

Avocado Nurseries and Rootstock Cloning

Westfalia Nursery (South Africa)

Avocado tree propagation has been formerly under control of the production unit, Westfalia Estates but has recently been moved across to Merensky Technological Services headed by Dr Stefan Köhne. Merensky Technological Services have appointed Ms Erica Faber (ericaf@hansmerensky.co.za) as their nursery manager and she brings considerable experience from both citrus tree and ornamental plant production to the job.

Since entering into clonal rootstock propagation the nursery has produced about 1 million grafted trees on cloned rootstocks which have been planted on its own estates or sold to South African growers. More recently nursery trees have been exported to Kenya for use in the developing avocado industry of this country. In years of strong demand for nursery trees Westfalia produce about 100 000 grafted trees on cloned rootstocks. Past demand has mainly been for ‘Duke 7’ but the nursery is currently changing across to ‘Merensky 2’ (a local selection) that has better Phytophthora root rot tolerance and improved productivity when compared with ‘Duke 7’.

‘Ettinger’ is the main source of “nurse” seed material being available early in the autumn (Table 1). Seed is picked from the trees when mature and removed from the flesh while the fruit are still firm. The fruit is cut around the equator with a sharp knife and broken open (Fig. 1). Due to the stage of unripeness the seed coat comes cleanly away from the seed which is then hot water treated at 50°C for 30 minutes.



Fig. 1 Removing ‘Ettinger’ seed from the fruit. Westfalia Nursery uses ‘Ettinger’ as a ‘nurse’ seed in the cloning process. Note the seed is removed from the seed when the fruit is firm and the seed coat comes away cleanly from the flesh.

On cooling the seed is dusted with a Benlate[®]/Dithane[®] mix (1:1) and then planted into folded nursery bags (Fig. 2).

The Westfalia nursery is using the “Brokaw” method for producing cloned trees wherein the “nurse” seedling is retained as a composite part of the nursery tree right through to point of sale. The cloned rootstock section is separated from the “nurse” seedling through the use of a “strangulation” washer placed at the base of the etiolated rootstock growth (Fig. 3).



Fig. 2 Nursery bags folded in preparation for planting a ‘nurse’ seed.

Once “nurse” seeds have been grafted to the elite rootstock they are placed in a dark room for etiolation. The room is held at approximately 27°C and well ventilated to prevent fungal growth on etiolating shoots. The etiolated growth is allowed to reach 20-30 cm before removal from the room. The base of the shoot is treated with 0.75% KIBA, a strangulation ring fitted and the fold bag pulled up and potting media added.



Fig. 3 Strangulation washer placed at the base of the etiolated rootstock to give separation from the “nurse seed”.

After about 100 mm of new growth has occurred on the cloned rootstock the fruiting scion is grafted to the tree. Following the hardening of new growth from the scion the young trees are transplanted into large (6-7 L) containers (Fig. 4) where they are grown through to the point of sale. The young transplanted trees are sprayed with Vapour Guard[®] to reduce transpiration and placed under 60-80% shade for 2-3 weeks before shifting to a higher light regime.



Fig. 4 Preparation of transplanting sites in nursery bags (left) in readiness for transplanting young, grafted cloned rootstocks for growing on the saleable size.

Allesbeste Nursery (South Africa)

Allesbeste Nursery is located on the outskirts of Tzaneen in South Africa and is owned by Dr André Ernst (ahernst@mweb.co.za) who completed his PhD at the University of Pretoria studying avocado propagation. The nursery manager is Gerhard (Gerry) Erasmus. About 70% of the nursery trees produced by Allesbeste Nursery are on clonal Duke 7 rootstocks whilst the balance are on seedling rootstocks, mostly 'Zutano'.

The nursery has strict hygiene standards throughout its operation (Fig. 5) with the nursery divided into zones. Disinfection of the hands and feet of personnel moving between these zones is mandatory. Sodium hypochlorite (2.5%) is used daily to sterilise paths and work areas (very corrosive).



