In Vitro Factors Affecting Plant-Out Performance of Micropropagated Plants

Frank Benson
Benson Micropropagation Pty Ltd, 175 Wadeville St, Pallara, Queensland, 4109

INTRODUCTION

There is a direct relationship between plant-out success and the quality of the product coming out of the micropropagation laboratory. As with other forms of plant propagation (cuttings, grafts and seeds), success rate is dependent on the health and pre-treatment of the stock plants.

With any new technology, it takes a while to identify the factors affecting quality and then how to go about manipulating these variables to maximize quality. In this paper I will present what I consider some of these variables, and my personal observations over the eight years I have been in commercial plant micropropagation.

My observations will be confined to three topic areas: 1) growth rate, 2) light intensity, and 3) relative humidity.

GROWTH RATE

Plant growth *in vitro* requires a carbon source (sugar) in the medium. When plants are transferred to a medium without an added carbon source they do not grow and are usually dead within a few weeks, indicating they have a negative carbon balance. Grout and Millan (1985) found that with strawberry plantlets "carbon fixation by leaves produced in vitro is low, insufficient to sustain growth autotrophically, and does not increase significantly during the acclimitization period following transplanting. Leaves that developed after transplanting fix relatively high levels of carbon 7 days after emergence, allowing continued whole plant growth, and show a significant increase in fixation subsequently" This shows that *in vitro* leaves serve as an energy store until the plants had new leaves capable of maintaining the carbon balance. One of their conclusions for improved transplanting was to maximize the rate of new leaf production following plant-out.

Similar conclusion can be made for roots. Even if the plants have produced functional roots in culture, all the root hairs will be removed with the agar. So if we have actively growing root tip meristems prior to plant-out, within a short period of time the plants will have functional roots.

My application of these conclusions has led to the production of plants such as the *Grevillea* in Figure 1, left. This plant, 10 days after plant-out, will have 1 to 3 new leaves and 10 mm of new root growth with root hairs, as in Figure 1 right.

Time on Delivery Media. There is a finite quantity of nutrition in micropropagation media. The effect of this on plant growth is best summarized by Figure 2. This shows an initial acclimitization phase, then rapid growth until nutritional deficiency starts, then a gradual reduction in growth rate until, finally, zero growth. What we need to do is identify the time to the peak in the curve, and plant-out then.

Figure 1. The ideal *Grevillea* 'Robyn Gordon' to plant-out (left), and 10 days later.

