- Attacks by pathogenic fungi or bacteria. These attacks may occur in spite of all precautions taken, and the infection site should be removed and the tray drenched with a fungicide.
- Attacks by fungus gnats (to the prothalli roots). Once the maggots of these gnats are in a tray of prothalli they are very difficult to control. It is best to ensure prevention by effective sterilisation and sealing.
- Algae and mosses may smother the newly grown ferns. This may be an indication of incomplete sterilisation of the media before sowing or later contamination.
- If spores are sown too thickly the prothalli will become crowded, misshapen, and weakened. They are therefore more susceptible to damage and the entry of disease.

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Propagation of Apple Rootstocks by Tissue Culture

Belinda S. Hazell
Forest Home Nursery – Hazell Bros Agricultural Division, 799 North Huon Road, JUDBURY TAS 7109

Tasmania has historically been recognised as a prime apple growing region of the world. Traditionally, commercial apple orchards have grown fruit on large seedling trees. In recent years, the trends have been for trees grown on dwarfing rootstocks to cater for new planting techniques and return higher yields per hectare. There is considerable demand in the marketplace for the supply of elite dwarfing rootstocks which are difficult to conventionally propagate quickly in large numbers.

INTRODUCTION

Forest Home Nursery's business operations began in 1985. Since its inception the nursery has delivered quality apple and stonefruit trees annually, primarily to Tasmania and several mainland states.

The nursery has conventional field stoolbeds of several *Malus* cultivar rootstocks. The quantity and quality of these stocks over recent years has varied. A decision was made in 1991 to establish a tissue culture facility. The purpose of Forest Home Laboratory is to meet the first class rootstock requirements of cultivars to meet market needs, i.e., disease free, robust, and high production levels. A fully qualified biotechnologist was employed to establish the laboratory and get production under way. Training of a second staff member commenced immediately and in 1992 two more staff were employed. The laboratory now has three skilled staff trained in basic micropropagation techniques and an additional two people managing the hardening-off stage. The operation is housed in a basic structure modified to a clean laboratory environment.

Forest Home Laboratory produces quality *Malus* rootstocks using tissue culture techniques, primarily on dwarfing *M*. Malling 26 (M26) stock. Research is currently being undertaken in the commercial development of the cultivar Malling 9 (M9) and a new introduced dwarfing stock, Budagovsky 9.

During the past 15 years major advances have been made in micropropagation. Conventional rootstock beds take approximately 5 years to reach commercial production yields. The nursery management decided to adopt tissue culture because of the potential to achieve high yields in a shorter timeframe. Additionally, because the cultivar explants are multiplied vegetatively whether by traditional cutting practices or by micropropagation, high quality and clean material can be selected. Using tissue culture all explants from a single parent plant are clones, because their genetic make-up is identical to that of the parent. This allows the nursery to select superior cultivar material for mass clonal production.

MATERIALS AND METHODS

At Forest Home Laboratory, apical and axillary buds are harvested from clean *Malus* mother stock grown in an optimal environment to promote growth. This material is disinfested in the laboratory prior to placement in vitro (in glass). This method is superior to past trials which used dormant buds from a normal orchard environment. These buds were put through a harsh cleaning process and large losses were incurred. Those that did survive were difficult to stimulate into shoot proliferation.

More importantly the microcutting method allows the laboratory to initiate stocks from a guaranteed clean source of material. The final product, a 2-year-old apple tree, is a high-quality, sought-after product. The current method of harvesting explants allows the laboratory to keep *Malus* stocks dormant under refrigeration and introduce new material into the system when required.

Since the laboratory's inception, many hours have been dedicated to finding the best possible mix of ingredients for the culture medium. The laboratory currently has four basic mixes in use. Two formulations are used for shoot proliferation and two for root initiation. Each medium has its own individual identification code and each batch made has an identification number to ensure traceability. This identification is labelled on each container made.

In the initial stages of research in-vitro explants were placed in polycarbonate test tubes with 10 ml of medium. Once rapid shoot proliferation has occurred, microcuttings are taken and transferred under sterile conditions. It was quickly realised that in vitro the *Malus* stocks were prone to vitrification.