Fig. 5 Main entrance to Allesbeste Nursery, Tzaneen; note covered copper fungicide footbath.

5.5 g m⁻³ (Fig. 6). Fumigated soil is covered for 2 days and then aerated for about 2 hours before filling nursery bags. The seed are planted in bags the next day without any phytotoxicity problems from methyl bromide being observed.

Allesbeste Nursery has found that the best results with rooting cloned rootstocks are achieved going into late summer and autumn (no reason for this was apparent). For clonal production the 'nurse' seeds are planted in February/March (as soon as available) with grafting commencing as soon as seedlings are large enough to take the

Allesbeste Nursery uses composted pine bark as the main constituent of potting media's for the production of avocado nursery trees. The pine bark is supplemented with dolomitic lime to supply Ca and Mg and to raise the pH to around 6.0. Although the material is bought premixed with added macro and micro nutrients the nutritional content of the media is unreliable and is supplemented with additional fertiliser by Allesbeste Nurseries. Granular elemental sulphur (95% formulation; Sulfur 95™ from Ocean Agriculture Pty Ltd at www.oceanag.co.za) is added to the potting media at the rate of 1.5 kg m⁻³. The composted pine bark is mixed with coarse river sand and soil at the ratio of 60/20/20. This media is used in the 'nurse' seed tubes for the production of seedling trees used to graft to rootstocks for cloning and for the large planter bags where trees are grown through to saleable size.

Potting media's are sterilized with methyl bromide at



Fig. 6 Potting media being prepared for sterilisation at Allesbeste Nursery, Tzaneen, South Africa. The soil in the concrete beds is covered with a plastic tarpaulin and fumigated with methyl bromide at 5.5 g m⁻³

rootstock scion wood. Grafting this union is carried out with PVC tape rather than using Parafilm[®] as this is believed to stimulate rooting of the clone after etiolation (constriction at the union as the stem diameter increase with growth of the plant). Allesbete believes that clonal production is best carried out during the late summer/autumn months as this is when they get the greatest success with rooting. Shade is 60% on seedlings and 40% on the main production area.

‘Zutano’ and ‘Ettinger’ are used as “nurse” seed by Allesbeste Nurseries for propagation of clonal rootstocks (Table 1). Fruit for seed use is harvested from the tree but as a precautionary measure the surface of fruit is sterilised by treatment with 1.5% sodium hypochlorite when it is brought to the nursery. When the fruit has softened the seed is extracted, washed and then air-dried for a couple of days to crack the seed coat. The seed is then hot water treated (30 min at 50°C), dipped in prochloraz and planted into black poly nursery sleeves. As temperatures get cooler going into autumn/winter, seed is placed in the heated etiolation room to assist germination but is moved to a greenhouse shortly after shoots appear (can graft etiolated growth from seedling). When “nurse” seedlings are large enough they are grafted with the elite rootstock selected for cloning (to date mostly Duke 7 for commercial production in South Africa) (Fig. 7).



Fig. 7 Duke 7 grafted to “nurse” seedlings prior to etiolation.

As buds on the rootstock scion begin to break plants are transferred to the etiolation room where they are held at 25°C until about 300 mm of growth has been made (Fig. 8). Plants are occasionally sprayed with copper oxychloride to manage fungal growth and insecticides to control mealy bugs and aphids. Nursery stock is managed on a computer database and annual batches of plants are always kept separate. The etiolation room used by Allesbeste is built with concrete blocks with an insulated ceiling but is also well ventilated.

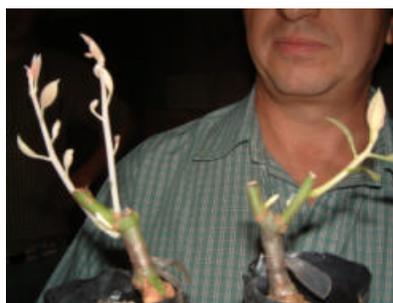


Fig. 8 Etiolation room at Allesbeste Nursery (left) with strong etiolated growth being produced from the Duke 7 rootstock material (right).

