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Dwarfing citrus trees using viroids

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Background

In the 1940s and 1950s the Australian citrus industry was decimated by phytophthora root rot which affected trees on sweet orange and rough lemon rootstocks. This prompted the citrus industry to focus on the use of *Poncirus trifoliata*, which was resistant to phytophthora root rot. However, *P. trifoliata* at that time was an unreliable stock producing some trees which were dwarfed and unthrifty, and sometimes showing symptoms of butt scaling.

These symptoms were attributed to a number of strains of citrus exocortis viroids (CEV). The various strains were classified as mild, moderate or severe, depending on the extent of tree stunting, decline and bark scaling. Later it was found that exocortis is caused by a viroid (it does not have a protein coat like a virus does) and that the dwarfing budlines (in the absence of bark scaling) were caused by other viroids, unrelated to citrus exocortis viroid.

The viroid dwarfing lines in *P. trifoliata* in Australia were found to contain only one or two viroids. These were Viroids CVd-IIIb and CVd-IIa.

A viroid very closely related to CVd-IIa is CVd-IIb. CVd-IIb causes the bark gumming and pitting symptoms of cachexia or xyloporosis and consequently in more recent times only dwarfing budlines containing CVd-IIIb have been distributed by NSW DPI. Because these budlines are graft-transmissible, they are known as 'graft-transmissible dwarfing budlines' and they contain a 'GTD viroid'.

In the absence of a proven dwarfing rootstock for citrus, GTD can be used in high density citrus plantings where controlling tree size is essential for success. Future research may eventually provide a dwarfing rootstock but for now the industry can take advantage of the effect of GTD for particular rootstock/scion combinations in high density plantings.

Overseas research

Research overseas on the use of GTD for dwarfing has been carried out in Israel, South Africa and the United States of America.

In Israel, the use of dwarfing viroids was not as selective as in Australia. There have also been problems with poor tree performance, especially under drought conditions (water stress) and where water salinity levels were high.

South Africa had trialled the use of dwarfing viroids but is now moving away from it in favour of other management techniques to reduce tree size, such as rootstock selection and the open hydroponic growing system.

The Californian Citrus Clonal Protection Program currently supplies RNA-IIIb and RNA-IIa dwarfing viroids for use on *P. trifoliata* rootstocks. They are currently undertaking trials to assess the use of dwarfing viroids on other rootstocks.

At present only the Australian and Californian citrus industries are using dwarfing viroids in commercial citrus plantings.

Australian trials

A number of Australian dwarfing research trials using strains of the GTD viroid have been carried out by NSW DPI since the 1950s. These trials included:

- A trial planted at the Somersby Research Station on the Central Coast of New South Wales in 1955. It included 'Bellamy' nucellar navel orange, 'Washington' navel, Valencia orange and Marsh grapefruit grown on *P. trifoliata* rootstock. The trees were inoculated with nine dwarfing budlines. It was only the Bellamy navel which was inoculated with all 9 dwarfing budlines. Other varieties were inoculated with other budlines or a lesser number.
- A series of trials were then established at the Yanco Agricultural Institute in 1961, 1963, 1964 and 1986, and at the Dareton Research Station in 1966 and 1987. In these trials, navel and Valencia orange trees on *P. trifoliata*, Troyer citrange, Carrizo citrange and Rangpur lime were inoculated with either the severe CEV or the best performing (yield/tree size) strains of GTD viroid from the Somersby trials.

Results from Australian research trials

Irrigation

Irrigation had a major effect on growth and productivity of high-density dwarfed plantings. This was related to application interval, frequency of fertigation and size of the wetted root zone. Total water consumption and nutrient removal was higher on high-density plantings because of the greater root density per unit area. However, high-density plantings were more efficient in their use of water. The response to GTD inoculation is more evident in drip irrigated trees (where there is more uniform growth), than those trees using microjet irrigation. Drip irrigation in combination with continuous nitrogen fertigation further enhanced water use efficiency, compared with systems using microjet irrigation.

