

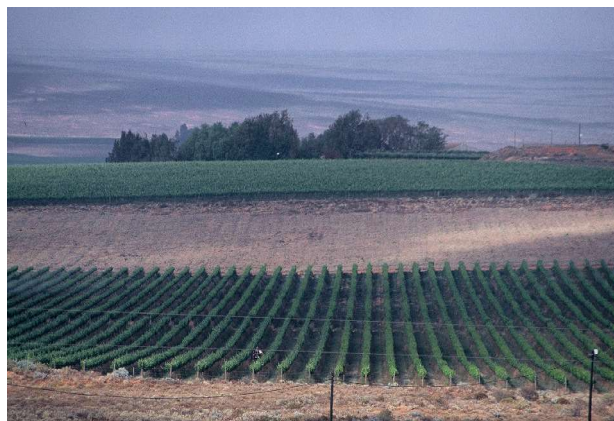
# PLANT MATERIAL QUALITY

A compilation of research

**JJ Hunter**

**CG Volschenk, DJ Le Roux, GW Fouché & L Adams**

ARC Infruitec-Nietvoorbij, Private Bag X5026, 7599 Stellenbosch, South Africa



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CONTENTS

<b>INTRODUCTION</b>	<b>3</b>
<b>CULTIVATION FACTORS IMPACTING ON THE QUALITY OF GRAFT MATERIAL</b>	<b>3</b>
<b>Seasonal growth pattern, starch accumulation, and mineral absorption by rootstock mother material</b>	<b>4</b>
<i>Growth pattern</i>	4
<i>Mineral content</i>	4
<i>Reserve content</i>	4
<b>Density and shade in rootstock canopies</b>	<b>5</b>
<b>Fertilisation of rootstock cultivars</b>	<b>5</b>
<b>Time of collecting graft material</b>	<b>5</b>
<b>Graft cane circumference</b>	<b>6</b>
<b>Graft cane length</b>	<b>6</b>
<b>Position of graft scion</b>	<b>6</b>
<b>Desiccation and water submersion of graft material</b>	<b>7</b>
<b>Other morphological, anatomical and physiological factors to be considered during cultivation</b>	<b>7</b>
<i>Morphology and anatomy</i>	7
<i>Physiology</i>	8
<b>GRAFTING AND CALLUS FORMATION</b>	<b>9</b>
<b>Events during the formation of a graft union</b>	<b>9</b>
<b>Factors necessary for a good graft union</b>	<b>9</b>
<b>Factors impacting on callus formation</b>	<b>9</b>
<b>Some physiological observations concerning callusing</b>	<b>10</b>
<b>Clogging of xylem vessels, callus ability and composition of xylem sap</b>	<b>10</b>
<b>Relationship between starch, mineral composition of graft material, mineral composition of soil and callus formation</b>	<b>10</b>
<b>Hot water treatment of scion cultivars</b>	<b>11</b>
<b>NURSERY ENVIRONMENT FACTORS</b>	<b>11</b>
<b>Climate</b>	<b>11</b>
<b>Effect of plastic covering</b>	<b>11</b>
<b>Effect of irrigation</b>	<b>12</b>
<b>STARCH AND MINERAL REFERENCE SOURCE</b>	<b>12</b>
<b>ROOT FORMATION – GENERAL INFORMATION</b>	<b>12</b>
<i>Auxins</i>	12
<i>Cytokinins</i>	12
<i>Gibberellins</i>	12
<b>COMPATABILITY AND AFFINITY – GENERAL INFORMATION</b>	<b>13</b>
<b>REQUIREMENTS FOR FIRST CLASS STOCKS – GENERAL INFORMATION</b>	<b>14</b>
<b>MATERIALS AND METHODS DEVELOPED AND USED DURING THE INVESTIGATIONS</b>	<b>15</b>
<b>Method for starch analysis</b>	<b>15</b>
<b>Mineral analysis</b>	<b>15</b>
<b>Proteins in graft material</b>	<b>15</b>
<b>Simple method for determination of starch at farm level</b>	<b>15</b>
<b>Seasonal growth pattern, starch accumulation, and mineral absorption by rootstock mother material during the growth season</b>	<b>15</b>
<b>Density and shade in rootstock canopies</b>	<b>15</b>
<b>Fertilisation of rootstock cultivars</b>	<b>15</b>
<b>Time of collecting graft material</b>	<b>15</b>
<b>Graft cane thickness</b>	<b>16</b>
<b>Graft cane length</b>	<b>16</b>
<b>Graft scion position</b>	<b>16</b>
<b>Desiccation and water submersion of graft material</b>	<b>16</b>
<i>Long graft canes (not cut) – simulate desiccation after collection (lie in mother block)</i>	16
<i>Short graft canes (cut) – simulate leaching of stocks before storage</i>	16
<b>Clogging of xylem vessels, callus ability and composition of xylem sap</b>	<b>16</b>
<b>Relationship between starch, mineral composition of graft material, mineral composition of soil and callus formation</b>	<b>17</b>
<b>Hot water treatment of scion cultivars</b>	<b>17</b>
<b>Plastic covering and irrigation in nursery</b>	<b>17</b>
<b>Starch and mineral reference source</b>	<b>17</b>
<b>LITERATURE CITED</b>	<b>18</b>

## PLANT MATERIAL QUALITY

### A compilation of research results

#### INTRODUCTION

Nurserymen, grape producers and winemakers have a joint responsibility to ensure that wine of the highest quality is possible and indeed made. Poor callus development, nursery performance and affinity of scions and rootstocks annually result in huge financial losses for both nurserymen and grape producers. The factors leading to poor performance of graft combinations during callusing and in nurseries and vineyards are not well known and are often ascribed as being of morphological, physiological and/or environmental origin. The quality of graft material can be defined as the ability for callusing, root formation, budding and growth during both the callusing period and in the nursery. The objectives of the research were to improve callus formation and affinity/growth of graft combinations and to contribute to the improvement of the cultivation of stocks to be delivered to the grape producer. Here, we present a compilation of results obtained over a 10 year period. In addition, some general information on important physiological and morphological aspects of plant material is included. A complete document, including all tables and figures, is available upon request and is titled: “The physiological and morphological quality of plant material – a compilation of research”.

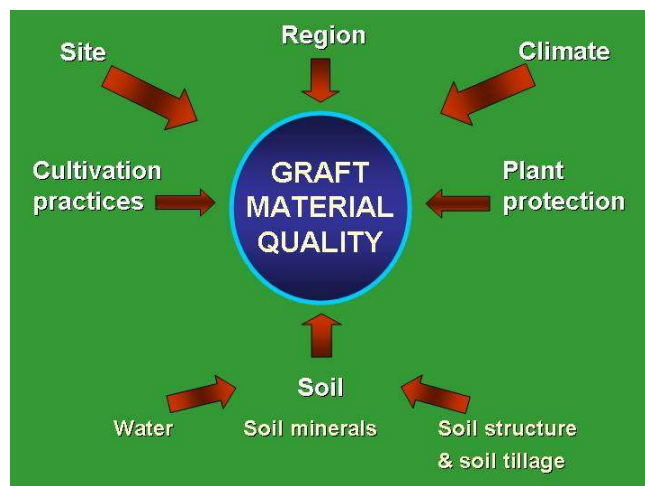


#### CULTIVATION FACTORS IMPACTING ON THE QUALITY OF GRAFT MATERIAL

Strong growing, mature vines, judiciously fertilised, free of blemishes and with delayed abscission of leaves should be promoted during cultivation and eventually selected for grafting. Since the selection of graft material is a very important action in grapevine propagation, two questions should be answered:

- How can the quality of graft material be recognised and
- What are the factors that affect the quality of graft material

To answer this, knowledge of morphological, anatomical and physiological characteristics is necessary. Successful grafting can be expected when minimum morphological/anatomical requirements are met and no physiological stress is experienced.



