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# Graft-transmissible Diseases

Budwood is the primary inoculum tissue used for most inoculations, but bark and leaves may also be used. Budwood should not be collected during excessively hot weather because some CGTPs in the perimeter branches of field trees can be temporarily inactivated or severely suppressed by heat. When the season changes and temperatures become cooler, however, the pathogen will usually return from its reservoir location in the roots or shaded parts of inner branches.

An ice chest should be used for budwood storage when collecting. Clippers should be disinfected, when moving from tree to tree, by dipping or spraying in a 1 percent sodium hypochlorite solution. Bark samples can be placed in a small plastic tube, but the tube should not be sealed. Immediately after collecting the tissue samples, they should be put into polythene bags to prevent their drying and immediately put into an ice chest.

All samples should be labelled clearly at the time of collection. Upon arrival at the plant laboratory, they should be put directly into a refrigerator at 5-6°C. Avoid freezing the inoculum. Budwood can be maintained under refrigeration for two weeks or longer but should preferably be used as soon as possible. If a field tree is selected as a primary candidate, a budstick should be taken below or proximal to a welldeveloped and typical fruit. A bud propagation is then made, and the propagation held in the greenhouse. This propagation will then become the primary plant, and budsticks can be taken anywhere from this plant for initial indexing, for heat-treatment, for shoot-tip grafting, or for use as positive control tissue to test the effectiveness of the heat-treated or shoot-tip grafted plant.

### INOCULATION METHODS

The most frequently used method for inoculating indicator plants for the detection of most CGTPs is by "bud"-graft inoculation. The term "bud"-graft includes buds with

"eyes", stem pieces without "eyes" (sometimes called blind buds), and also chip-buds. The blade is first slashed through the inoculum tissue, and then a single slash is made in the stem of the receptor plant. This procedure is repeated ten to 25 times per plant. The slashed area of the receptor plant is then wrapped with budding tape. Citron is an excellent donor host as well as a receptor host for mechanical transmission by knife or razor-blade slash. In general, seedlings are preferred as receptor or indicator plants. However, if propagated clonal buds derived from seedling lines are substituted for seedlings, they should be tested and compared against the seedling for their performance as indicators since their performance as budlings may be different from that of seedlings.

Although "buds" are used as inoculum for most inoculations, other tissue and techniques, i.e. leaf, bark, root, or side grafts, should be continually tried and tested to find the most effective means of bringing out maximum symptom expression. This is especially true for any initial indexing of new diseases or diseases of unknown etiology. Specific clonal selections used as scion propagations rather than seedlings have been found superior as indicators for indexing of certain pathogens, i.e. the cachexia, exocortis and exocortis-like citrus viroids.

A vigorous rootstock such as rough or Volkamer lemon is recommended as a rootstock under the clonal bud. The forcing of clonal buds is recommended where tristeza is endemic and tristeza-susceptible indicators may show too strong a tristeza reaction, thereby masking symptoms of other pathogens. In many cases tristeza can be filtered out by inoculating trifoliate orange seedlings and using shoots of trifoliate as inoculum.

A modification of this technique is to graft an indicator scion bud on a trifoliate or citrange seedling, inoculating the seedling and forcing the indicator bud. In most cases tristeza will be filtered from the new growth of the developing indicator shoot. Some isolates of tristeza can pass through trifoliate or citrange, but most do not. When testing for the bud-union effect of citrus tristeza virus using a sweet orange scion budded on a sour orange rootstock, or for the bud-union crease of certain scions on trifoliate or citrange rootstock induced by the tatterleaf virus, propagation of the scion and inoculation of the rootstock can be done simultaneously and the sour orange or trifoliate rootstock seedling is then bent just above the scion bud to promote rapid forcing of that bud.

### POSITIVE AND NEGATIVE CONTROLS

It is extremely important that both positive and negative controls be incorporated in each index test. A collection of infected source plants containing mild and severe CGTPs

should be developed and maintained as a "virus bank". Sweet orange has been found to be an excellent holding or reservoir plant for almost all CGTPs. These reservoir or bank plants should be periodically indexed to ensure that the pathogen is present or has not changed. It is important that the mildest CGTP sources be collected and preserved in the "virus bank", and these should be used as positive controls for each index test. These positive controls will provide the determining factor as to when an

or may develop poorly in indicator plants. Also, citron reservoir plants used for PAGE detection of citrus viroids may not build a high titre of viroid under cool conditions. These plants must be held at warm temperatures.