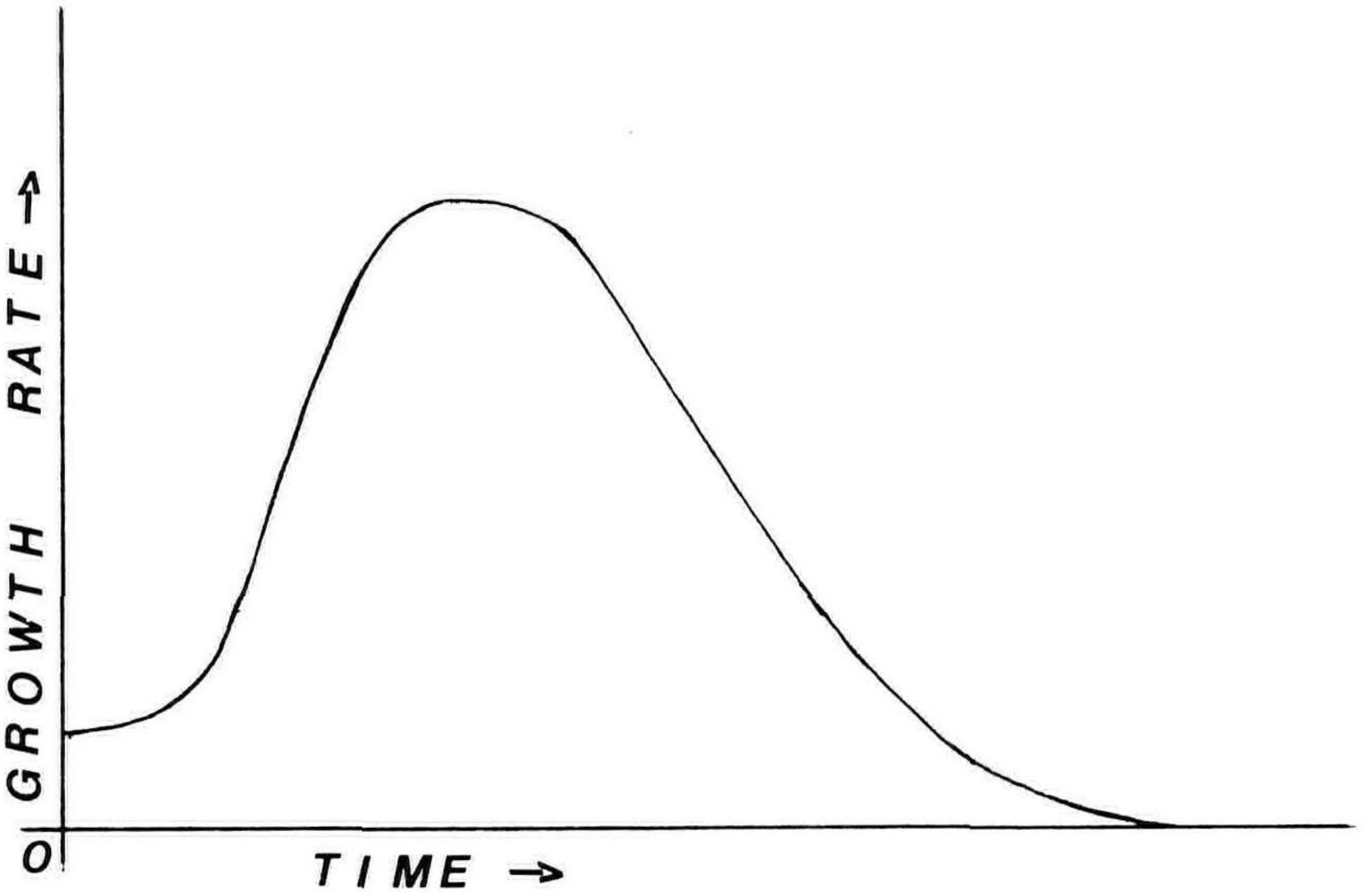

Figure 2. Growth rate verses time, for plants grown in finite nutrition.

I did an experiment with *Syngonium podophyllum* 'White Butterfly' to examine the effect of length of time spent on the delivery medium on plant-out performance.

The experiment consisted of 96 plants at each of three ages on delivery media (3, 5 and 7 weeks). They were planted into a peat and fine polystyrene mixture, (1:1, v/v) placed on a 25° C bench, with mist for the first 5 days.

Figure 3 shows the plants just before plant-out. The following were noted in this research:

1) Plants on the medium at 3 weeks have new leaves unfolding.

2) There is a considerable difference in size and root length between the 3 and 5-week plants while plants grown for 5 and 7 weeks are similar in size.

3) All the 7-week plants have 1 or 2 of the lower leaves yellow and dead, indicating that they have run out of nutrients and have a negative carbon balance.

Four weeks after plant-out we went through and counted the losses, from the 96 of each age - none for 3 weeks, eight at 5 weeks and fifteen at 7 weeks. Another significant observation was the number of "runts" where the plants were smaller and the new leaves were not much larger than the ones before. The number of "runts" were two, eleven and twenty respectively. The "runts" were obviously not in good physiological health, and just managed to make it through the shock of plant-out.

Figure 3. Appearance of *Syngonium podophyllum* 'White Butterfly' after varying time on delivery media. Left to right: 3, 5 and 7 weeks.

From these results it appears that the best time to plant-out *Syngonium podophyllum* 'White Butterfly' under our laboratory conditions, would be at 3 weeks.

Contamination. Nutritious sugary micropropagation medium, as well as being good for growing plants will, also grow most microorganisms. These compete with the plants for nutrients in much the same way as weeds compete with plants in pots (except that they can not be manually removed).

The effects of a contaminant microorganism varies with the organism, severity of the infestation, and whether or not it is producing a toxin. As a basic generalization, if the contaminated *in vitro* plants are smaller than non-contaminated

ones, incubated for the same period of time, the contaminating organism has reduced the growth rate.

Age of Motherstock. In the same way as poorly watered and fertilized plants make poor sources of cutting material, cultures left too long between subcultures produce inferior plant-out material.

I have noticed that cultures left unworked for several months usually require 2 to 3 subcultures before they perform as well as those that are regularly worked. This I have put down to the loss of juvenility.

LIGHT INTENSITY

Although photosynthesis is not an important source of energy for the plants, light levels do have a marked affect on their morphology *in vitro* Haramaki (1971), using gloxinias, found that increasing light levels up to 3,200 lux produced progressively larger plants with greater leaf size, while at 10,700 lux there was little growth, and leaves were smaller and discolored.

I have noticed similar responses in my laboratory. Figure 4 illustrates the effect of light levels on *Gerbera jamesonii in vitro* and, as you can see, my results agree with those of Haramaki. When these plants were planted out as per the *Syngonium podophyllum* 'White Butterfly' above, after 4 weeks none out of the 32 plants incubated under 1500 lux had died, while 18 of 32 at 300 lux were dead.