Worldwide, rootstock mother material is either trellised or grown flat on the ground. Trellising is done by means of slanted stakes or by means of a T-system covered with wire netting. When rootstocks are not trellised, the flat shoots mostly form a thick carpet on the ground. Some shoots are therefore fully exposed to sunlight, some only receive diffused sunlight and others are growing in complete shade. The shoots differ in the extent of exposure to sunlight. This has a direct effect on the reserve and ripeness status of shoots. Shoots therefore do not ripen uniformly and this may eventually lead to varying callus success. The susceptibility of flat-growing shoots for pests and diseases will also increase as a result of more difficult control, higher temperature and humidity, and possible damage. It was found that trellising increased shoot mass, whereas longer shoots of higher quality and more uniform diameter could be obtained (Ambrosi & Kriel, 1958).



Scion cultivars having good yields, but balanced vegetative:reproductive growth ratios, on well-prepared soils that allow a well-distributed root system, should be created and selected for use in grafting. It is essential that the canopy be well exposed to sunlight in order to increase reserve accumulation in the canes to be collected. This can be achieved by judicious application of canopy management practices such as efficient trellising, suckering, shoot positioning, topping and leaf removal (Hunter *et al.*, 1995).

#### **Seasonal growth pattern, starch accumulation, and mineral absorption by rootstock mother material**

The seasonal growth pattern, starch accumulation, and mineral consumption by trellised rootstock mother material (99R and 101-14 Mgt) were investigated over three seasons (1996/97 – 1998/99 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij). Data from Ramsey, 110R and US 8-7 were also collected in February for two seasons. Fresh and dry mass of vegetative growth (leaves, petioles, primary shoots, secondary shoots and the rest) and primary shoot lengths, soil mineral content (0 – 30 cm, 30 – 60 cm, 60 – 90 cm), mineral content of different plant parts (primary leaves, petioles and shoots, secondary shoots and the rest) and starch content of different plant parts (primary leaves, petioles and shoots, secondary shoots and the rest) were monitored monthly (from November until July) during the growth season.

##### *Growth pattern*

Graft cane production of the different rootstocks differed clearly. A reasonably stable growth pattern occurred until January/February, after which it decreased. Leaf fall occurred earlier for 101-14 Mgt (April) than for 99R (May). In contrast to production vineyard experience, 101-14 Mgt grew more vigorous than 99R in the mother block and again point to a mutual effect of scion and rootstock under vineyard conditions. The results highlighted the positive effect of secondary (lateral) shoots on the growth of rootstock mother material. Primary shoot growth decreased in the order of Ramsey, 110R, 101-14 Mgt, 99R, and US 8-7. Mean primary shoot lengths of more than 500 cm were generally found for Ramsey, less than 500 cm for 110R, less than 400 cm for 101-14 Mgt and 99R, and less than 300 cm for US 8-7.

##### *Mineral content*

The mineral content of the different rootstocks showed little variation and leaf and petiole levels were mostly comparable to those of bearing vines. The mineral levels in the primary and secondary shoots were, except for low N and Ca contents, also comparable to the leaf norms found for bearing vines by Conradie (1986). As no obvious deficiency symptoms occurred, these mineral levels can probably be considered normal. Calcium and N are important for shoot growth and for callus development and should these aspects nevertheless be investigated. Transport of minerals from the roots to shoots should be stimulated by judicious management practices. An analysis of the mineral requirements of rootstocks for the production of one ton of graft shoots and of a 28 cm length graft shoot showed that Ramsey, 110R and 101-14 Mgt had the highest mineral levels among the rootstocks investigated. Ramsey displayed very high potassium levels, whereas 99R, 110R and 101-14 Mgt had high N requirements and Ramsey and US 8-7 would seemingly benefit from high Zn availability. The results indicated that soils may be over- or under-exploited, depending on the rootstock used. That will eventually lead to differential fertilisation in order to ensure optimum soil utilisation and cost efficiency and to restrict soil and water pollution. Continuous monitoring of the soil is necessary for the prevention of luxurious and haphazard fertilisation and thus imbalances in mother material.

##### *Reserve content*

Secondary shoots contain only two thirds to three quarters of the amount of starch in the primary shoots; this may play a role in callus success when both primary and secondary shoots are used as graft canes. This can also play a huge role in the annual variation in callus success. On the basis of starch content, the results nevertheless indicated that secondary shoots may also be used as graft canes in the case of 99R, 101-14 Mgt, Ramsey and US 8-7. In the case of 110R the risk of using secondary shoots

may be higher. Secondary shoots of the aforementioned rootstocks should still be used with care, particularly under unfavourable growth, callus and nursery conditions.

### **Density and shade in rootstock canopies**

In an effort to elevate density and shade in the canopies of the rootstocks and to increase the starch content of primary shoots, secondary shoot removal was done. That led to a decrease in the starch content of 5% in the case of 99R and 15% in the case of 101-14 Mgt primary shoots. The secondary shoots therefore contributed, as in the case of production vineyards, to photosynthetic activity and carbohydrate production capacity during particularly the second phase of the growth period when primary leaves already started senescing. The percentage class I stocks was also decreased (at least 5%) by secondary shoot removal.



### **Fertilisation of rootstock cultivars**

During the 1995/96 season the relationship between starch content, mineral composition of graft material, and callus development, was investigated during the callus period. Based on this, K, Zn, and K-Zn fertilisation was applied to 99R and 101-14 Mgt and the effect on starch content, callus development and nursery performance determined. Analyses included starch contents of scion and rootstock before and after callus, root and shoot mass, as well as mineral contents of graft canes and grafted stocks before and after callus and after the nursery period. Means of the two years showed that, in spite of little change in starch content, K and Zn (for 99R) and K, Zn and K + Zn (for 101-14 Mgt) fertilisation increased callus performance (1997/98 – 1998/99 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij). The effect will probably be greater under less favourable soil conditions. The results also indicated that the rootstock had an effect on scion metabolism from as early as the callus period. Poor callus formation is probably the result of physiological abnormalities in both the rootstock and scion cultivar. The Zn and K + Zn fertilisation for 99R and Zn fertilisation for 101-14 Mgt increased the root and cane mass of the nursery vines. The results indicate that supplying in the fertilisation needs of the rootstocks can have a positive effect on callus and nursery success.