Tree Growth and Performance

After GTD, there is a reduction in scion trunk cross-sectional area, tree height, canopy volume and yield. There is a positive relationship between tree yield and canopy size. There is no effect on cropping efficiency or yield efficiency.

Viroid dwarfed trees have smaller summer and autumn growth flushes, and appear less damaged by frost. There are fewer spring shoots, and less annual vegetative growth. The timing and duration of flushes is unaffected.

The timing and intensity of flowering is unaffected. The ratio of leafy to leafless inflorescences is unaffected.

There are significant effects on annual tree yield at six years. This is directly related to the reduction in canopy size. There is no effect on canopy development until 4–5 years after inoculation, whilst effects on yield are delayed for a further 1–2 years. Tree performance is unaffected for the first 4–5 years after inoculation for *P. trifoliata*, and 7–8 years for citrange rootstocks.

Viroid dwarfing has an advantage over a dwarfing rootstock, such as Flying Dragon, because a tree canopy can be developed relatively fast before tree growth slows. On the contrary, a dwarfing rootstock is slow growing in the nursery and vigour is still low after establishment in the orchard.

The use of viroid dwarfing in a replant soil may reduce tree size more than anticipated, especially with *P. trifoliata* which could be reduced to less than 50% in replant soil.

Fruit Characteristics

There are no effects on fruit quality factors such as fruit size, fruit growth diameter, colour, percent juice or acid.

Rootstocks

P. trifoliata is better suited to GTD high-density plantings than the citrange rootstocks.

The size of trees is reduced by about 50% on *P. trifoliata*, 25% on Troyer citrange, and 20% on Carrizo citrange.

Tree Management

In Australia, dwarfing viroids have not been associated with either a higher incidence of disease or mutations. Ideal growing conditions and management are required, especially the suitability of the rootstock to the soil conditions.

Inoculation

The time of inoculation influences the extent of the dwarfing response, the earlier it is done the greater the dwarfing response. If trees are inoculated five years after planting, there is no apparent reduction in tree size.

Inoculation can be done using either the bud or bark from the inoculated bud stick. The use of bark shields is preferred by growers, because no subsequent removal of shoots is required.

High Density Plantings and Viability

Substantial increases in productivity are achieved early in the orchard's life in high-density plantings, compared to conventional plantings.

Financial viability depends on the cost of trees – whether they are purchased from a nursery or they are propagated by the grower. Financial viability is highest when the grower produces the nursery trees. High-density plantings of 1,000 trees/ha on *P. trifoliata*, and 800 trees/ha on citrange rootstocks are recommended. Cropping efficiency is greater in high-density plantings but investment costs are higher.

Advantages of GTD high-density plantings are early income on investment and production efficiencies. Disadvantages are the higher cost of planting, and the greater need for optimum orchard management.

Guidelines and recommendations

- GTD inoculation is only recommended with *P. trifoliata*, Troyer or Carrizo citrange rootstocks. You may get a response with Rangpur lime, but Rangpur is very susceptible to phytophthora.
- GTD inoculation is recommended for use only with navel and Valencia oranges. It may be used with other orange varieties but at present there is no research data available. The CVd-III viroid originally came from grapefruit and there were some early trials with CVd-III on grapefruit at Somersby.
- GTD inoculation is not recommended for lemon or mandarin varieties.
- The use of GTD inoculation is recommended only on trees (scion and rootstocks) that have a known high health status. That is, the scion and rootstock have been sourced from a certified agency such as Auscitrus (formerly the Australian Citrus Propagation Association).
- GTD inoculation should not be undertaken in the nursery. Trees should be inoculated when they are planted in the orchard. This is to reduce the possible transmission of the viroid from inoculated trees to non-inoculated trees through pruning/budding tools.
- GTD inoculation is best done in the first year of planting in the field, before trees are 18 months old.
- GTD inoculated trees require a very high level of orchard management. Trees should not be stressed, but well maintained.
- GTD inoculated trees are not recommended in sites that may be subject to water shortages or high salinity irrigation water.
- The use of GTD inoculated trees is recommended for high-density plantings, not normal low-density plantings.
- GTD can be transmitted from inoculated to normal trees through mechanical means such as when pruning, hedging or budding. All care should be taken when working on GTD inoculated trees and blocks to carefully sterilise all pruning/budding equipment before it is used in non-inoculated trees or blocks.