Following etiolation the base of the shoot is sliced longitudinally with a sharp knife on one side of the stem removing a sliver of bark thereby exposing the cambium tissue. The cut region is then treated with 0.7% IBA in the first crop of shoots (0.5% IBA in the second crop of shoots) using one drop of solution from an eye-dropper (Fig. 9). The “nurse” seedling is kept growing

through the application of a slow release fertilizer to the top of the sleeve and plants are watered daily.



Fig. 9 Cutting the base of the etiolated shoot in preparation for the IBA application.

On removal from the etiolation room the plants are held under heavier shade for 3-6 weeks during which rooting usually occurs. The fruiting scion is grafted 15-30 cm above soil level. The apical bud is removed from the scion when grafting both the candidate rootstock and fruiting scions to encourage branching. The cut surfaces are tipped with Pancil-Plus, Sanachem Pty Ltd, PO Box 1454, Durban 4000, South Africa.

The grafted, rooted clone is clipped from the nurse seed when 3-4 small leaves have been produced on the fruiting scion (Fig. 10). Clipping is carried out at this stage to reduce the transpiration shock following removal from the nurse seed. Once cut from the nurse seed the young, rooted cloned plant is grown on under greenhouse conditions. If force-grown it takes 8-12 weeks before planting out in the big pots.



Fig. 10 Micro-cloned Duke 7 rootstock grafted to 'Hass' at the stage plants are clipped from the "nurse" seedling (left); and a grafted micro-cloned rootstock showing root development in potting mix.

Micro-cuttings can be managed in the small tubes for up to one year. When growth in micro-tubes is being forced plants are fertigated twice weekly with urea/KNO₃/NH₄SO₄ taking care that the fertigation solution doesn't exceed 150 mS m⁻¹. Plants from the micro-tubes are transferred to 8 L nursery bags with extra holes cut in the bottom when the scion growth is fully expanded and hardened (Fig. 11). When transplanting the micro-tube tree a hole is made in the potting media with a coring tool large enough to allow the micro-tube plant to be slipped into place. After making the hole but prior to planting 10 g on slow release fertiliser is dropped in the hole and

covered with potting media so as the roots of the micro-tube tree do not come into direct contact with the fertiliser.

Budwood supply for grafting is managed by cutting trees back to stimulate regrowth beginning in January. A group of trees are successively pruned back on a monthly basis so the mature wood with vegetative buds only is available throughout the grafting season. Scion wood is not generally stored under refrigeration apart for immediate needs. 'Hass' trees grafted to cloned 'Duke 7' are usually sold for 22-50 Rand (about \$4.00 AUD) or 14 Rand (about \$2.50 AUD) for trees on seedling rootstocks (Fig. 12).



Fig.11 Transplanting micro-cloned 'Duke 7' rootstock grafted to a 'Hass' scion into 8 L nursery bags to grow through to a saleable size: left – making and fertilising the hole; middle – removing the micro-cloned from the nursery tube; right – transplanted tree.



Fig. 12 'Hass' grafted to cloned 'Duke 7' rootstocks ready for sale (left and right) showing the root development in pots (centre).

Table 1 'Nurse' seed varieties used by nurseries producing cloned avocado rootstocks.