### **Time of collecting graft material**

Although long storage periods can also result in changes in graft material, respiration of carbohydrate reserves in winter can be restricted by collecting graft material as soon as possible. This will also result in better labour distribution. In an investigation regarding the optimum time (between April and July) for graft cane collection (1997/98 – 2001/02 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij), starch (before and after callus) and mineral analyses as well as grafted stock callus values indicated that the realisation of high starch contents and callusing would require that collection of material preferably be completed before June. The % Class I stocks (as well as root and shoot mass values) obtained after the nursery phase showed that 101-14 Mgt, US 8-7 and US 2-1 should be collected as early as possible, 110R, 140 Ruggeri, SO4 and 143 B should preferably be collected before June, and 99R, Ramsey and 1103 Paulsen any time between April and July. Starch contents largely decreased during the storage period. Proper management must ensure maximum starch levels in the material before storage in order to increase the buffer capacity of the material against unfavourable conditions during callusing and in the nursery and to increase the number of high quality nursery vines.



### **Graft cane circumference**

A circumference of 6.5 – 12 mm is currently the norm. The behaviour of thinner versus thicker shoots, bundled together, in the nursery was questioned. The presence of more or less thick or thin stocks in a group (bundle) had no effect on the callus ability

of others or their growth (cane and root mass) in the nursery (1994/95 WW12/03 Research Report, ARC Infruitec-Nietvoorbij). Thin stocks showed the highest increase in total mass. It seemed that the morphological dimensions of graft material have no relationship with callus ability of the graft combination.



### Graft cane length

A length of 26 – 28 cm with a nodium at the base is the norm [Too short internodia indicate poor growth, whereas long internodia indicate vigorous growth. Both can affect callus formation as a result of stress factors associated with poor (e.g. malnutrition) or vigorous growth (e.g. shade)]. During cutting, approximately 5 mm must be left below the basal nodium and approximately 4 cm above the apical nodium (graft cut side). An investigation was done to determine whether reserve nutrients of the rootstock, notably starch, play a role during the callus period, whether the full metabolic capacity of the rootstock (as quantified by the length of the rootstock cutting) is used during the callus period, whether the formation of callus tissue is a localised process, and whether the scion grows on its own reserves or simply because conditions are favourable (1994/95 WW 12/03 Research Report, ARC Infruitec-Nietvoorbij; Hunter *et al.*, 1996). Two-bud graft scions (Cabernet Sauvignon) grafted onto a normal full length (25 cm) rootstock (99 Richter) cutting or to only a section (5 cm) of the rootstock cutting were compared after 0, 1, 2, 3 and 5 weeks of callus. It was found that:

- Optimum callus occurred after three weeks for both treatments.
- Callus of the full-length stocks was more complete, whereas root and shoot growth were increased.
- Scions showed a higher starch content than rootstocks.
- Starch of full-length rootstocks was markedly lower after week 3, whereas that of short rootstocks was already significantly lower after week 2.
- Starch from both scion and rootstock was used solely for callus formation only in week 1. From week 2 onwards it was probably mobilised to support shoot and root growth in particular as well as further callus formation.
- The whole rootstock contributed metabolically during the callus period.
- The full-length rootstock is necessary to sufficiently support both callus formation and vegetative growth.
- Starch content of both scion and rootstock played a prominent role during the callus period and the callus process is therefore not a localised event.
- Starch metabolism plays a major role in producing a quality nursery vine.



### Position of graft scion

Sourcing graft scion material from scions is a very important action that depends on morphological dimensions, physiological quality and economical considerations. An experiment was done for two growth seasons using material from Grondves mother plantations in Stellenbosch (2002/03 – 2003/04 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij). Graft scion material from apical and basal positions on the shoot was sourced to determine differences in callus performance. Rootstock 110R was used. Results showed that callus performance was not affected by the position of the graft scion. The recovery percentage of C. blanc and Merlot did not differ with the use of graft scions from different positions. The recovery of S. blanc was, however, increased with the use of basal graft scions, whereas that of C. Sauvignon was increased with the use of apical graft scions.

Canopy appearance of the different source material is not known. Canopy light exposure plays a role in starch accumulation, which involvement in callusing and growth had already been confirmed.

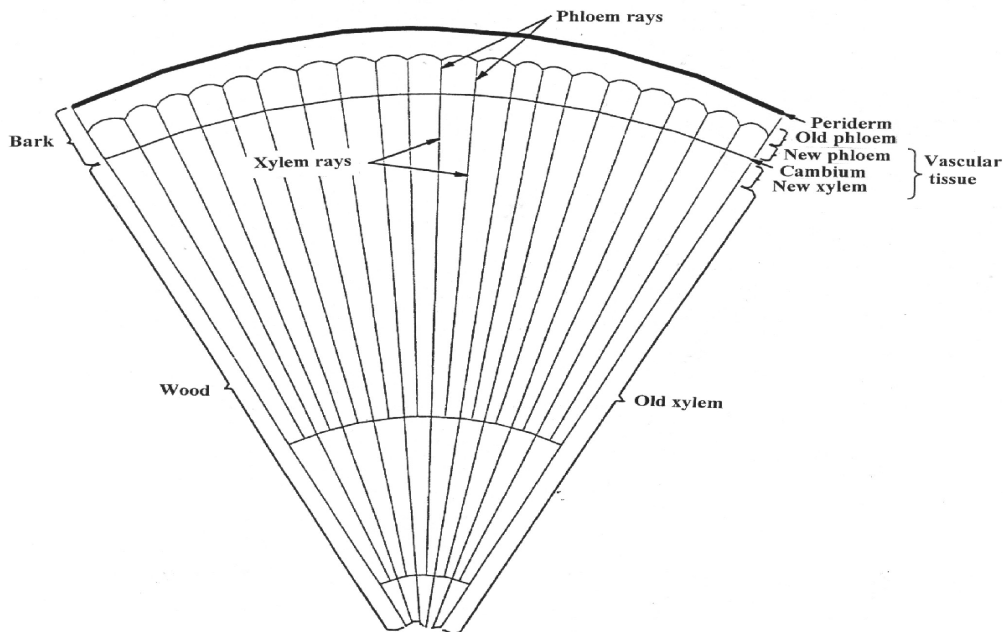
### Desiccation and water submersion of graft material

An investigation (2000/01 - 2001/02 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij) was done to determine the effect of desiccation (before cutting) and water submersion (after cutting) on callus and nursery success. In the first investigation on 99R and 101-14 Mgt, desiccation resulted in a loss of water, but this had been reasonably recovered by an overnight period in water. Desiccation as well as water absorption resulted in water loss from 110R and 140 Ru graft canes during the storage period. A loss of possible water loss restricting compounds (e.g. fatty acids, tannins, slime, protein, dormancy callus in the phloem) during desiccation and water submersion may have played role. For 99R, 101-14 Mgt and 140Ru, callus percentage was increased by a period of desiccation (of up to three days), followed by an overnight water submersion period. For 110R, callus performance was stimulated only by desiccation. The results were difficult to explain. Nevertheless, it is clear that different genetic material react differently to treatment. In general, drastic treatment of up to three days of desiccation, three days of water submersion or up to three days of desiccation followed by submersion in water, apparently had no effect on the performance of the vines in the nursery.

