and canopy symptoms may develop without obvious damage to the aboveground portion of the trunk.

Phytophthora also infects fruit, causing a firm, light brown rot. In the orchard, fruit near the ground become infected when splashed with soil containing the fungus. Later if conditions continue to favor infection, the disease spreads to fruit throughout the canopy. Most infected fruit soon abscise, but some may enter the packing house and create a postharvest decay problem.

The primary means by which *Phytophthora* species are spread through citrus orchards are infested nursery plants, water logged conditions or floods and irrigation water. Surface water following heavy rains may carry the fungus as it drains from the orchard. The fungus is also carried in soil on farm equipment when vehicles move from infested to uninfested orchards or nurseries.

Most of the common planted scion varieties are moderately to highly susceptible to bark infection. Lemons, limes, sweet oranges, and grapefruit are very susceptible. Valencia orange is generally more tolerant than navels and other early and mid-season oranges. Most tangerines and their hybrids are more tolerant than oranges but are sometimes seriously affected.

Most of the commercially used rootstocks are somewhat tolerant to *Phytophthora* diseases. Trifoliate orange is nearly immune to infection. Swingle citrumelo, alemow, and to a lesser extent Cleopatra mandarin and sour orange possess a high degree of resistance. Troyer and Carrizo citranges, Rangpur lime, and most selections of rough lemon are tolerant to bark infection and can be planted on well-drained sites. Sweet orange rootstocks and some sources of rough lemon are highly susceptible to infection.

Many problems due to *Phytophthora* in orchards can be avoided by beginning with *Phytophthora* -free nursery stock. Citrus seeds should be treated with hot water at 52^0 C for 10 minutes to eliminate *Phytophthora* spp. Seed beds and nurseries should be steamed, fumigated, or should be located on virgin soils. Soil drenches of metalaxyl or foliar sprays of fosetyl-Al are useful for preventing root rot and foot rot in the nursery. Sanitary measures and certain precautions should be taken to avoid introduction of the fungus into nurseries. Soil can be contaminated by transplanted citrus seedlings or plant material from infested sites; soil or runoff water from existing orchards; tillage equipment used on infested sites; and the movement of people or animals.

One of the most effective and widely recommended control measures is the practice of budding trees well above the soil line and planting them so that the bud union in the orchard is not buried. Injuries to the trunk by implements should be avoided. Foot rot and gummosis can also be prevented in the orchard by painting the trunk with copper fungicides or applying systemic fungicides, such as metalaxyl or

fosetyl-Al, which can be used as soil drenches, foliar sprays, trunk paints, or applied through irrigation systems, as appropriate to cure existing lesions as well as prevent infection. Effective treatment of infected trees requires early detection, and this is difficult in large orchards. Thus, effective control of foot rot and gummosis depends mainly on preventative measures involving sound cultural practices supplemented by chemical control

Laboratory diagnosis:

Assay methods for the detection of *Phytophthora* species:

Leaf baiting in soil or water samples: Representative water and soil samples, thoroughly mixed, are divided into 5-gram subsamples in the compartments of an ice cube tray. The compartments are then filled with distilled water, which is mixed with the subsamples. Four leaf disks, ¼ inch in diameter, are floated on the surface. The disks are incubated at 82^0F in the dark for 48 hoursand then plated on PARPH selective medium and incubated for 72 hours. *Phytophthora* colonies on the medium are identified by comparison with known cultures.

Leaf and fruit baiting is the most sensitive technique for the detection of *Phytophthora* species and is especially useful for detecting contamination of irrigation water in nurseries and for checking nursery crops for infestation. The assay does not give information on the population density or the level of damage to citrus crops.

Soil dilution plating: Representative soil samples are thoroughly mixed in a resealable plastic bag. The mixed soil is poured into a 6-ounce Styrofoam cup with drainage holes in the bottom. The soil in the cups is watered until it is saturated and then is allowed to drain. After 24 to 48 hours of incubation, 10 cubic centimeters of soil is removed and mixed with 90 milliliters of 0.25% water agar. Five to 10 samples (1.0 milliliter) of the mixture are individually plated on PARPH selective medium and incubated for 48 to 72 hours at 82^0F. *Phytophthora* colonies on the medium are counted, and the counts per plate are totaled and expressed as propagules per cubic centimeter of soil.