Variety	Nurseries					
	ACW	Allesbeste	Brokaw	C&M	Greenleaf	Westfalia
'Ettinger'		■				■
'Lula'			■	■	■	
'Reed'	■					
'Zutano'	■	■		■	■	

Pine Island Nursery (Florida)

Pine Island Nursery is one of the larger avocado nurseries in Florida servicing the commercial requirements of growers. The nursery is managed by Colleen Boggs and Eric Tietig. Avocado production in Florida is based on seedling rootstocks with some 25 different cultivars grown commercially. West Indian varieties do not store well on trees when mature so a longer season is obtained through choosing cultivars with different maturity times. Seeds of 'Waldin' are used for propagation of all varieties. However, there is no comparative data on the use of seedling 'Waldin' as a rootstock with other potential seedling material. Seeds of 'Lula' have been used in the past but the seed size is small resulting in low-vigour nursery trees and it is difficult to graft to some varieties hence it is not popular with the nursery industry. 'Waldin' seed costs about 24 cents US each with a further 32 cents US to remove the seed from the fruit. No scarification is carried out although the seed coat generally is removed when extracting the seed from the fruit. The seed is planted directly following removal from the fruit and has a 95% strike rate. Avocado trees are grown in large containers (12 L) and are sold for \$8-50 to \$12-00 US depending on tree size when collected from the nursery. The main planting period is from August through to October (autumn).



Fig. 13 Avocado production at Pine Island Nursery, South Florida

The nursery itself was basic with all of the production being carried out on weed mat laid directly on the ground (Fig. 13). Soil was not sterilised and granular pre-emergence weedicides were applied to the top of the pots for weed control. Nutrition to trees was applied via a granular slow-release fertiliser with an NPK ratio of 18-8-15.

ACW Nursery (California)

The ACW nursery is located near Fallbrook in southern California on the commercial avocado property of Dr Bill Arteberry but the nursery operation is supervised by Mr Reuben Hofshi (rhofshi@cts.com). The nursery was established to primarily service the requirements of the ultra-high density orchards being established on the property. ‘Zutano’ is most commonly used as the “nurse seed” and the largest are selected and are not deliberately scarified following heat treatment, although seed coats generally come off during subsequent handling. At times ‘Reed’ is used as a “nurse seed”. During propagation, the greenhouse is maintained at 20-25°C and all stages of the cloning process are carried out on heated propagation tables that are held at about 27°C. Once reaching sufficient size, the ‘nurse’ seedling is grafted with a scion of the selected rootstock and allowed to grow until a shoot begins to develop (approximately 14 days). The tree is then placed in darkness and the shoot etiolated until reaching about 400 mm in length (7-14 days) (Fig. 14).



Fig. 14 Etiolation of the rootstock shoot following grafting to the nurse seedling. Once new growth begins following grafting, plants are placed in darkness for 7-14 days until the new shoot reaches about 400 mm in length.

After the plant is removed from the dark, the stem is vertically scraped completely around for approximately 100 mm along the etiolated section and the area treated with a 1% IBA/0.5% NAA solution (Fig. 15). A sliced, clear plastic cup (240 ml) or clear plastic sleeve is taped around the stem with the scored area about one-quarter of the distance below the top of the cup/sleeve. The cup/sleeve is filled with sterile rooting medium and taped to a stake for support (Fig. 16a). The transparency of the container allows visual inspection of root development so that progress can easily be monitored. Under optimal conditions the cup/sleeve is usually filled with roots within 4-6 weeks (Fig. 16b). When sufficient top growth has been made the scion cultivar is grafted and when 60-100 mm of new growth has been made the rooted, clonal tree is removed from the nurse seed. The grafted, rooted

cutting still in the clear container, is placed on the heated propagation table in the greenhouse for an additional 4-6 weeks to harden-off. Subsequently it is replanted into a large nursery bag and transferred outside and grown on to the required size for field planting. This takes an additional 8-12 months. Following detachment of the rooted, grafted cutting from the “nurse seed” a second cloned rootstock can be produced. However, in this case the cloned rootstock may not be grafted to the scion variety until after removal from the “nurse seed”. This latter stage depends on the residual vigour of the “nurse seed” and the prevailing climatic conditions (time of year). Following propagation of the second clonal rootstock, the “nurse seed” can be used as a standard seedling rootstock.



