LEAFROLL CONTROL STRATEGY

8. CREATION OF HEALTHY VITIS PLANTING MATERIAL WITHIN THE SOUTH AFRICAN WINE GRAPE CERTIFICATION SCHEME

8.1 Grapevine leafroll-associated virus 3 (GLRaV-3) elimination from an infected plant

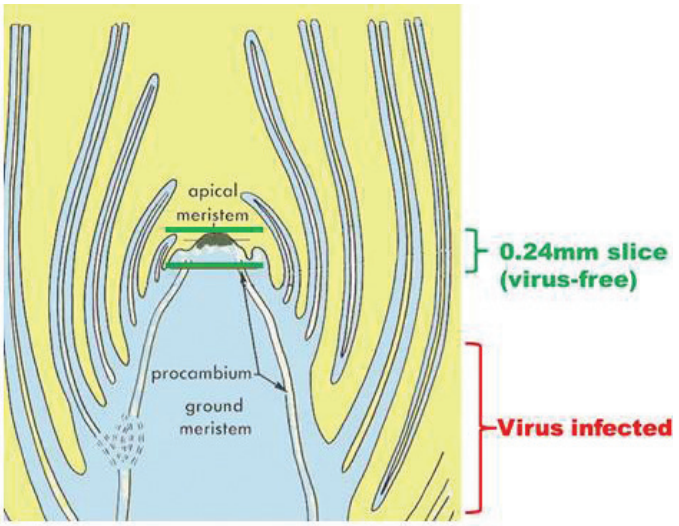
Chemicals to kill viruses in plants on a large scale, equivalent to fungicides to treat fungi in agriculture, are not currently available. Viruses can however be eliminated, at significant cost, from individual plants by various means from which healthy plants can then be propagated by vegetative means.

In South Africa, virus is eliminated from desirable *Vitis* cultivars and clones primarily by heat therapy and meristem tip culture.

This involves placing the *Vitis* plant from which virus must be eliminated in a growth room at 36-38°C for 100 days (Fig. 1). Under these conditions the virus replication and movement in the grapevine is suppressed and a virus-free, actively growing shoot tip of the vine obtained (Fig. 2).

Figure 1: Vitis plants from which virus-free meristems are to be harvested kept at 36-38oC for 100 days (Image: Vititec)

Using a light microscope, a 0.24 mm slice of the shoot tip is made (Fig. 2), and this is placed in tissue culture, from which a whole plant is regenerated (Fig. 3).



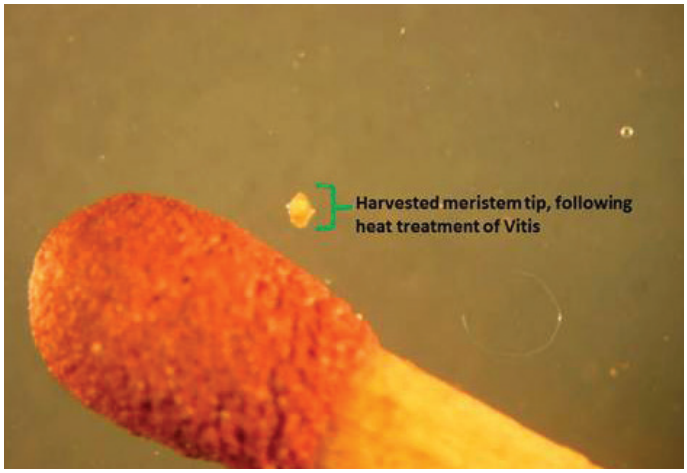


Figure 2: Schematic diagram of a section of meristem of Vitis plant harvested after heat treatment. (Image: Adapted by G. Pietersen, Original Image: Vititec)

Figure 3: Regeneration of a whole plant (nuclear plant) from the meristem of a plant subjected to heat treatment. (Image: Vititec)

This process takes between 18 to 24 months to complete.

The tissue culture plantlets are hardened off and a number of replicates of the regenerated plant are produced in insect-free greenhouses (Fig. 4) and these become the nuclear plants of that cultivar/clone.

Figure 4: Nuclear plants being propagated under insect-free greenhouse conditions. (Image: Vititec)

To ensure that this plant does not become infected by external sources of virus, nuclear plants are maintained in insect-proof greenhouses under very strict sanitary conditions with prescribed access prohibitions (Fig. 5).