### Other morphological, anatomical and physiological factors to be considered during cultivation

*Morphology and anatomy (Schenk, 1978)*

- Sap present in the phloem (just below the cork layer) (Fig. 1 – Kester, 1965).
- Green and non-blemished phloem.
- Firm xylem (wood).
- Pith:wood ratio: The perception exists that the size of the pith does not increase after shoot growth stopped. The woody part, however, continues to increase (depending on the growth conditions). That led to the use of the pith:wood ratio. As a general rule, the surface area of the pith should not be more than a quarter of the surface area of the diameter of the shoot (cane in winter).
- A protected cambium.
- A periderm (cork) with several layers that protect the shoot against desiccation (starch and sugar are stored in the periderm and affect the ripeness and grafting capability).



**Fig. 1. Schematic representation of the different tissue layers in the graft material (Kester, 1965).**

### Physiology

- An increase in nitrogen fertilisation from 30 – 90 kg/ha decreased the starch content of graft material. In such cases, growth is normally increased and special care must be taken to accommodate the growth properly.
- In Hungary, wood containing a minimum carbohydrate (sum of glucose, fructose, sucrose and starch) content of 13% is considered well-ripened (Zanathy *et al.*, 1996).

- With micro-stocks it was found that leaf and root development is dependent on the translocation of water and carbohydrates (Guerrier-Julien *et al.*, 1996). This indicates the very important role of sufficient water and starch accumulation in graft material and grafting success.
- In Germany it was found that the accumulation of K, Ca, Mg and P in one-year-old wood is dependent on the cultivar and season (Fardossi *et al.*, 1996). The following variation was found for 26 rootstock cultivars over a 4-year period:
 

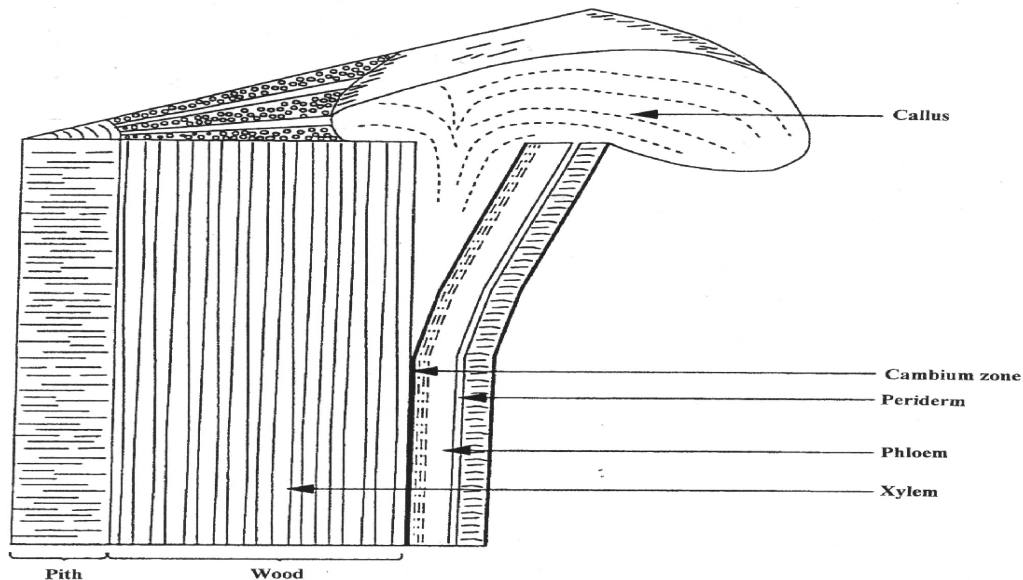
K:	0.6 – 0.8%
Ca:	0.35 – 0.7%
Mg:	0.075 – 0.135%
P:	0.085 – 0.139%
- Light conditions, temperature, water, and fertilisation will affect the mineral level in the material. The mobility of different minerals will also affect their relative impact. The mineral content of the wood will eventually affect growth of stocks in the nursery.

### GRAFTING AND CALLUS FORMATION

When a plant is wounded, as occurs during grafting, many physiological processes follow in order to heal the wound. This differs from other types of wounding (e.g. during pruning) in the sense that a different plant part is “placed” upon the wound, wound tissue must be formed, and the union must be successfully fused. This is essential for successful grafting and vine development.



Callus tissue can be defined as a non-uniform mass of parenchyma cells in different stages of lignification. It usually develops on the basal side of a cutting from young cells in the vicinity of vascular cambium, although different cells of the cortex (on the outside of the phloem) and pith can also contribute to development (Fig. 2). The callus cells are thin walled and turgid and can easily dry out and die.



**Fig. 2. Schematic representation of the development of callus tissue from vascular tissue.**

#### Events during the formation of a graft union (Kester, 1965)

- The wound heals. Exposed living cells die and brown. The number of cells depends on the length of exposure and extent of desiccation.
- Living cells (xylem and phloem) below the dead layer divide to form callus. This is a prerequisite for a successful graft union.

- Exposed, dead cells are sealed (with gum/resin). Fast sealing prevents excessive water loss. Some cultivars react slower than others.
- A periderm or cork layer forms towards the outside of the wounded area.
- A connection between the water conducting tissue (xylem) of the scion and rootstock forms. This is priority of the scion part of the combination. New xylem and cambium tissue form from the callus tissue. Except for the involvement of the natural plant hormones auxin and cytokinin, the physiological explanation for the formation of callus is relatively unclear. The stimulus apparently comes from existing vascular tissue. It is therefore very important that tissue from both scion and rootstock corresponds in terms of a tight graft union, and graft cane and graft scion of similar thickness.
- The nutrient translocation elements (phloem) of the graft cane and graft scion are connected. This apparently forms later than the xylem.
- Strengthening tissue (fibre elements) forms inside the graft union, contributing to the development of a rigid stem.
- Successful fusion of scion and rootstock will promote growth and development in the vineyard.



#### Factors necessary for a good graft union

- Tight fit of scion and rootstock.
- Similar thickness of graft scion and graft cane.
- Smooth graft cut.
- Moisture-rich material which is dry on the outside to allow for the clinging of wax.
- A graft union that is well covered by wax to prevent desiccation and fungal infection.
- Callus development originating from both graft scion and graft cane.
- Correct polarity of material.
- Graft scion and cane correspond in terms of dorsiventrality. Since more vascular tissue occurs at the dorsal and ventral sides of canes, callus formation in these areas is better than at the two flat sides of the shoot.
- Affinity of the genetically different material.



#### Factors impacting on callus formation

- Genetic composition. Some cultivars are prone to poor callus production.
- Supply of endogenous growth regulators. Callus is a function of growth substances such as auxin and cytokinin. Auxins such as indole acetic acid, naphthalene acetic acid and indole butyric acid stimulate callus formation. Different ratios of auxin and cytokinin regulate growth, e.g. combinations of high or intermediary auxin and high or intermediary adenine induce callus, high auxin and low adenine induce roots and low auxin and high adenine induce shoots. The balance between the different growth regulators is therefore critical and possibly one of the reasons why externally applied growth regulators have not regularly shown a practical advantage. This indicates that natural balances in the plant should rather not be disturbed or that growth regulators must at least be used with great care.