The liberal inclusion of mild- and severe positive controls gives a working indication of the proper time and temperature for symptom appearance. The lack of any symptom development in plants inoculated with these mild-positive controls would invalidate the index. Vigorous growth is important for production of good leaf and stem-pitting symptoms. Stem pits are poorly produced in poor unthrifty plants.

#### CHECKING INOCULUM SURVIVAL

Two to three weeks after inoculation, the wrapping tapes should be removed, the inoculum examined for survival, and the survival recorded. If tapes are cut with a knife or razor-blade, these tools should be disinfected in a 1 percent sodium hypochlorite solution between each cut. When buds are taken from mature wood of a dark-coloured budstick, or when bark inoculum is used, it is sometimes difficult to tell if the inoculum tissue is dead or alive.

A small slice or cut made into the brown bark surface of the inoculum will reveal the bright green colour of living tissue beneath, thus indicating that the inoculum is alive. If both inoculum "buds" are dead, the plant should be reinoculated, or new inoculations made to another plant. Generally, if one of the two inoculum "buds" is alive, the plant need not be reinoculated provided there are sufficient replications.

### Records

A record sheet for each index must be kept. This should include: the experiment number, date of inoculation, source of the inoculum, indicator plants used, inoculum survival rate, reading dates, and a large space reserved for notes on observations. Records should preferably include temperatures and light conditions under which indexing was done, and any use of artificial lighting.

### Indexing Using Field Trees

Certain indexes require a longer term for completion of the expression of the mildest symptoms. At such times the inoculated index plants growing in the plant laboratory (greenhouse) need to be set out in the field, or field trees need to be inoculated and observed. For example, in the long-term index for cachexia the mild-positive controls may show no symptoms in the greenhouse even after one year. Therefore, it is best to move the indicator plants to the field and plant them at close spacing until the mild controls show positive symptoms. Similarly, certain strains of exocortis or related citrus

viroids may require a field test to show mild bark cracking on their trifoliate or Rangpur lime indicator rootstocks.

The testing of sweet orange on sour orange rootstocks for the classical quick-decline tristeza reaction may also require an extended period of time for typical tristeza decline symptoms to develop. The testing for cristacortis also requires long-term observation of plants or trees in a screenhouse or in the field. These indexes should be carried out in an environment where temperatures are conducive to best symptom expression. Again, mild- and severe-positive controls should be present. For certain diseases, trees in the field may have to be tested or inoculated to observe specific symptoms, i.e. testing for blight, or observing fruit for symptoms of impietratura.

### TRISTEZA

Tristeza is possibly the most destructive disease of citrus. Many millions of trees on sour orange rootstock have been destroyed in Argentina, Brazil, California, Florida and Spain. The disease continues to spread into new areas, e.g. Israel and Venezuela, destroying citrus plantings where sour orange is the predominant rootstock.

There are many strains of tristeza, causing various field symptoms on different scions and rootstocks. Isolates selected from field trees may induce a reaction only in Mexican lime. Some will cause bud-union failure of certain scions on sour orange rootstock, and others can induce severe pitting or stunting and yellows in a variety of indexed seedlings. Tristeza stem pitting can severely injure scions of grapefruit and sweet orange directly, regardless of rootstock, by inducing a severe pitting and enlarged cheesy bark in the scion, resulting in smaller fruit, loss of production and debilitation of the tree. Tristeza stem pitting also seriously affects lime and may limit production in many areas.

Extensive slide and text reviews on many aspects of tristeza are given in *Description and illustration of virus and virus-like diseases of citrus*, edited by Bové and Vogel, and these are highly recommended for reviewing the tristeza and tristeza seedling-yellows diseases. The plant index is still invaluable for detection of CTV and its many isolates. Graft inoculation to indicator plants can detect tristeza when virus titre is very low.

The severe seedling-yellows and stem-pitting forms of tristeza can currently be distinguished from mild forms only in plants. Garnsey et al.proposed a standardized host-range analysis for evaluating the severity of tristeza isolates by rating decline, stem pitting and seedling yellows on different hosts. There are strains of tristeza that are difficult to detect in seedlings of Mexican lime but that can be easily detected by ELISA. A California tristeza isolate (T-519) is very difficult to identify in Mexican lime indicator plants grown under temperature regimes conducive to good symptom development, but it is readily detected by ELISA. This illustrates the value of, and need for, using