Figure 5: Nuclear Vitis plants maintained in insect-free greenhouses. (Image: Vititec)

Planting material that has passed through thermotherapy and meristem tip culture for which all virus tests have not been completed is referred to as candidate registered nuclear material, but those for which all tests are completed and negative for viruses become registered nuclear material.

8.2 Tests to ensure the elimination of GLRaV-3

To ensure that the nuclear plant is indeed free of virus, and to register the clone , tests for leafroll-associated viruses and a number of other viruses are conducted, either with specific laboratory tests for the viruses, or by inoculation onto sensitive indicator grapevines, capable of showing clear virus symptoms.

Cabernet franc clone 1A is specifically used to test for leafroll disease. These indicator plants are maintained for three years, and monitored for symptoms annually (Fig. 6).

Figure 6: Leafroll symptoms on Cabernet franc, a cultivar used as indicator for this disease. (Image: Ontario GrapeIPM, www.omafra.gov.on.ca)

Laboratory tests for leafroll include a mandatory enzyme-linked immunosorbent assay (ELISA) test for GLRaV-1, -2 and -3 on candidate clones. Tests for registered clones include a mandatory immunosorbent electron microscopy (ISEM) test for GLRaV-1,-2, -3 and hardwood indexing within one year of the plants being placed in nuclear blocks.

Plant improvement organizations may also optionally test the candidate or registered clone for other viruses including GLRaV-1, -2, or -3 by the more sensitive but expensive polymerase chain reaction (PCR) test.

If the laboratory tests are positive for any of the viruses or the indicator plants show symptoms, the whole virus elimination procedure is repeated.

Plants that test negative for the prescribed viruses by ELISA tests, hardwood indexing, ISEM and have proven to be cultivar-true, become registered nuclear plants.

Nuclear plants are tested every five years for GLRaV-1, -2 and -3 (and GVA and GVB) by ELISA to ensure they remain free of these viruses.

Should any nuclear plant display symptoms of any disease, have any abnormalities or test positive in any of the laboratory virus detection techniques, they must be destroyed by incineration immediately.

8.3 Propagation of planting material in foundation- and mother blocks.

8.3.1 Foundation blocks

Buds are harvested from nuclear plants and grafted onto rooted rootstocks in foundation blocks (Fig. 7).

Figure 7: Field grafting of buds obtained from nuclear plants onto clean rootstocks in order to establish foundation blocks (Image: Vititec).

Foundation blocks may be greenhouses or in open field plantings and must be isolated from other *Vitis* or other plants by a prescribed distance of 3 m in order to prevent infection by leafroll of these vines from surrounding vineyards.

Foundation blocks in the field may not be established on soil that was subject to a fallow period of less than two years or previously planted to *Vitis,* unless it also had foundation block status. This avoids the danger of leafroll re-infection of these grapevines from infected remnant roots, volunteer plants or residual viruliferous mealybugs derived from a previous vineyard on that site.

Foundation block grapevines must be tested by ELISA to GLRaV-1, -2 and 3 regularly to confirm that they have remained free of leafroll.

Foundation blocks are inspected annually for leafroll symptoms in autumn by trained personnel (Fig. 8), prior to the harvesting of foundation-grade cane material. This is done in all red cultivars. All white-cultivar vineyards are tested on a grapevine for grapevine basis for GLRaV-1, -2 and -3 using ELISA tests. Infected grapevines are marked (Fig. 9) and cane material from all infected grapevines are removed prior to winter harvesting of foundation-grade cane material.

Figure 8: Visual inspection in autumn of foundation blocks for leafroll disease symptoms and other abnormalities. (Image: G. Pietersen, ARC-PPRI)

Figure 9: Leafroll infected plants are marked towards the end of autumn, by spraying the trunks with PVA paint. Sometimes immediately (Fig. 10), but more generally following leaf fall, canes are removed from such plants so as not to become mixed with canes harvested for planting material purposes. (Image: G. Pietersen, ARC-PPRI)

Figure 10: Leafroll infected plants are marked towards the end of autumn, by spraying the trunks with PVA paint. Sometimes immediately (this figure) but more generally following leaf fall, canes are removed from such plants so as not to become mixed with canes harvested for planting material purposes. (Image: G. Pietersen, LNR-NIPB)

Leafroll infected grapevines (either visually determined or by ELISA) must be rogued in these blocks to prevent further spread of leafroll in these vineyards.