- Reserve nutrients in graft material. High quantities of carbohydrate and nitrogen reserves such as starch increase the potential for callusing. Accumulation is enhanced by amongst others good sunlight exposure and judicious fertilisation of mother material.
- Polarity of the plant material is important for permanent success of the graft union and refers to basal movement of auxins and other compounds in the graft scion.
- Temperatures during callus are critical. If temperatures are too low, callus formation is inhibited, whereas if they are too high, soft callus that easily get damaged is produced. Day/Night temperatures of 27 - 28 °C are considered optimal.
- Light exposure during the second half of the callus period results in the obtaining of green shoots and leaves that are photosynthetically active. This makes a contribution to the developing graft union in the form of carbohydrates and natural growth regulators. The stocks are then also effectively hardening already during the callus period.
- High humidity prevents cork formation and induces callus formation. The graft union must be sealed and the water content of material maintained.
- Atmospheric levels of oxygen (16 - 18%) are needed during callus.

#### **Some physiological observations concerning callusing (Schaefer, 1981)**

- Callus tissue contains 8 - 17% carbohydrate, 12 - 18% N-compounds (of which 0.4 - 2.2% soluble proteins), and 6.8 - 17% phenolic compounds.
- Callus tissue contains the same % carbohydrate and three times more N-containing compounds than the rootstock. Callus tissue is therefore a strong sink during the callus period.
- More energy is used with better callus formation.
- In some combinations N-mobilisation increased with better callus and was delayed with poorer callus. Despite the negative consequences of excessive growth, it is necessary that adequate N is present in graft material.
- Although enzymes, such as acid phosphatases (involved in hydrolysis processes, i.e. the splitting of compounds) and peroxidases (involved in oxidation and reduction processes), have no direct relationship with callus success, the aforementioned was higher with better callus and the latter higher with poorer callus. The peroxidase apparently inhibits the activity of growth substances. Peroxidases and polyphenoloxidases can delay cell division and result in browning and death of tissue at the wound and can therefore affect callus formation. The peroxidase activity was also higher in the wood of stocks with poor affinity. Activity of acid phosphatases and peroxidases and conversion of proteins and carbohydrates were higher in callus than in wood. This resulted from, amongst others, active growth, oxidative processes, and lignification of callus tissue.
- Protein composition may present an indication of callus ability. In an investigation to determine the role of proteins in graft cane quality, differences in the profiles of 101-14 Mgt and 99R were found (1994/95 WW12/03 Research Report, ARC Infruitec-Nietvoorbij).
- Stocks may callus poorly because of delayed development. Practical possibilities to prevent this are e.g. to optimise the time of material collection, storage temperatures, length of the callus period and the temperature at which is callused.

#### **Clogging of xylem vessels, callus ability and composition of xylem sap**

To determine the relationship between the ability of graft material to callus and the clogging of xylem vessels, the composition of the xylem sap and the distribution of compounds in the graft combination, were investigated (1993/94 WW 12/03 Research Report, ARC Infruitec-Nietvoorbij). Morphological dimensions (circumference and lateral and dorsal-ventral diameter) and extent of xylem clogging in internodia or nodia of rootstock graft material (101-14 Mgt, 99R, 110R, Ramsey and US 8-7) showed no relationship (single or multiple correlations) with the callus of rootstock or graft combination. Similarly, although the carbohydrate content (sucrose, glucose, fructose) of clogged material was lower than that of non-clogged material (probably because of the formation of compounds such as fats, tannins, protein, dormancy callus), translocation of <sup>14</sup>C (as indication of translocation from the rest of the rootstock to the point of callus formation) and the composition of xylem sap (carbohydrate, organic acids, fungi, bacteria) apparently played no role in callus ability. The correct balance between growth regulators and carbohydrates (as source of energy) possibly plays a role. Considering all factors, only the callus ability of the rootstock showed a 76% correlation with that of the graft combination; it can also be used to predict callus of the graft combination with a 58% success rate.

#### **Relationship between starch, mineral composition of graft material, mineral composition of soil and callus formation**

An investigation into the relationship between starch, mineral composition of graft material, mineral composition of the soil, and callus formation of 15 different rootstock cultivar/clone/block combinations, grafted onto the same scion cultivar (Chenin blanc) and analysed (for starch, callus and mineral content) at intervals during the callus period (1995/96 WW 12/03 Research Report, ARC Infruitec-Nietvoorbij), showed the following :

- Starch is directly involved in callus formation and vegetative growth of the stock during the callus period.
- The rootstock cultivar effects the starch concentration of the graft scion, whereas the cultivar, clone, and block effect that of the rootstock.
- Rootstocks differ with respect to starch depletion during callus. This has implications for the length of the callus period.
- Starch of 101-14 Mgt showed the highest and that of Ramsey the least decrease from week 3 to 5.
- The starch depletion of 110R and that of 99R was similar.

- In the case of 101-14 Mgt, callusing for more than 3 weeks would therefore not make any further contribution to the amount of callus formation, because the original starch is already depleted.
- In the case of Ramsey, a 5-week callusing period would increase callus formation and stock development.
- The mineral elements generally reacted opposite to starch during the callus period.

#### **Hot water treatment of scion cultivars**

Application of hot water treatment (50 °C for 30 min.) resulted in a general loss of water (up to 40%) from the canes of scion cultivars (Chenin blanc, Malbec, Red Muscadel, Ruby Cabernet, Sémillon, Tinta Rorez, Tinta Francisca, Turiga Nacional, Viognier) (1998/99 – 1999/2000 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij). This practice will require more care during the callus and nursery periods in order to sustain the water content of graft material. Hot water treatment resulted in starch losses of more than 60%. The results implied that hot water treatment may affect the quality of graft combinations and lead to losses in the nursery, especially under conditions unfavourable for the accumulation of starch and because high starch losses also occur during the callus period. Results indicated that the starch content of scions was higher than that of rootstocks.

### **NURSERY ENVIRONMENT FACTORS**

During the initial growth phase of nursery vines, environmental conditions should be optimal in order to stimulate growth of shoots and roots and to prevent growth arrestment. An experiment was done over two growth seasons in the Kys nursery close to Vredendal to investigate the role of soil temperature (simulated by using different colours of plastic covering) and water status of the soil (simulated by irrigating by means of an under-plastic drip system in volumes similar to those supplied by the nursery on a standard basis, the standard plus one third and the standard minus one third) (2002/03 – 2003/04 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij).



#### **Climate**

From September to October, the highest soil temperatures occurred between 15:00 and 17:00. From November to May, the highest temperatures occurred between 16:00 and 18:00. From September to November, the uncovered soil displayed the coolest night and afternoon temperature, whereas from 09:00 to 15:00 it was similar to that of the plastic covered soil. From December to March, the temperature of the uncovered soil reached higher levels during the period between 11:00 to 15:00 than that of plastic covered soil. The different coloured plastic coverings resulted in only slight differences in soil temperature. Brown and black plastic led to slightly lower temperatures in the morning and black plastic to slightly higher temperatures in the late afternoon. The environment temperature and humidity data of all the months of the growth season showed that the temperatures often increased to above 30 °C, with concomitant low humidity. Overhead irrigation to decrease temperature and increase humidity was therefore needed.

