# Comparison of Different Brands of Petri Dishes for Use in Tissue Culture©

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**Two petri dish brands, Labserve™ (Biolab Scientific, New Zealand) and Greiner™ (Greiner Labortechnik, Germany) were compared for their effect on fresh weight growth of radiata pine embryogenic tissue. The Greiner™ brand gave less variation in tissue growth between dish replications than the current Forest Research standard brand, Labserve™. However, total fresh weight for two of the four surviving cell-lines was reduced in these dishes. It is recommended that more extensive testing of Greiner™ dishes take place before they are adopted as the new laboratory standard brand.**

## INTRODUCTION

Forest Research in Rotorua has been developing and refining plant-tissue-culture methods for *Pinus radiata* organogenesis and embryogenesis over a number of years (Reilly and Washer, 1977; Horgan, 1987; Smith, 1986; Smith et al., 1994). These tissue-culture methods have offered significant advantages over traditional nursery propagation approaches, allowing faster and greater amplification of superior material (Menzies et al., 1985). Tissue culture has also facilitated the maintenance of juvenile vigour by enabling tissue to be held in conditions of suppressed growth, such as in cool storage (Horgan, 1987), or no growth as in liquid nitrogen storage (Hargreaves and Smith, 1994a, 1994b). These protocols have shown sufficient promise to be commercialised by Fletcher Challenge Forests and Carter Holt Harvey Forests. Over the last decade at Forest Research, the emphasis has shifted from development of amplification and storage protocols to the application of tissue culture to tree breeding and biotechnology in areas such as gene mapping and genetic engineering (Walter et al., 1998).

When embryogenesis protocols were being refined, factors, which significantly added to the cost of the process, were examined more closely. Although labour was the most significant cost factor, the cost of petri dishes also had a significant impact due to the need to frequently (every 10 to 14 days) transfer embryogenic tissue to fresh medium. From the mid 1980s until the late 1990s, Falcon™ (Becton Dickinson Labware, New Jersey, USA) petri dishes (90 mm × 25 mm) were used exclusively at Forest Research and they were very expensive relative to other brands available. However, earlier comparisons with other brands had shown Falcon™ to be superior for tissue growth (Hargreaves, unpublished data). In more recent years, Labserve™ (Biolab Scientific, New Zealand) has produced a dish that proved to be comparable to Falcon™ (Grace, unpublished data). Currently, Forest Research uses Labserve™ petri dishes (90 mm × 25mm; costing half the price of the Falcon™) for all stages of conifer embryogenic tissue culture from initiation and maintenance through to embryo maturation.

Several factors are known to contribute to these differences observed in tissue growth in various brands of disposable petri dishes on the market; one being the type of plastic used for dish manufacturing. Some producers cannot guarantee the proportions of various plastic additives and impurities in their dishes, though they can often give an expected range. Toxic effects on growth observed in vitro have sometimes been traceable to a given dish batch number (Nairn, personnel communication; Hargreaves, unpublished data). Other anomalous growth has occurred in a more random manner; for example, inexplicable tissue response in a single replication in a given trial. Another known factor is ethylene build-up within culture dishes, especially where tissue is growing rapidly. Ethylene is known to inhibit embryogenic tissue growth and somatic embryo maturation (Warr et al., 1995; Grace, unpublished data). It should be noted that it is embryogenic tissue that is especially sensitive to dish effects, whereas larger pieces of organised tissue (cotyledons, adventitious, and axillary shoot material) and some of the later stages of somatic embryo development have been found to be insensitive to dish type. Most petri dish manufacturers use moulds that produce petri dish lids with three to four small wedges of plastic on the underside of the lid. These ensure that the lid does not sit flush with the dish base and provide opportunity for gas exchange with the ambient air conditions, even through the plastic film used to seal the dishes during use (this film keeps contaminants out of the cultures and slows water loss from media and tissue). The amount of ventilation may vary with dish brand, and inadequate ventilation may inhibit the release of volatile substances produced either before or during tissue growth. These volatile substances have been implicated in “toxic dish effects” (Hargreaves, unpublished data).