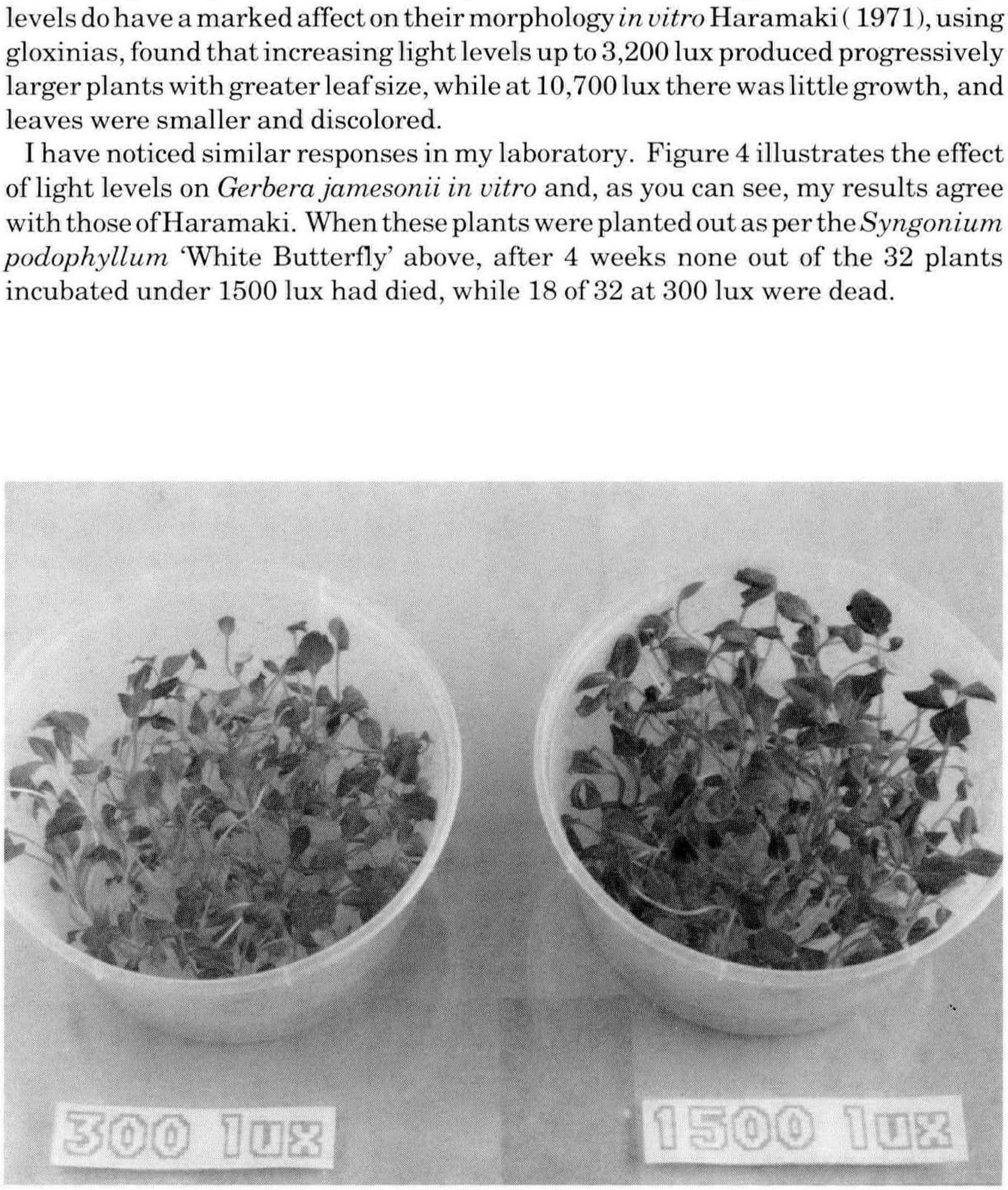

Figure 4. Appearance of *Gerbera jamesonii* cultures after incubation under varying light levels. Left to right: 300 and 1,500 lux.

I undertook the same experiment using *Spathiphyllum* 'Petite'. The best plants were produced at 300 lux, while those under 1,500 lux were smaller, with smaller, paler leaves, and less root development.

From these results it appears that optimum light levels for each crop need to be experimentally determined.

RELATIVE HUMIDITY

In the culture vessel there is very high relative humidity because it is a capped vessel with water in the bottom. This results in a number of deleterious physiological effects:

(1) Condensation droplets form on the container walls and top. When a leaf or shoot tip touches the container, it traps this condensation, which can lead (especially in woody species) to a drowning of this organ.

(2) This high relative humidity can produce vitrified (glassy) plants, where the stems and leaves are translucent, thickened and brittle. These plants are impossible to plant-out successfully.

(3) Woody plants have a major problem with shoot tip die-back (necrosis). Barghchi (1987) attributed this to the high relative humidity reducing transpiration by the leaves, which resulted in reduced water uptake and reducted uptake and translocation of certain minerals that are dependent on the transpiration stream for transport to the shoot tip.

