Kenya Avocado Industry Support Project (KAISP) - **Phytophthora-free Avocado Nursery Protocol**

RA Fullerton[1], John Campbell[1], BM Mwangi[2]

[1] The New Zealand Institute of Plant and Food Research.

[2] Olivado EPZ Ltd, Kenya

March 2023

This protocol is designed to deliver strong, vigorous, true-to-type grafted avocado seedlings whose roots are free from the root rot causing organism *Phytophthora cinnamomi.*

- *Phytophthora cinnamomi* is a soil borne organism (germ) that infects and kills the roots of avocado (and thousands of other plant species) which ultimately kills the tree.
- For simplicity we will refer to it hereafter as “Phytophthora”.
- It is the most destructive disease of avocado world-wide and is capable of destroying entire orchards.
- The most common way the disease is distributed to orchards is by nursery plants that are already infected by Phytophthora.
- Once present in an orchard it is impossible to get rid of and can spread from tree to tree in surface water and underground.
- Every precaution must be taken to ensure that plants taken to new areas are not infected by Phytophthora.
- The most effective way to avoid spreading the disease is to raise grafted seedlings in a nursery that is free from Phytophthora.

Phytophthora –free nursery production involves four simple steps:

1. Construct or upgrade the nursery to enable it to meet the needs of the Phytophthora-free production system.
2. Use of potting mix that has been heated by steam to 70°C or over for at least 20 minutes. This process, called pasteurisation and will kill any Phytophthora in the potting mix.
3. Introduce hygiene precautions during the raising of the plants to ensure there is no chance of reintroducing Phytophthora from unpasteurised soil.

4. Routine laboratory testing of a sample of roots and potting mix (1-2% of plants in the batch) for the presence of Phytophthora several times during plant development to ensure the nursery remains disease-free.

This protocol is appropriate for all tree crops being produced by commercial nurseries in Kenya.

1 Nursery requirements

Refer to the concept design plans for simple low-cost Phytophthora-free nursery.

- It is normal to divide the nursery into two zones:
    - Red Zone. This refers to the area of soil storage, preparation of the potting mix and pasteurisation (sterilisation).
    - Green Zone. This refers to the area after pasteurisation where bag filling, planting and all subsequent nursery operations (raising of rootstocks, grafting, plant care, hardening off, despatch) are carried out.
- The aim is to prevent any movement of unpasteurised soil or potting mix from the red zone to the green zone.
- A covered, secure area (preferably a shed) is needed for soil preparation, pasteurisation, and bag filling and planting.
- The shed is divided inside into red and green zones:
    - The unpasteurised mix and soil pasteurisation bins are in the red zone.
    - The pasteurised soil is then moved in clean ‘green’ wheelbarrows to the green zone.
    - The green zone in the shed is connected directly to the green zone plant raising area.
- The floor of both red and green zones must be kept clean to prevent the movement of non-pasteurised soil from the red to the green zone.
- All non-pasteurised soil must be regarded as potentially carrying Phytophthora.
- Bag filling and planting are done in the green zone of the shed and the planted pots moved to the growing area.
- Ideally healthy plants should be raised on benches on a concrete floor. The cost of this type of facility is prohibitive in most parts of Kenya.
- A low-cost alternative involves the use of a rock base/gravel pad raised at least 30 cm above ground level.
- The pad can be prepared by a base of quarry stones which are filled, covered by fine gravel then rolled to compact and level it.
- The plants can be placed directly onto the gravel pad.
- The edges of the pad should extend approximately 1m beyond the shaded area to prevent soil splashing into the nursery green zone.

- The whole green zone should be protected by a splash barrier of at least 1m high to prevent splash of non-pasteurised soil from entering the nursery area. This could be:
    - a block wall
    - a barrier of densely woven ‘weed mat’
    - a row of dense grass such as Napier grass at least 1m wide
    - the splash barrier should be at least 1 meter away from the first row of plants in the nursery.
- Access to the green zone should be restricted to nursery personnel who have been trained and understand the principles of Phytophthora-free nursery production.
- A foot bath containing a copper fungicide should be placed at the entry to the green zone.
- Personnel working in the green area should have a separate pair of clean (mud-free) boots for working in the nursery area. The boots can be stored in a clean cupboard of the red zone and operators put them on before passing through the footbath to the green zone.
- The solution in the footbath should be replaced daily.
- The green area should have both shaded and unshaded areas.
- Shade is required during the seed germination, rootstock growth, grafting and growing-on periods. The plants can be removed to the unshaded area of the pad for hardening off before planting.