Recently, a new petri dish brand (Greiner™) (Greiner Labortechnik, Germany) became available, which is slightly cheaper than the current Labserve™ product, and a trial was established to determine whether they would compare favourably with Labserve™ dishes.

## MATERIALS AND METHODS

Four embryogenic cell lines were chosen, having a range of known growth rates from slow to vigorous. These had all been maintained on embryo development medium (EDM6) (Smith, 1996) for a minimum of 10 culture cycles. Tissue is usually transferred at 2-week intervals. Tissue was suspended in embryogenesis medium (EM) medium plus 0.4 M sorbitol (Hargreaves and Smith, 1994a), 1g of fresh tissue to 4 ml of liquid. Suspended tissue (0.5 ml per dish giving a tissue weight of 0.125 g per dish) was dispensed into the two dish types containing the same batch of EDM6 medium (25 ml per dish). Several replications (2 to 7) were made of each cell line per dish type. Tissue was aired, by removal and replacement of the lid in sterile conditions after 3 weeks to vent off any ethylene build-up. Tissue was incubated at 24±1°C under low light conditions 5 $mmolsom^{-2} \cdot s^{-1}$. A destructive assessment of fresh tissue weight was made at 7 weeks.

Comments were made on individual replications and contamination, though this data is not presented here. Data was analysed by ANOVA.

## RESULTS AND DISCUSSION

The fresh weights of embryogenic tissue in the two dish types after 7 weeks growth is given in Table 1.

**Table 1.** Fresh weights (g) of embryogenic tissue after 7 weeks growth.

| Cell line | Dish type Greiner™ | Labserve™ | Mean for cell line |
|---|---|---|---|
| 98-38 | 0.399 | 0.232 | 0.315 |
| Int 99-76 | 9.498 | 10.290 | 9.924 |
| E825-40 | 1.945 | 1.892 | 1.919 |
| Int 99-145 | 3.337 | 3.423 | 3.380 |
| Mean for dish type | 4.652a* | 5.229a | |

*Means with the same alphabetical letters are not significantly different (P = 0.05).

The average fresh weight of tissue was greater in Labserve™ dishes for two of the four cell lines although the differences were not significant (P = 0.05) (Table 1). However, in general, the largest differences in fresh weight of tissue were between the different genotypes rather than the dish types (Table 1). This was expected, as genotype is often the overriding factor in all experiments with embryogenic tissue (Smith et al., 1994; Hargreaves, unpublished data). Cell-line Int 99-76 had the highest fresh weights recorded overall and 98-38 was the slowest growing of the 4 lines. Int 99-76 had a bigger range in fresh weight (0.900 to 16.857 g) between replications on Greiner™ than on Labserve™ dishes (6.616 to 14.101 g). However, the other 3 cell-lines had greater ranges on the Labserve™ treatments. If variability of replications is an indication of product inconsistency, then Greiner™ would be the better product for 3 of the 4 lines (Table 1). It may be that the more vigorous cell lines are more sensitive to the dish internal environment and batch inconsistencies and the slower growing lines are not so affected. Earlier work testing different brands of cryopreservation vials on embryogenic tissue storage in liquid nitrogen had shown the opposite effect, with the different brands giving significant differences in re-growth if the lines concerned were less vigorous (Hargreaves et al., 1995). Int 99-145 had one Greiner™ replication that failed to grow, as did 98-38, which also had a Labserve™ replication that did not grow. In total, 3 of the 43 dishes used in this experiment were contaminated (7%), two were Labserve™ treatments and one Greiner™.

## CONCLUSIONS

Greiner™ dishes were not toxic to the growth of radiata pine embryogenic tissue and gave less variation in growth compared with the Labserve™ brand. However, total fresh weight for 2 of the 4 surviving cell-lines was reduced on Greiner™ dishes although this was not significant. More extensive testing of the two dish types is needed to establish whether or not the Greiner™ dishes can be adopted as the standard petri dish, perhaps including the more expensive Falcon™ brand as a control, using a minimum of 10 genotypes with 6 replications per treatment.

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