#### **Warm/cold callusing and plastic covering**

It was found that cold callused stocks generally had a higher callus percentage than warm callused stocks when 110R was used as rootstock. When using 101-14Mgt, no differences were found between the callusing procedures. No differences in growth percentages four and eight weeks after planting occurred between the different callusing procedures. However, it seemed that root growth was favoured with cold callusing, whereas shoot growth was favoured with the warm callusing procedure. From September to November, plastic covering increased soil temperature during the night and afternoon. From December to March, the temperature of uncovered soil was higher from 11:00 to 15:00. Among the silver, brown and black plastic coverings investigated, black plastic seemed to only slightly increase the temperature during late afternoon. Plastic covering had no effect on the number of stocks recovered. Growth of both S. blanc and C. Sauvignon was retarded when grafted to 110R; this did not occur when 101-14Mgt was used. This effect was more pronounced with plastic covering than without plastic covering. In spite of this, no differences in root and shoot mass between rootstocks, scions or with or without plastic covering occurred. The colour of the plastic covering also had no effect. Plastic covering of the soil had no apparent effect on the performance of the nursery vines.

#### **Irrigation**

The different irrigation treatments (normal, normal + 1/3 & normal - 1/3) had similar effects than the plastic covering (mentioned above) on the length of the growth period of the different graft combinations. The highest volume of water tended to increase shoot and root mass. Concerning the parameters investigated, the results showed no advantages of plastic covering over uncovered soil. If it is to be used, any of the colours investigated seems suitable. Irrigation is important and increased irrigation seemed to be favourable for growth.

### STARCH AND MINERAL REFERENCE SOURCE

A need for the existence of a reference source that may be referred to in cases when problems with graft material are experienced was identified. Results of this investigation (1997/98 - 1999/2000 WW 12/03 Research Reports, ARC Infruitec-Nietvoorbij) indicated higher starch levels in scions (Chardonnay, Sauvignon blanc, Pinotage, Shiraz, Merlot, Pinot noir, Sémillon, Ruby Cabernet, Colombar and Cabernet Sauvignon) than in rootstocks (101-14 Mgt, 99R, 110R, Ramsey, US 8-7, SO4, 3306 Couderc, 1103 Paulsen, 140 Ru and 143 B). The starch content of all the scion and rootstock cultivars investigated decreased from February to July. That of Ramsey, SO4, 3306 C, Ruby Cabernet, Merlot, Pinotage and Sauvignon blanc did not decrease sharply. The starch content of the rest of the cultivars, however, decreased drastically and implies that these cultivars must be collected as soon as possible after the growth season. Scions and rootstocks had similar mineral contents which indicate that fertilisation requirements probably would also be similar.

### ROOT FORMATION - GENERAL INFORMATION

Because of the involvement of growth regulators with root formation, and growth and development in general, a few factors on the importance and active mechanism of some of these compounds should be considered.



#### *Auxins*

Natural auxins, such as indole acetic acid (IAA) and synthetic auxins, such as indole butyric acid and naphthalene acetic acid, have a definitive effect on root formation. Not much is known about the factors that regulate IAA *in vivo*. Auxin is formed in meristems and growing tissue. The shoot tips are a huge source. Transport from this source is normally not through the phloem tissue, but apparently through basipetal downwards excretion from cell to cell in the cytoplasm - at a rate of approximately 10 - 20 mm/hour. Transport is mainly dependent on tissue orientation and concentration gradient. Inhibiting factors are e.g. O<sub>2</sub>- and energy shortages. Indole butyric acid solutions are commonly used to increase root formation.

#### *Cytokinins*

Cytokinins are produced in the roots. It moves along with the transpiration stream. Together with their role in the delay of leaf senescence, it also results in the mobilisation of nutrients. The latter is important for callus formation, e.g. the mobilisation of starch, which acts as energy source.

#### *Gibberellins*

Gibberellins are produced in the shoot tips (leaf primordia) and roots. Wounded tissue is also a source of gibberellin. In this regard, it is important to remember that the graft cut is also wounding and gibberellins would therefore also be produced here when conditions are favourable. Gibberellins move up and downwards in the plant without polarity. It is therefore present in both the xylem and phloem.

As already mentioned, the regulatory mechanism of growth regulators is dependent on specific ratios and it is therefore of the utmost importance that disturbance of endogenous levels outside the natural be prevented.

*Root formation is dependent on the following factors :*

- Cultivar - some root naturally better than others.
- Reserves - rooting and carbohydrate mobilisation in graft canes take place parallel to each other (Bartolini *et al.*, 1996). The starch content of graft scions decreases during rooting (Buttrose, 1965).
- Virus infection - this can affect graft material to such an extent that no roots are formed. Mother material must therefore be inspected continuously for harmful viruses.

- Balanced mineral composition - this must be monitored on a regular basis.
- Leaching of stocks apparently increases the quantity and quality of roots - an endogenous inhibitor of root formation is probably present in the grapevine.
- Polarity of the graft cane.
- Presence of growth regulators.
- Temperature.
- Leaf development and growth tips - there exists a synergistic mechanism between the two components in the stimulation of root development.
- Good light conditions.

*Rooting of graft material can be improved by considering the following factors during selection :*

- High N-levels - this leads to poor root formation.
- Zn is favourable to root formation (Zn is a requirement for the formation of triptofan, which is a precursor for auxin).
- High carbohydrate levels favour root formation.

*A moderate N-high carbohydrate balance in graft material can be obtained as follows :*

- Monitoring of the mineral status of the soil and the mother material on a continuous basis to prevent imbalances.
- Focus on moderate N fertilisation and expose shoots to be used as graft canes to good light exposure in order to prevent etiolation, reduce shoot growth and increase carbohydrate accumulation.
- Graft material should conform to physical requirements (basal shoot parts contain more starch, contributing to rooting).

### COMPATABILITY AND AFFINITY – GENERAL INFORMATION

Compatability is defined as the ability or inability of scion and rootstock components to combine successfully during the nursery phase (Van Schalkwyk, 1987). During incompatibility, callus tissue is mostly formed, but because of anatomical and physiological differences no or very little physical fusion takes place. The term affinity refers to the success of a graft combination in the long term. It may happen that incompatibility of a combination does not surface during the nursery phase, but only after a number of years in the vineyard in the form of deterioration and eventual death of the vine - this will surface faster when the vineyard is exposed to stress conditions.

*How can incompatibility be recognised?*

- Stocks show no growth.
- Stocks have short shoots with yellowish leaves and die eventually back.
- Stocks break when the bend and turn test is applied.
- Thickenings on, above or below the graft union - in the case of mature vines a thickened scion above the graft union is a characteristic sign of affinity problems.
- Poor root formation.