Fig. 15 Scraper used to prepare the etiolated section of the stem for treatment with a 1% IBA/0.5%



Fig. 16 In the rootstock cloning method of Hofshi (1996) the (a) application of a 'clear plastic 240 ml cup' filled with a rooting medium placed on a pre-treated portion of the etiolated shoot to induce rooting; and (b) root development in the cup after 4-6 weeks and the subsequent grafting to a scion variety and detachment from the 'nurse' seed.

Prior to our visit ACW had mistakenly etiolated seedlings and then promoted rooting using the same procedure as that for cloning elite rootstock. On inspection of this material it was noted that the IBA/NAA-treated, etiolated section of the seedlings had produced strong, uniform roots which were superior to the rooted sections of elite rootstocks that were being produced using the same technique (Fig. 17). This comparison clearly illustrated the differences in response between physiologically juvenile and mature plant tissues with respect to their rooting ability.



Fig. 17 Comparison in the rooting of etiolated stems of physiologically mature (left) and juvenile (right) plant tissue (ACW Nursery, California).

Research into growth manipulation using different spectral light composition during nursery production was beginning at the time of the visit. Investigations in Israel have shown that with ornamental species different spectral light composition, modified by using coloured shade cloths, can have a marked effect on growth. Red and Pearl shade cloth (manufactured by Polysack in Israel) have been found to promote vegetative growth while Grey enhances side branching in some ornamental species. Results of this research will be made available by ACW when the experiment has been completed.

Greenleaf Nursery (California)

Greenleaf Nursery is located at Bonsall in southern California and is owned by Dave Brodersen and Wendy Katze (wendy@gleafnursery.com). The nursery is relatively new to avocado production and is targeting 40 000 avocado trees on cloned rootstocks in the 2002/03 production

season. The owners believe that production of cloned rootstocks where the “nurse seed” has been removed will give them the most reliable product to sell to their clients and to achieve this they are using a similar production technique to that seen at Allesbeste and ACW nursery’s.

Greenleaf Nursery has put its own interpretation on cloned rootstock production with some modifications of the Allesbeste/ACW production methods. There was little difference in the production of “nurse seedlings” between Greenleaf and ACW in that they were both using polystyrene drinking cups as their nursery pot. However, Greenleaf had moved away from using ‘Zutano’ as a “nurse seed” and were using ‘Lula’ imported from Florida. They claimed greater success in producing cloned rootstocks when using ‘Lula’. Greenleaf was using clear drinking containers (16 fluid ounces) with a central hole punched in the base as a container to hold the potting media around the etiolated shoot (Fig. 18). The etiolated stem was pre-treated prior to potting by scraping either side of the shoot for 4-5 cm and then sprayed with 1% IBA/0.5% NAA.



Fig. 18 Inserting clear containers over the etiolated stems prior to filling with

The most notable innovation of Greenleaf Nursery is the changed pot dimensions and shape for finishing off plants prior to sale. They are using pots with a vertical ribbing utilising forestry research which has demonstrated that this type of pot design produces trees with vertically straight root systems reducing the potential of the roots to curl and the plant to become root-bound (Fig 19).



Fig. 19 Vertically-ribbed, 5 L pots used by Greenleaf Nursery to grow plants through to saleable size (left). The pots were held vertical by a frame and base that grouped them into packs of six which facilitated handling in the nursery. An inverted plant shows the straight root system produced by the vertically-ribbed pots (right).

to give a different root system when trees are turned out for field planting. Greenleaf Nursery started using ‘Zutano’ as the nurse seed but is now using ‘Lula’ as the nurse seed which is imported from Florida. The sourcing of nurse seed for production of nursery trees is a major flaw in the Californian production of nursery trees due to the unknown status re: Avocado sunblotch viroid. The use of ‘Lula’ as a “nurse seed” gives much more vigour in rootstock scion growth which carries through to the grafting of the fruiting scion before removal from the nurse seed.