Inoculating trees

The budding or grafting techniques for field GTD inoculation are similar to those for the production of nursery trees. If you have the level of propagation skill required for nursery tree production, you should be able to field inoculate your own trees. If not, employ professional budders to inoculate your trees.

When to inoculate

Citrus trees should be inoculated within 6–18 months of field planting using the technique described below. The dwarfing effect is reduced when inoculation occurs after this 18-month period. There will be little dwarfing effect, if any, on trees inoculated 4 or 5 years after planting.

GTD budwood is only available in spring and autumn, from the NSW DPI Yanco Agricultural Institute. Contact the Farm Supervisor, Joe Valenzisi on 6951 2540 or fax 6955 7580 for an official order form. Alternatively phone the District Horticulturist, Griffith on 6960 1300. Orders should be placed as early as possible (August) as budwood is limited. Research suggests the best transmission occurs from an autumn inoculation as the level of the dwarfing factor is then higher in GTD source trees. A suggested strategy would be to field plant in spring, inoculate in autumn and re-inoculate any failures the following spring.

Receival and storage of budwood

Inoculation budwood is cut and despatched like normal citrus budwood. Leaves are removed and the budsticks placed in plastic bags and cool stored at low relative humidity prior to despatch. They are forwarded direct by registered or express post.

When the budsticks arrive, check them and immediately store them in a refrigerator at 5°C. Inspect them periodically and remove any loose leaf stalks. Budwood can be stored for up to six weeks if held at the recommended storage temperature, but it is better to use the budwood as soon as possible.

A recent trend in storage has been to place budwood in long-life vegetable bags, obtainable from supermarkets. This has extended the storage life for budwood held in refrigerated conditions.

Equipment required to inoculate

You need a sharp budding knife and budding tape for inoculation. An esky with ice or freezer blocks can be used to keep the budsticks cool during the field budding. Remember to cover the cooling material with damp newspaper or cloth so the budsticks are not in direct contact with a freezing surface during this field operation.

Any cutting or budding equipment used on the dwarfing material or young trees should be kept separate and only used for this purpose. There is a high risk of mechanical transmission of the viroid on equipment such as secateurs and budding knives. Disinfect your equipment with a 10% solution of commercial bleach (12% available chlorine) before you start and at the end of each inoculation session to prevent cross-contamination.

The inoculation technique

1. The T or shield bud is the preferred inoculation technique (Figure 1), though the chip or patch bud has been used successfully by some growers.
2. A normal eye bud or bark shield at least 1 cm long can be used for inoculation. Bark shields are preferred by growers. The advantage of using only bark shields is that no subsequent removal of any shoots will be required. If buds are used they may shoot, so if the bud begins to grow it should be rubbed off; if it remains dormant the tree bark will eventually callus over the wound.
3. The bud or bark shield is budded direct into the scion about 10–15 cm above the union with the rootstock (Figure 2). In older trees (12–18 months after planting) this shield could be placed in a major scaffold branch.
4. A double inoculation is preferred to a single inoculation. This increases the probability of viroid transmission and reduces the need to re-inoculate trees in the following spring or autumn if the buds or bark shields don't take. If at least one of the inoculations is successful, this is considered adequate for GTD transmission.

   Double T buds are usually placed relatively close to each other on the same side of the tree. This allows wrapping with the one piece of budding tape and easy checking of bud take. Transmission of the viroid is considered to have occurred if the bud or bark shield has remained green and alive for at least four months from budding.

   Spring inoculations can be left wrapped for 4–6 weeks and then the tape should be removed to check the initial bud take. Autumn inoculations can be left wrapped for a longer period (usually over the winter months), but should be checked by late winter in case replacement material is needed for reinoculation in spring.
5. The budstick supplied provides you with both buds and bark patches (Figure 3).