Physiologically, it was found for fruit trees that starch apparently builds up in the scions of incompatible, but not in compatible combinations - the graft union probably has a girdling effect. Ostensibly, carbohydrates accumulate in the scion because of a phloem (transport) malfunction across the graft union (Breen, 1974). This can also occur for just-grafted stocks when the graft union tissue is damaged during or before grafting. In addition, in the case of minerals it was found that zinc translocation through the graft union of incompatible combinations is blocked. This was also found for other elements such as Ca, K, Mg, Fe and Mn. Apparently, scions and rootstocks of incompatible combinations have quantitative differences in sugar and free-amino-nitrogen composition (Kolesnik, 1963).

### REQUIREMENTS FOR FIRST CLASS STOCKS – GENERAL INFORMATION

- The graft union must be completely fused right around.
- The bend and turn test must be resisted.

- At least one undamaged, well-ripened one-year old shoot with minimum length of 15 cm must be present.
- Well-developed roots distributed in all directions and of which at least two are strong roots.



It is clear from the investigation that the graft scion, graft union and rootstock all react with each other in terms of nutrient distribution and utilisation, translocation of nutrients and water, and changes in endogenous growth factors and the environment. These interactions determine the behaviour of the nursery stocks in the vineyard and their performance in terms of e.g. cold resistance, disease resistance, growth, and eventual yield and grape quality. It therefore is logical that judicious management practices (in the mother block and nursery) and grafting techniques must be followed to eventually provide a quality stock to the grape producer.

## MATERIALS AND METHODS DEVELOPED AND USED DURING THE INVESTIGATIONS

1. **Method for starch analysis**  
Extract soluble sugars from plant material. Suspend dry or fresh plant material in plant material in MeOH-CHCl<sub>3</sub>-0.2M HCO<sub>2</sub>H. Extract at 20 500 rpm. Filter homogenate. Extract further with 80 % ethanol. Freeze residue at -20 °C. Freeze-dry and keep for starch analysis. Add 80 % acetone to the dry plant material, mix, put in ultrasonic water bath, leave in refrigerator for 6 hours, centrifuge and decant supernatant. Add 80 % ethanol, mix, put in ultrasonic water bath, centrifuge and decant supernatant. Add H<sub>2</sub>O, mix, put in ultrasonic water bath, centrifuge, decant supernatant, freeze residue at -20 °C and freeze-dry overnight. Add water, mix, put in ultrasonic water bath, leaf for 60 min. in refrigerator and centrifuge (remove control sample). Add enzyme-buffer solution, mix and incubate for three hours at 40 °C with constant shaking. Centrifuge, dilute with water H<sub>2</sub>O, mix a aliquot with ABTS-enzyme-buffer reagent. Leaf 30 min. for colour development. Read absorbancy and express result in mg starch/mg plant material (with the aid of a glucose standard range).
2. **Mineral analyses**  
Graft material was dried and then analysed with a nitrogen analyzer and inductive coupled plasma spectrophotometer for individual minerals.
3. **Proteins in graft material**  
Graft canes of 99R and 101-14 Mgt were used (collection in July). Both SDS-PAGE and A-PAGE methods were used to determine and quantify the protein profiles of the material.
4. **Simple method for determination of starch at farm level**  
An iodine trichloride solution was added to discs of oven dried graft canes and left in the dark. After filtration, the unused iodine was titrated with sodium thiosulphate. The starch content is calculated from the titration figure. A higher starch content of the sample led to a lighter reaction solution. The normal enzyme method was used to verify the results as found with the iodine method and to determine the relationship between the two methods.
5. **Seasonal growth pattern, starch accumulation, and mineral absorption by rootstock mother material during the growth season**  
Trellised rootstocks 99R (clone RY141) and 101-14 Mgt (clone AA219A) at Grondves, Stellenbosch, were used. No treatments were applied. For 99R, secondary shoots were removed as part of normal farm practice. One shoot/vine was sampled from 6 vines per rootstock and represented three replicates per rootstock. The shoots were divided into primary shoot leaves, primary shoot petioles, primary shoot, secondary shoots and the rest of the vegetative growth (the latter included secondary shoot leaves, secondary shoot petioles and tendrils). All of these plant parts were analysed for mineral and starch content and fresh and dry mas determined. Samples were taken during the growth season and during winter. Duplicate soil samples were taken at three depths (0 - 30 cm, 30 - 60 cm, 60 - 90 cm) at every stage. The duplicate samples per depth were mixed and analysed together for mineral content. Additionally, rootstocks Ramsey, 110R and US 8-7 were also monitored in February for total mineral and starch content.
6. **Density and shade in rootstock canopies**  
Trellised rootstocks 99R (clone RY141) and 101-14 Mgt (clone AA219A) at Grondves, Stellenbosch, were used. The vines (5 per treatment) were treated on a regular basis in the first part of the season. Two treatments were included, a control (without secondary shoot removal) and removal of all the secondary shoots. Graft canes were harvested in July, stored and then grafted. The starch and mineral contents were determined and callus development and growth characteristics (in the nursery) evaluated.
7. **Fertilisation of rootstock cultivars**  
K and Zn fertilisation of 99R (clone RY141) and 101-14 Mgt (clone AA219A) was applied at Grondves, Stellenbosch. The rootstocks were trellised. In the case of K, 150 kg/ha (approx. 67.5 g/vine) was applied by means of band placing in March (according to the after harvest period for vineyards) in the form of 300 kg KCL in one payment. In the case of Zn, leaf sprays were applied at a concentration of 1.6 kg/500 l/ha (approx. 2.87 g/vine) in the form of ZnSO<sub>4</sub>·7H<sub>2</sub>O at two stages (in November and in December). Combinations of K and Zn fertilisation were also applied. The K, Zn, and K-Zn fertilisation treatments were each applied on three parcels, each having 5 vines per parcel. Buffer rows were included. Secondary shoots of 99R were removed as part of normal farm practice. The material was collected in June and grafted in August to Chenin blanc. Three replicates of 10 graft canes per replicate were used.
8. **Time of collecting graft material**  
Graft material of 99R, 101-14 Mgt, Ramsey, 110R, US 8-7, 140Ru, 1103 Paulsen, 143B and US 2-1 at Grondves, Stellenbosch, was used. The material was collected in April, May, June and July and analysed for minerals and starch. After storage, minerals and starch were again analysed, after which the material was grafted, callused and planted in the nursery. Stock performance in terms of class, and shoot and root growth was determined after the nursery phase.
9. **Graft cane thickness**

Experiments were done at two commercial nurseries. Chenin blanc was grafted onto two rootstock cultivars, 99R and 101-14 Mgt. The grafted canes were divided into two groups, 6.5 – 7 mm and 7 – 12 mm, on the basis of internodium thickness. Stocks of 12 mm were marked to determine their performance against the others. Twenty stocks, comprising different compositions (5 % - 95 %; 10 % - 90 %; 20 % - 80 %; 30 % - 70 %) of the two groups (6.5 – 7 mm and 7 – 12 mm) were randomly replicated for each cultivar.

**10. Graft cane length**

Graft scions of Cabernet Sauvignon and graft canes of 99R (RY 13A) were used. Graft scions were grafted to a short (apical 5 cm) and a normal full length graft cane. After grafting, the combinations were analysed for starch and organic acid content at weeks 0, 1, 2, 3 and 5 during the callus period. The full length graft cane was divided into five 5 cm pieces from apical to basal; these pieces were individually analysed.