Mealybug, the vector of leafroll, is controlled in foundation blocks by a prescribed insecticide application program of one application of a systemic insecticide (Imidacloprid) within the first season of establishment and every two seasons thereafter (for scion and rootstock material). Annual contact insecticide (Chlorpyrifos/Prothiophos) winter applications (for scion material only) are also part of the prescribed program.

Insecticides with differing active ingredients, of which one is an organophosphate, must be applied alternately to prevent mealybug resistance to the insecticide.

Contact insecticides must be applied with a foliar spray applicator at high water rates to ensure full coverage of the grapevine.

Material from foundation blocks may not contain any sign of mealybug infestation upon harvesting of the planting material.

Ant control is also prescribed in foundation blocks as ants tend to protect mealybugs against natural enemies of the mealybug.

Foundation blocks are kept free of weeds, so that these do not serve as alternative hosts for mealybugs to the insecticide treated grapevines.

To prevent the movement of mealybugs from other vineyards to the foundation blocks on implements, these must all be washed prior to use in the foundation block. Secateurs must be disinfected and laborers must wear new protective overalls or clothes before entering a foundation block.

Foundation blocks lose their status if 3 % leafroll infection has occurred, or once the producer no longer rogues infected grapevines.

Rootstock foundation blocks have the same pre-requisites as scion foundation blocks, except that contact insecticides do not need to be applied as it is impractical to enter the rootstock blocks which are not trellised.

8.3.2 Mother blocks

Mother blocks may only be established from foundation-grade material and may be greenhouses or open field plantings.

If a vineyard had previously been planted on that site, all remnant roots and previous *Vitis* material must be removed over a one season fallow period.

Mother blocks are isolated from other *Vitis,* or planted a prescribed distance of 3 m away in order to reduce infection of these grapevines by leafroll from surrounding vineyards.

The reduced isolation distance for mother blocks is due to the practical imperative that most mother blocks are actually active commercial vineyards, from which mother block status cane material is just harvested. Mother blocks of all cultivars are inspected annually in autumn for leafroll symptoms. This is done by trained personnel prior to the harvesting of mother block-grade cane material. Infected grapevines are marked (Fig. 9) and cane material from all infected grapevines and one on either side of the infected grapevines are removed prior to winter harvesting of mother block status cane material.

Leafroll infected grapevines are rogued in the first two seasons after establishment and thereafter producers on estates where mother blocks occur are requested to rogue infected grapevines in these blocks to prevent further spread of leafroll in these vineyards, but this is a voluntary activity and not conducted by all growers.

Canes may no longer be collected from mother blocks if the leafroll incidence in such blocks exceeds 5 % and then the mother block status is lost. In practice however, canes are no longer collected at leafroll incidences of 3 % or higher.

Monitoring of mealybugs in mother blocks is done by visually inspecting the fruit-bearing zone of all the grapevines (usually five) in 20 bays, randomly spread through every 2 ha or pro-rata if larger, of the mother block, and the presence or absence of any mealybug life-stage recorded.

Mealybug must be controlled in mother blocks immediately following observation of mealybugs. Systemic and contact insecticides must be used as per label specifications over the entire mother block.

Contact insecticides (Chlorpyriphos) must be applied with a foliar spray applicator at high water rates to ensure full coverage of the grapevine.

Material from mother blocks may not contain any sign of mealybug infestation upon harvesting of the planting material.

Ant control is also prescribed in foundation blocks as ants tend to protect mealybugs against natural enemies of the mealybug.

To prevent the movement of mealybugs from other vineyards to the mother blocks via implements, these must all be washed prior to use in the foundation block. Secateurs must be disinfected and laborers must wear new protective overalls or clothes before entering a mother block.

Rootstock mother blocks have all the same pre-requisites as scion foundation blocks, except that contact insecticides do not need to be applied because it is not possible to achieve this in the un-trellised rootstock blocks.