   A double budding strategy could be:

   - use two bark patches. The full utilisation of the budstick will allow the maximum number of trees to be inoculated.

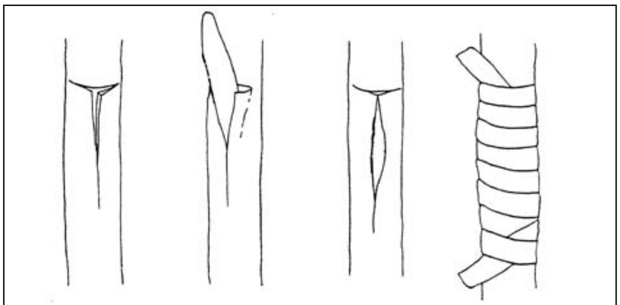

Figure 1. The T or shield bud is the preferred inoculation technique.

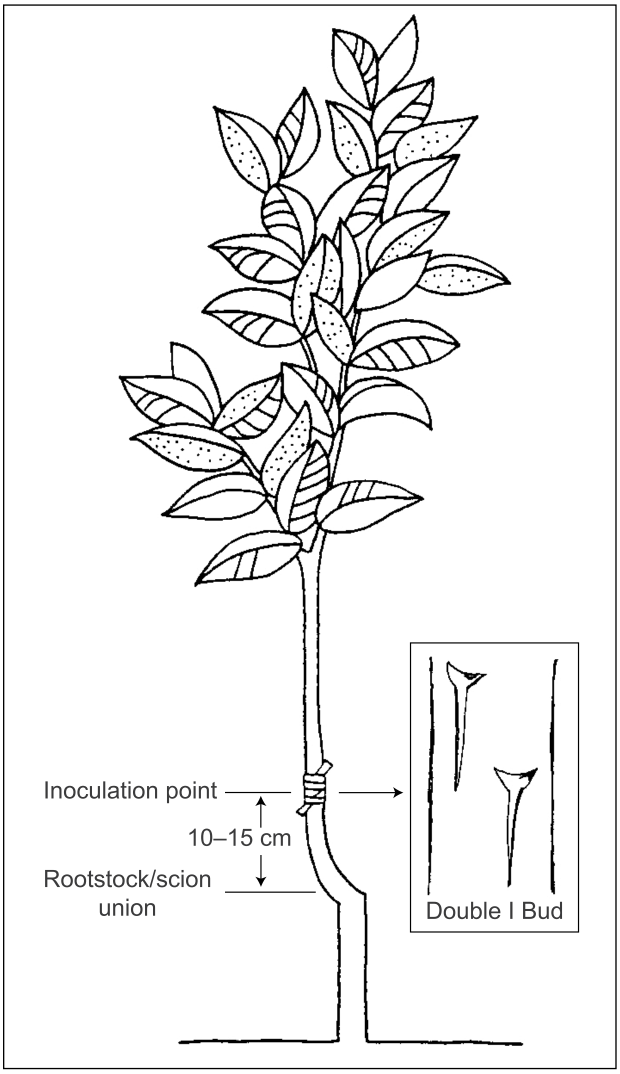

Figure 2. Inoculation position is about 10–15 cm above the union with the rootstock.