**11. Graft scion position**

Graft graft scions from basal and apical parts of canes and from different cultivars (Chenin blanc, Sauvignon blanc, Cabernet Sauvignon, Merlot) onto 110R. The material was sourced from Grondves, Stellenbosch, and the stocks planted at Stargrow Nursery, Citrusdal. Three replications comprising 30 stocks per replicate were used.

**12. Desiccation and water submersion of graft material**

Graft canes of 101-14 Mgt and 99R were used in 1999/2000 and graft shoots of 140Ru and 110R in 2000/01. The following protocol was used:

*Long graft canes (not cut) – simulate desiccation after collection (lie in mother block)*

Material was desiccated for 0 hours (control), 3 hours, 6 hours, 24 hours, 48 hours and 72 hours at 35 – 40 °C. It was then cut, placed in Kaptan solution (disinfectant) (30 min.) and stored. After storage, the material was placed in water overnight, the water content determined and analysed for starch. It was then grafted to Chenin blanc, callused, callus formation evaluated and planted in the nursery. Another quantity of material was, instead of placing it directly after cut into Kaptan, placed in water for 12 hours, after which it was disinfected and stored.

*Short graft canes (cut) – simulate leaching of stocks before storage*

Material was placed into water for 0 hours (control), 3 hours, 6 hours, 24 hours, 48 hours and 72 hours. It was then stored, placed in water overnight, the water and starch contents determined, grafted to Chenin blanc, callused, callus formation evaluated and planted in the nursery.

**13. Clogging of xylem vessels, callus ability and composition of xylem sap**

Forty-two different rootstock/clone/sub clone/block combinations were used. The scion cultivar was Cabernet Sauvignon, clone CS 16. Extraction of xylem sap (to determine whether the xylem was blocked or not) was done as follows: Twenty graft canes per clone-block combination was used (orientation of graft canes was kept). From every graft cane a 7 cm internodium and a 7 cm nodium part was cut and the dorsal-ventral diameter, lateral diameter and the circumference of the internodium and nodium, respectively, were measured. Three to four cm of the bark (phloem) of the basal part of every cane part was removed. Five cm<sup>3</sup> water was sucked through the xylem at constant suction and the xylem sap-water mixture kept for later analyses. The time for sucking the 5 cm<sup>3</sup> water through the xylem was noted.

The callusing of graft combinations, rootstock clones/sub clones and graft scions was done as follows: Twenty graft canes per rootstock clone/sub clone were grafted. After absorption of water and disinfection, omega cuts were made by machine (also to rootstocks and graft scions separately). Stocks were then dried, waxed, packed and callused at 26 °C to 28 °C for 3 weeks. The callus tissue formed was then evaluated and scored out of 4. To determine the distribution of nutrients (and reserves) and positions in the graft combination where the biggest clogging occurred, U-<sup>14</sup>C-Sucrose was used. The same rootstock clone, originating from different blocks and from which the xylem clogging and callus ability differ, was used in the investigation. This was done as follows: Five replications of the stocks were used directly after callusing. U-<sup>14</sup>C-Sucrose (3.75 µCi) was applied to the basal part of the rootstock. Fixation is allowed for 24h. Stocks were then removed, rinsed with water and placed in a glasshouse. The translocation period was 4 days. The stocks were then divided into 6 parts, namely the graft scion, graft union, apical internodium, apical nodium, basal internodium and basal nodium. The parts were dried, ground and the radio activity quantitatively determined. Intact stocks (5 replicates of each) were also dried and kept for the development of auto radiograms in order to determine the qualitative distribution of the radio activity through the stock. The xylem sap as obtained during the sucking of the xylem of the different rootstock/clone/sub clone/block combinations was frozen at -20 °C, freeze dried and analysed for sugar and organic acid content. The xylem sap was also evaluated for the presence of fungi and bacteria. Sugar and organic acid analyses were done on the same rootstock clone from different blocks and from which the xylem clogging differ. The frozen xylem sap was dissolved in 1 cm<sup>3</sup> 50 % Acetonitrile and the sugars and organic acids separated by means of anion resin. Both fractions were diluted and analysed by means of high pressure liquid chromatography. Fungus and bacteria counts were done on the xylem sap of the same rootstock, originating from different blocks and from which the xylem clogging and callus ability differ.

- 14. Relationship between starch, mineral composition of graft material, mineral composition of soil and callus formation**

Graft scions of Chenin blanc, clone 1064B, were grafted to 12 different rootstock clones from 15 mother blocks. The graft scions and rootstocks were analysed for starch and mineral content at week 0, and after week 1, 2, 3 and 5 of the callus period. Callus formation was evaluated after week 1, 2, 3 and 5.
- 15. Hot water treatment of scion cultivars**

Nine cultivars, Chenin blanc (clone SN220C), Malbec (clone MC279), Red Muscadel (clone RM111E), Ruby Cabernet (clone RC1A), Sémillon (clone GD315A), Tinta Rorez (clone TR1A), Tinta Francisca (clone TF2B), Turiga Nacional (clone TN1-9K), and Viognier (clone VR1-5K) at Grondves, Stellenbosch, were used. These vines (3 replicates of 5 vines each) were two thirds defoliated in December. The objective was to decrease the starch content of the shoots. It was compared to control vines (without defoliation). Canes were sampled in July and stored. Before grafting, starch and water contents were determined and the material subjected to hot water treatment (50 °C for 30 min.). After this, starch and mineral contents were again determined. Graft scions were grafted to the same rootstock and evaluated for callus development. The stocks were planted in a nursery and stock performance determined at the end of the nursery phase.
- 16. Plastic covering and irrigation in nursery**

Material was obtained from Grondves, Stellenbosch. Warm and cold callused stocks from Sauvignon blanc and Cabernet Sauvignon grafted onto 101-14 Mgt and 110R, were used. The stocks were planted under plastic (brown, silver and black) and without plastic soil covering at Kys Nursery, Vredendal, on a sandy soil. Three water levels were also applied by means of a drip system (normal, normal minus one third of normal and normal plus one third of normal). The normal drip irrigation (2.2 L drippers spaced 30 cm on ridges that were 80 cm apart) was scheduled as follows: For one month after planting, 30 min./day (1.05 L/day = 4.4 mm/day); for two weeks after that, 10 min./day (=1.46 mm/day); for three months after that, 20 min./day (= 2.92 mm/day); for one month after that, 30 min./day and after that for one month 30 min./every second day. Additionally, during warm periods of the day (normally from 11:00 for 10 min.) overhead irrigation was applied to all treatments. Three replications comprising 30 stocks each were used for every treatment combination.
- 17. Starch and mineral reference source**

Graft canes of rootstocks 101-14 Mgt, 99R, 110R, Ramsey, US 8-7, SO4, 3306 Couderc, 1103 Paulsen, 140Ru, 143B and scions Chardonnay, Sauvignon blanc, Pinotage, Shiraz, Merlot, Pinot noir, Sémillon, Ruby Cabernet, Colombar and Cabernet Sauvignon were harvested in February and July at Grondves, Stellenbosch. One cane per vine was sampled from 6 vines per rootstock and represented 3 replications per rootstock.

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