Vitrification is mainly evident in the leaves produced in vitro. Traditionally vitrification has been defined by visual symptoms such as a glassy, water-soaked appearance and irregular growth. Plantlets with these characteristics cannot survive stress after transplanting, they require a very gradual transition period to regain normal morphology and ultimately survival (Yang, 1999). The laboratory established that the vitrified shoots transferred through without change which resulted in greater losses. Several experiments were undertaken to reduce the vitrification phenomenon including using liquid media, Vita film™, membrane rafts, and imported gamma-irradiated trays. As is sometimes the case in research, by accident it was discovered that simply loosening the lids of the culture vessels enabled sufficient gas exchange to prevent vitrification.

Excellent sterile technique and selection protocols (e.g., removal of nonviable explants such as vitrified material and old growth) ensure that contamination rates are negligible and success rates high.

The labour costs of micropropagation are extremely high. Continual review of methods occurs with a view to increasing efficiency. An example of this is the conversion from polycarbonate tubes to 250-ml polycarbonate jars. A more recent introduction into the process have been polycarbonate "takeaway food" type containers, which have improved both efficiency in labour and use of space. It is anticipated that a large financial saving will be made because of this innovation in the cost of the container alone.

All polycarbonate products are sterilised and reused wherever possible. Explants are placed in jars or tubs in vitro for a minimum of 4 weeks to enable rapid shoot proliferation. A set protocol for transfer and multiplication is followed. After 4 weeks larger shoots (plantlets) are selected for rooting and placed onto media designed to initiate root development, whilst the smaller plantlets are placed back onto shooting media for further transfers.

Simmonds (1983) studied how the length of time spent on rooting media affects root production and ultimately plant establishment. The results showed that although only 34% of M26 shoots had rooted after 3 weeks on rooting media, compared to 69% rooting after 6 weeks, the post-culture establishment and growth of plantlets in the 3-week group was significantly greater than that of the 6-week group. Forest Home Laboratory explants are on rooting media for at least 3 weeks before they are removed to the humidifier to commence hardening off.

The hardening-off process has proved to be the most challenging. Over 50% losses have occurred due to unfavourable environmental conditions. This results in increased susceptibility to disease infection or desiccation of plantlets, which causes irreversible tissue damage and death. During 1997 Forest Home Laboratory approached the University of Tasmania to conduct research into the hardening-off stage. The Laboratory supplied the infrastructure and the University of Tasmania the research skills. In 1998, as partial fulfilment of the requirements for the degree of Graduate Diploma in Agricultural Science (Honours), Simon Yang completed his research towards the thesis titled *Growth and Survival of Tissue Cultured Apple Plantlets after Transplanting*.

A review of relevant literature found that exposing plantlets to an environment of reduced relative humidity without disturbing or injuring the delicate root system increased wax development in the cuticle and therefore survival rate (Fuchima et al., 1981; Ziv, 1986). Changes to the environment in vitro may produce better quality plantlets. Environmental factors which can be manipulated include: light (intensity and day-length), temperature, relative humidity, and media (mainly rooting media). It is possible to harden off plantlets while they are still in vitro or at least gradually adapt plantlets to the outside environment after transplanting.

Yang (1999) found that minimising water loss via transpiration is crucial to the survival of plantlets. The first 1 to 5 days spent in the humidifier at Forest Home are essential, firmly establishing that a consistent environment in the earliest stages of the hardening off process is vital. This, together with a proactive program to prevent infestation by fungal or insect pests, ensures a viable survival rate is achieved.

In October 1998 the laboratory won the Inaugural Tasmanian Farm Business Development Award. This funded the purchase of a cement mixer to improve the incorporation of nutrients through our potting medium and supported a study tour to a major tissue culture facility in New South Wales.

All of these factors combined resulted in the modification of the hardening-off process, converting a greater than 50% loss rate to a 90% survival rate.

CONCLUSION

The main advantages to producing apple rootstocks by tissue culture are; high yields, ability to source superior explants, and effective timeframes for production. This has to be balanced against the cost of research and development.

Small private enterprises will find the going tough unless they have adequate start-up capital. Obviously corporate and government-run facilities will have a better chance of success, as they can set up the infrastructure to allow production en masse from the outset. The challenges faced by Forest Home Laboratory have been great, however from a modest beginning the rewards are now being realised and expansion is planned. Those private enterprises that also have the courage and fortitude to persevere will see the rewards of their endeavours.

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