This assay is useful for monitoring soil populations of *Phytophthora* in mature orchards. The number of propagules per cubic centimeter of soil is related to the level of root damage in nursery soils and the reduction of fibrous root density in orchards. The decision to treat an orchard for root rot can be based, in part, on propagule counts. Generally, with fewer than five propagules per cubic centimeter, no need for treatment is indicated, but a count of more than 10 to 20 suggests the potential for root loss and tree response to fungicide treatment.

ELISA (Enzyme-Link Immunosorbent Assay): Fibrous roots are cut into 0.5-inch lengths and mixed thoroughly. A 1:5 dilution (weight/weight) of roots with extraction buffer is placed in a thick, resealable bag, and a hand-held roller is run over the roots to grind them into pulp. The extract is dispensed into precoated immunoassay wells. The assay is performed as directed by the kit instructions. The concentration of *Phytophthora* protein in each sample is calculated from a standard curve of values produced by a standard preparation of soluble protein from *P. nicotianae.*

ELISA is useful for rapid detection of *Phytophthora* species at low levels, if the sample of roots is large enough to be representative. The preparations of root samples is labor intensive, but the assay is very sensitive, and results are obtained the same day. Commercial kits for single or multiple assays provide an effective measure of the activity of *Phytophthora* species in roots. However, ELISA is much more expensive than other methods. A simple method for detecting *Phytophthora* species on-site, using no specialized equipment, is available.

VIII. Protocol for seed handling

A. Handling of citrus seed

Collection of citrus seed should begin by the collection of sound fruit from registered trees. After extraction of seed from the fruit, the seed should be thoroughly washed with soapy water to free the seed from pulp.

To reduce the risk of *Phytophthora* spp. heat treat the seed in water which is 52^0C (127^0F) for 10 minutes, then immediately immerse in room temperature water. Surface dry the seed under shaded conditions. Optional seed treatment is with a dip of sodium hypochlorite at 4% for 10 minutes, then rinse thoroughly with water. After drying treated with a powdered fungicide, such a thiram.

If seeds are to be stored for a while the seed may be further treated with a 3 minute dip in a 1% solution of 8-hydroxyquinoline to reduce the occurrence of molds and improve shelf life.

Store the seed in a refrigerator 3^0 – 7^0C (35^0 - 45^0F), but do not freeze. Storage in plastic cans or cartons often lead to sweating, and the accumulated moisture can start moulding. Check the seed occasionally to avoid this from happening. Under carefully controlled conditions seed viability can be preserved for several months. Periodic germination tests should be conducted to insure seed quality.

Citrus seed must be properly labeled as to the variety, source of seed, and date harvested.

Citrus seed is highly perishable and care must be exercise at all stages to prevent damage. Upon purchase or receipt of seed and will not be planted immediately, they must be placed into cold storage at 3^0 – 7^0C (35^0 - 45^0F). However, it is recommended to plant seeds as soon as possible after receipt. Temperature extremes are fatal to citrus seeds, do not expose to freezing or high temperatures. Avoid exposure to direct sunlight at the time of planting

Planting instructions: There are a number of methods used to germinate citrus seeds. Selection of the system used depends largely on the availability of

materials. There are several important factors that must be considered regardless of the system:

1) Soil should be pre-moistened or drenched with a fungicide solution
2) Planting depth 2-3 centimeters (1 inch) max.
3) Cover the seeds with a thin layer of sand to prevent drying.
4) Soil must be moist, never allow standing water. Frequent light irrigations are best (4 times per day). Reduce frequency as seedlings begin to grow.
5) Seed bed should be protected from direct sun maintain a 50% shade.
6) Seed germination is best at 30^0 - 35^0C (86^0 - 95^0F).