Huerto California Nursery (Chile)

Huerto California is one of several nurseries producing avocado trees for the industry in Chile and is owned by the Maghdal family (cmagdahl@safex.cl). The Chilean avocado industry has grown rapidly over the past 10 years with currently 20 000 ha planted and expansion still occurring at the rate of about 1000 ha year⁻¹. The industry is entirely based on seedling rootstocks with about 90% of these being 'Mexicola', which is highly susceptible to *Phytophthora* root rot. While disease pressure in Chile is relatively low the tree population is relatively young with many orchards being planted on heavy clayey soils which could create problems in the future.



Fig. X Although disease pressure is low in Chile *Phytophthora* root rot is beginning to appear in 6-7 year-old orchards.

'Nabal' is also used as a seedling rootstock in some situations where water quality is poor and salinity levels are high as this rootstock has greater tolerance than 'Mexicola'. A small orchard of 'Hass' grafted to seedling 'Velvick' trees was also inspected and the trees found to be performing very well despite the cool, Mediterranean climate.

The Huerto California nursery uses a potting mixture of 70% composted pine bark; 15% coarse sand; and 15% soil (described as clayey). One kg of S m⁻³ is added to lower the pH along with 180 g of superphosphate.

The soil is steam sterilised using the standard pasteurisation methodology. Seeds are extracted from the fruit and scarified by clipping the tops of the cotyledons prior to planting to improve the uniformity of germination. The floor of the nursery is concreted and copper fungicide footbaths are placed at strategic entry points to ensure traffic into the nursery does not carry disease. Trees are grown on wooden slats about 100 mm off the floor level (Fig. 20). This would not comply



Fig. 20 Grafted 'Hass' trees on 'Mexicola' seedling rootstocks at Huerto California. Note the wooden benches supporting the plants.

with ANVAS standards. The nursery is covered by black 35% shade cloth but research is intended to investigate different light spectrum modified by coloured shade cloths. The nursery trees are irrigated using 1.2 L h⁻¹ micro-tube with a 10-15 min daily watering period. Nutrition is also delivered via the irrigation system with plants receiving 1 g of urea, 0.5 g of KNO₃ and 0.2 cc of phosphoric acid plant⁻¹ month⁻¹.

Huerto California uses a grafting technique different to that commonly in practice by other nurseries visited. Scions are side-grafted into trees about 150 mm above the soil level (Fig.

21). About 10 days after grafting the tree is headed back to 4 leaves above the graft which encourages the grafted scion to grow. The claim is that trees grafted this way grow quicker as

they have a greater photosynthate base with the leaves retained above the graft. The trunk extending above the graft is also used to support the growth from the new scion.



Fig. 21 Side grafting into the trunk of young nursery trees (left). The tree is headed back to four leaves above the scion about 10 days after grafting when the buds begin to swell (right).

Huerto California has begun a cloned rootstock programme initially with Duke 7. The nursery is currently using the Brokaw technique and have about 2000 trees on cloned Duke 7 ready to plant in spring 2002. ‘Zutano’ is currently used as the “nurse seed” and etiolation is carried out in an air-conditioned dark room with the temperature held at around 25°C (Fig. 22). Plants in the etiolation room are sprayed with a mixture of copper oxychloride (300 g L⁻¹) and Benlate 50[®] (70 g L⁻¹) to control fungal pathogens and are normally in the room for 20-25 days.



Fig. 22 Plants in an air-conditioned, etiolation room at Huerto California



Fig. 23 Constriction ring placed on the plant to encourage rooting and to sever the clone from the “nurse seed”.

On removal from the etiolation room a metal washer is placed over and moved to the base of the etiolated growth (Fig. 23) and immediately above the washer the bark is slit vertically and painted with 0.75% IBA. The treated area is covered with potting media to encourage the development of roots. Experience to date suggests that it will take 18-20 months to produce a saleable tree on a cloned rootstock. Grafted trees on seedling rootstocks sell for \$6 AUD and it is expected that grafted trees on cloned rootstocks will sell for about \$10 AUD.