8.4 Low risks foundation and mother blocks (Star rating system)

Since foundation and mother blocks are often open field planted vineyards and often within the commercial grape production areas, grapevines in these vineyards often become re-infected with leafroll. While ELISA tests, visual inspection of symptoms to identify the virus infection, roguing of infected grapevines (in foundation blocks) and infected grapevine cane dropping prevent the majority of infected planting material not to be used further, early infections by leafroll associated viruses do not show symptoms and the virus is at too low levels to be detected by ELISA. During this so-called latent phase of infection, planting material may be collected from an infected grapevine and used for the establishment of new vineyards.

To overcome the problem of leafroll re-infection of foundation block grapevines and the resultant possibility of undetected latent infections occurring, a number of foundation and mother blocks have been established since 2006 in regions distant to commercial grape production (low risk areas) (Fig. 11).

Figure 11: Foundation blocks established in low risk areas (distant from commercial grapevine production and with no/little vine mealybugs). (Image: Vititec)

These so-called low risk units are given a star rating with three star blocks having the lowest risk of leafroll infection and concomitantly also the highest levels of control and one star blocks having higher levels of risk of leafroll infection albeit lower than standard foundation or mother blocks.

The low risk blocks have all the prescriptions of the regular foundation or mother blocks, but improved monitoring for mealybug is prescribed, a considerably lower tolerance of mealybugs is allowed and virus tests are conducted differently.

In the low risk foundation blocks mealybug monitoring is mandatory by hanging out one *Planococcus ficus* -specific pheromone sticky trap per hectare in the summer months from 15 November to 15 January, throughout the lifespan of the block (Fig. 12). Sticky traps are replaced monthly during this period and inspected for mealybugs every two weeks.

If more than 10 mealybug males are detected on a sticky trap, monitoring for mealybugs on the grapevines themselves must be conducted by visually inspecting the fruit-bearing zone of all the grapevines (usually five) in 20 bays, randomly spread through every 2 ha or pro-rata if larger, of the foundation block. The presence or absence of any mealybug life-stage must be recorded.

If a mealybug is found during this inspection, the foundation block drops to a lower star rating.

Control is based on presence of male mealybugs in pheromone traps followed by finding mealybugs in the grapevines. Application of a systemic insecticide and contact insecticides must be initiated immediately on the whole foundation block according to label prescriptions. In the case of rootstock, low risk vineyards, this need only be a systemic insecticide (Imidacloprid) as it is not possible to enter rootstock blocks which are un-trellised with spray applicators.

Figure 12: Example of a delta trap, with a sticky pad and a pheromone capsule inside to monitor mealybug male numbers. (Image: G. Pietersen, ARC-PPRI)

For all scion cultivars and US 8-7 and 143 B rootstocks, every grapevine in the vineyard is tested individually for GLRaV-1, -2, and -3 by ELISA in the first season of planting and every three years thereafter and all infected plants must be immediately destroyed by incineration.

A low risk unit that has lost a star status due to the presence of mealybugs, may regain its higher status if the prescribed spray program for regular foundation or mother blocks with mealybug monitoring as prescribed for low risk units is conducted for the two seasons following downgrading and no mealybugs are found in this period. Furthermore if any visually or laboratory tested leafroll infected grapevine is removed and no additional leafroll infected grapevines are found in the subsequent two seasons.

Certified planting material obtained from such vineyards are given a 3 star rating (Fig.13) and can be considered guaranteed leafroll associated viruses-free.

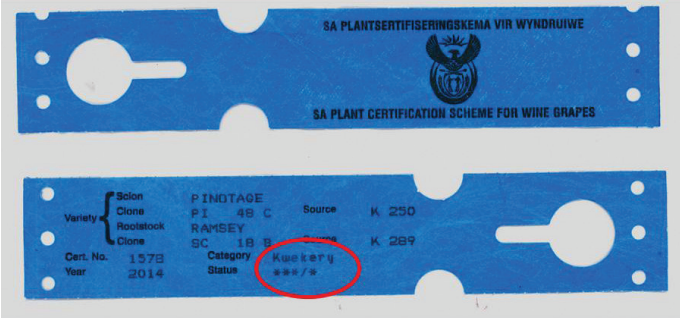


Figure 13: South African Plant Certification Scheme for Wine Grapes blue ticket showing (highlighted in red) the three star status of the scion/rootstock combination. (Image: Adapted by G. Pietersen, Original image: Vititec)

Mealybug monitoring by pheromone traps (Fig. 12) is mandatory only in low risk areas.

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