2 Preparation of potting mix

Composition

- Potting mix must be free-draining to prevent waterlogging which can kill roots at the bottom of the pot.
- A mix of 60% sand and 40% soil will provide a free-draining mix.
- The soil should be collected from a site that has previously not been used for avocado or other tree crops (preferably grazing land).
- If organic manure is used it should be free of lumps and added to the soil /sand mix prior to being pasteurised.
- The soil, sand and manure should be thoroughly mixed on a concrete pad before being pasteurised.

Steam pasteurisation

- Steam pasteurisation is the fastest, cheapest and safest way of killing Phytophthora in the potting mix.

- Steam can be produced by a wood fired, purpose-built boiler or a simple two drum boiler discharging steam generated in one drum through a pipe into the bottom of a second drum of potting mix.
- Heat the potting mix to at least 70°C for at least 30 minutes. Higher temperatures and longer times will not harm the mix.
- It is important to ensure that all the mix reaches the target temperature for the required time. The temperature can be measured by inserting a thermometer in the top of the mix.
- Pasteurised potting mix should be moved immediately into the secure nursery area (green zone) in clean wheelbarrows that have not been used for unpasteurised mix.
- Any contact of the pasteurised mix with unpasteurised mix or soil may reintroduce Phytophthora.

3 Preparation of Planter Bags (pots)

Planter bags

- Use biodegradable planting bags if available.
- Check that the bags have enough drainage holes at the bottom and sides to ensure good drainage.
- If there are only a few holes, add more using a paper punch to ensure the holes stay open. Holes will drain better than knife cuts in the bag.
- If bags do not have a gusset bottom, cut off about 20mm from the corner of each bag.

Filling of planter bags (pots)

- The pasteurised mix should be wheeled from the pasteurisation site (red zone) to the filling and planting area in the green zone as soon as possible after steaming.
- Pasteurised mix can be used for filling and planting as soon as it has cooled.
- Bags should be filled to approximately 5cm from the top to allow easy planting of seed.

4 Raising of rootstocks

Seed preparation

- Preferably collect seed from a certified mother block.
- Otherwise collect seed from reliable source to ensure it is true to variety.
- For orchards to be used for seed, check every tree very carefully when it is carrying fruit for symptoms of sunblotch viroid.

- If sunblotch symptoms are found on any tree in the orchard do not use that orchard for seed. The disease is transmitted by pollen from infected trees and the seed developed from that pollen will be infected.
- Fuerte and Puebla seed give the strongest seedlings for grafting. Puebla is preferred because it is stronger and faster growing than Fuerte.
- Collect fruit for seed from the trees only – do not collect from the ground to avoid infestation by avocado seed moth.
- Ripen fruit and remove seed.
- Wash seed of adhering pulp and allow to dry.
- When dry, seed can be stored in plastic bags, crates or bins.
- Plant as soon as possible after being cleaned and dried.

Planting and maintenance

- Before planting, cut or grate approximately 5-10mm from the top of the seed. This will break the dormancy and encourage rapid and even germination.
- Treat seed with a copper-based fungicide before planting.
- Plant one seed per planter bag and cover with approximately 5cm of pasteurised mix.
- Inspect regularly as seeds germinate.
- Rearrange planter bags as seedlings emerge and remove plant bags where germination failed. The potting mix from those bags can be returned to the soil preparation area, added to fresh mix, pasteurised and used again.
- Ensure that all planter bags are kept moist but not saturated.
- Ensure the even distribution of irrigation across all planter bags.
- Continue to rearrange pots during the germination and early growth to keep plants of the same size together. This will facilitate grafting.

Nutrition

- If animal manure is used in the potting mix the plants will normally have adequate nutrients for at least the first month. Additional fertiliser may be needed after that time to keep the grafted plants growing vigorously.
- If animal manure is not used chemical fertilisers must be applied.
- No chemical fertiliser should be added to the pots until the seeds have germinated.
- When rootstocks are between emergence and 10cm high each pot should be fertilised with N:P:K 17:17:17 at 3g per planter bag. Use an accurate measuring container for convenient and speedy fertilising.

- The fertiliser should be sprinkled evenly over the whole surface of the pot and care taken to prevent fertiliser pellets touching the shoot or stem to prevent burning the young growth.
- Fertiliser must NOT be put all in one place or buried in a hole in the soil. The nutrients must be able to move evenly down into the mix with water.
- Apply 3g N:P:K 17:17:17 per planter bag monthly or more often if required judged by plant colour and vigour.
- Phytophthora-free plants intended for organic orchards can have chemical fertilisers added as it will be three years before there is significant yield by which time they will have qualified for organic status. Fruit set on younger plants can be sold or processed as conventional fruit.
- Similarly, pesticides can be used in the nursery to protect them from pests and disease.
- Seedlings destined for organic compliance will commence their process towards Organic Certification status from the time of planting in the field (3 years).