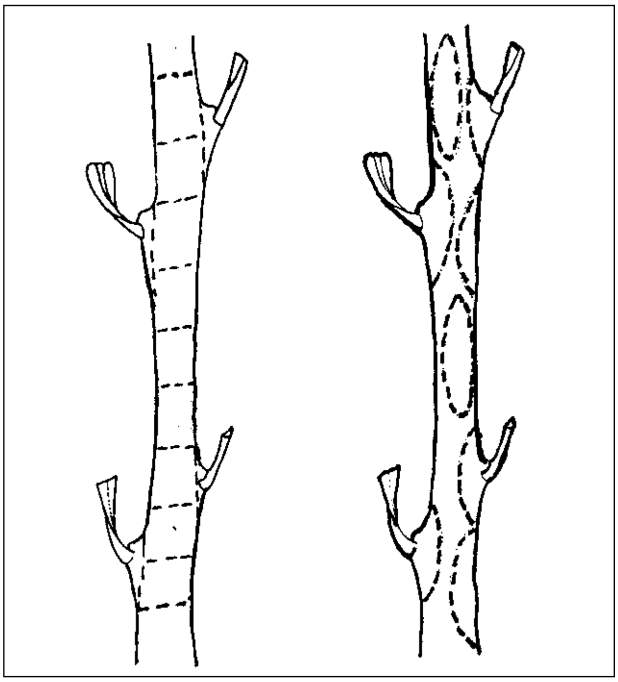

Figure 3. Using the buds and all available bark on the budstick will enable you to double inoculate many trees with the one bud stick.

- use two buds, or
- use one bud and one bark patch, or
- Contract budders are removing long strips of bark from the budstick and cutting them into short lengths for budding.

Care of young trees after budding

GTD inoculated trees must not be put under stress following field inoculation of the dwarfing budwood into the trunk. The stress factors can be wind, heat, lack of moisture or a combination of these factors.

Heat stress

Field inoculation should be timed to avoid high temperature periods. Problems with poor bud take have coincided with high temperatures during or shortly after GTD inoculation. Soil moisture conditions may appear adequate, but high temperatures combined with wind can desiccate young trees. A short period of this type of stress can cause bud failure. If inoculation has to be done during hot weather, place the T buds on the shadier, southern side of the tree.

Wind stress

If the citrus planting site is subject to high winds, it is worthwhile to consider inter-row windbreaks. Rye corn is suitable as a temporary windbreak for young trees and Jumbo sorghum a more permanent, taller alternative for wind protection.

Moisture stress

It is essential that inoculated trees not be water stressed following budding. For dwarfing transmission to occur, there must be a successful union between the bud or bark piece and the trunk. This union will fail if the tree is subjected to water stress, and you will have to re-inoculate at additional cost.

It is recommended that young trees be allowed to establish for 4–5 months before field inoculation. During this time they have a chance to develop a larger root system better able to tolerate varying soil moisture conditions. If inoculation has to occur immediately after planting in early summer or autumn, cut back the tree canopy by about 1⁄3 to reduce stress. The trees should be basin watered for the first few weeks after planting (the basin formed around the tree at planting is filled with water). Depending on the weather conditions, watering may be required at least twice a week for the first 3–4 weeks. A drip emitter, microjet or microsprinkler located close to each tree is effective for irrigation.

Ongoing tree care

Young GTD trees need to maintain a strong growth rate in their early years to develop a canopy. Effective irrigation, soil, nutrition and pest/disease management programs are essential for successful GTD tree development.

Young trees are also susceptible to stress from insect pest infestations, such as soft scales, aphids and citrus leaf miner. Special care should be taken to monitor and treat pests on GTD blocks.

Poorly managed young GTD trees will increase the dwarfing effect and could result in undersized trees which do not fill their allotted space.

References

Citrus high density management: Dwarfing guidelines for commercial use. Agnote DPI-76. December 1993. J.B. Forsyth, P. Barkley, R.J. Hutton and K.B. Bevington. NSW Agriculture.

Viroid Dwarfing for High Density Citrus Plantings. R.J. Hutton, P. Broadbent and K.B. Bevington. Horticultural Reviews, Vol. 24, 2000.

Citrus high density management: Field inoculation with GTD. Agnote DPI-107. October 1995. G. Sanderson and R. Withey. NSW Agriculture.

Acknowledgement

This Primefact replaces three earlier Agnotes and uses information from them:

- DPI-76, *Citrus high density management: Dwarfing guidelines for commercial use*, J.B. Forsyth, P. Barkley, R.J. Hutton and K.B. Bevington.
- DPI-107, *Citrus high density management: Field inoculation with GTD*, G. Sanderson and R. Withey.
- DPI-480, *Dwarfing citrus trees using viroids,* S. Hardy, G. Sanderson, P. Barkley and N. Dovovan.

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