Pruning rootstocks

- As the seed germinates a single and strong stem should be selected for growing on and grafting.
- Side shoots should be removed to produce a single straight stem for grafting.
- All other shoots emerging from the seeds should be removed.

5 Scion collection and storage

- Scions should be:
    - True to type (variety)
    - Taken from high yielding healthy trees preferably from a certified mother block.
- Scion mother blocks should be inspected for signs of sunblotch when holding a crop.
- If symptoms are seen on any trees do not take scions from that block.
- Select scions for stem size to closely match rootstock sizes.
- Remove leaves from the scion shoots to prevent dehydration.
- Keep cool and out of direct sunlight.
- Scions should be collected early in the day and grafted the same day if possible.
- Clearly label scions with the variety name and transport them in ice boxes with cool packs.
- The scions should be kept cool in ice boxes during the grafting process.
- Any unused scions from the first day can be kept in cool storage and used the following day.

- If they are not used the second day they should be discarded – the success rate of grafts declines as the scions age.
- Scions more than two days old will not take well after grafting.

6 Grafting

All grafting operations should be done in the green zone.

Grading of rootstocks

- Because the seeds germinate at different times the rootstocks will be of different sizes.
- As the rootstocks grow they should be sorted according to size to assist with grafting.
- Rootstock should be a minimum of a pencil thickness for grafting.
- Where possible the diameters of scion and rootstock should be the same.
- If they are of different sizes the cambiums should be aligned on one side for the greatest length possible.

Making the graft

Grafting can be done by:

- Traditional cleft graft using a scalpel blade or sharp grafting knife.
- Use of a grafting tool which cuts both the scion and the rootstock to matching shapes and allows a very fast and accurate graft.

If using a grafting tool, set up a grafting “flow” process for the best results in quality of graft and for the speed of throughput:

- Do the grafting on a table of comfortable working height.
- Place the bags of avocado rootstock plants on the table.
- Prepare the rootstock by removing the top to a standard height and removing leaves.
- Make the rootstock graft cut with the grafting tool at a consistent height below the previous cut.
- Cut the scion with the grafting tool.
- Align the scion into the rootstock.
- Grafts can be tied with plastic tape or preferably a biodegradable tape (biodegradable tapes do not need to be removed)
- Place the completed grafted plant into the growing area.
- Keep the working area tidy at all times– use bins to collect, remove and dispose of plant trimmings.

- Grafting tools and equipment should be cleaned before use and cleaned regularly throughout the grafting period.

7 Plant care after grafting

Inspection

- The whole facility should be inspected at least weekly using a check list.
- Check that Phytophthora-free requirements are being met and any issues of cleanliness, pest, disease, nutrition, weeds or irrigation are identified.
- Any problems should be documented on the weekly check list and corrective actions taken promptly.
- Retain weekly check lists for auditing purposes.

Plant management

- If plastic tape is used it should be removed **no later than 4 weeks** after grafting. The graft will be strongly bonded by that time.
- If tapes are left on, they will constrict of the stem as it enlarges and plants may break off when handled
- Remove side shoots as the scion grows to leave a single strong leader. Commence this early to promote the growth of the leader.
- Remove any shoots developing from the rootstock and any additional seedling shoots that emerge from the seed.
- Keep pots free of weeds at all times.

Early pruning of the growing scion to develop a single strong leader

Irrigation

- Plants should be inspected daily to ensure the potting mix is maintained damp but not saturated.
- Irrigation timing should be adjusted according to the weather to avoid over-wetting or drying out.

Nutrition

- Grafted plants should receive 3g N:P:K 17:17:17 at least monthly to promote strong vigorous growth. The frequency can be changed according to observed plant vigour.

General plant care

- Regular inspections should be made to identify and resolve pests, diseases, nutritional or weed issues.
- All activities shall be recorded in detail e.g. agrichemical and fertiliser applications recorded in the nursery diary.

8 Testing to ensure the nursery and plants are Phytophthora-free

Phytophthora testing

- Random pots in the nursery should be tested to ensure the nursery remains free from Phytophthora.
- Tests should be made of potting medium and roots on at least 2% of the grafted plants of each batch two or three times during their growth.
- If Phytophthora is detected in any batch, more extensive sampling of the nursery should be carried out to determine the extent of the contamination.
- An intensive inspection and audit of the nursery operation must be made to determine the source of contamination and corrective actions taken.

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For further information please contact:

RA Fullerton
Plant & Food Research Auckland
Private Bag 92169
Auckland Mail Centre
Auckland 1142
NEW ZEALAND

Tel: +64 9 925 7000
Email: bob.fullerton@plantandfood.co.nz