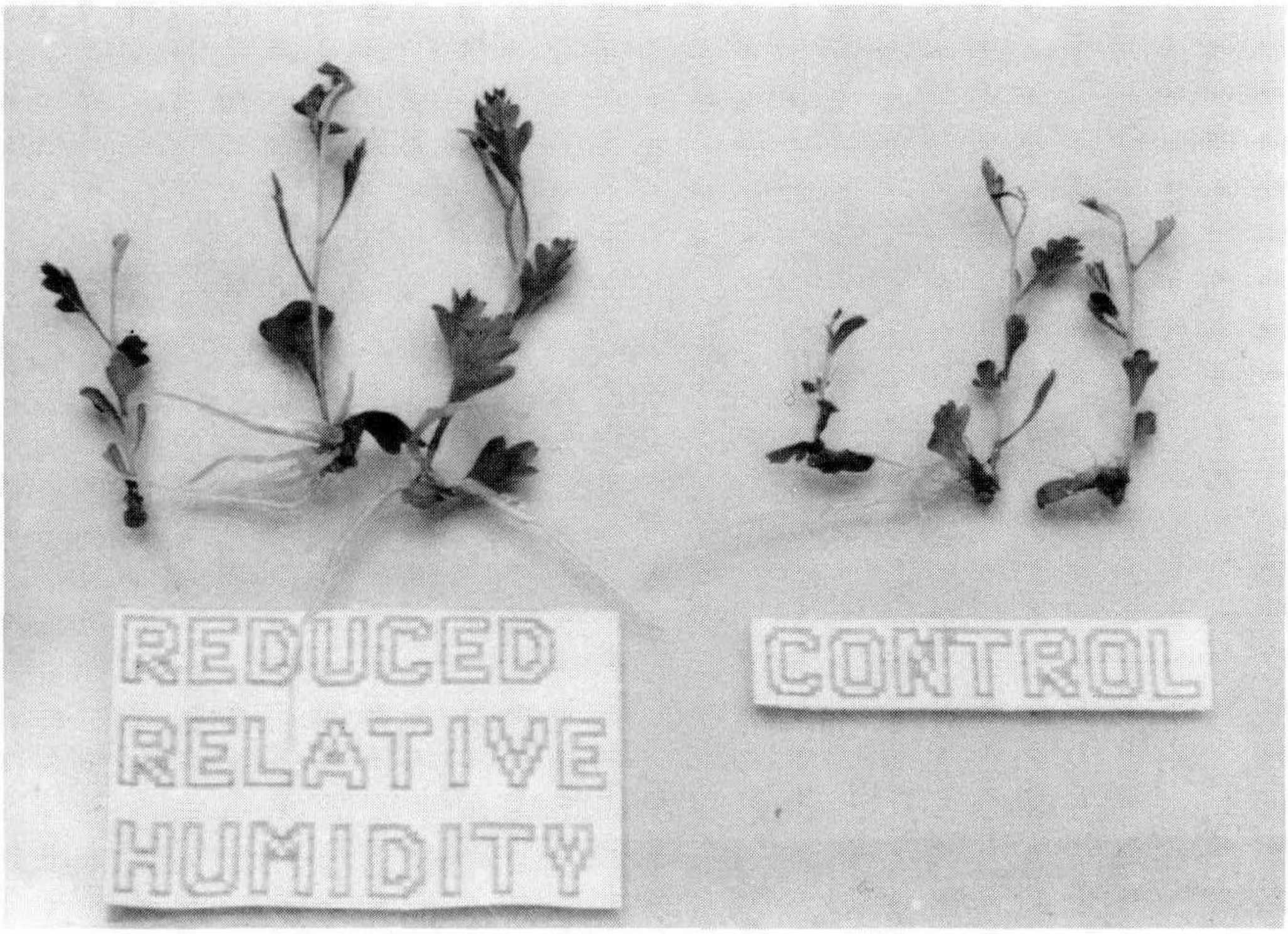

Figure 5. Appearance of *Grevillea* 'Robyn Gordon' after incubation under reduced relative humidity, compared with control.

A technique for reducing relative humidity, by placing the culture vessel on a surface 3 to 5°C cooler than the incubator temperature was tested (P. Debert, Personal Communication). The water vapor condensed on the bottom of the container, with a gradient of decreasing relative humidity up the container. Using this technique we have been able to almost completely remove any of the above problems. Figure 5 illustrates the effect of reduced relative humidity on *Grevillea* 'Robyn Gordon'. The plants and their individual leaves are larger, and there is no tip necrosis.

CONCLUSION

A good simile for the transition stage between *in vitro* and *in vivo* is a level road with a fully laden truck building up speed to get up a hill. This is our plant ready for the planting out. If it reaches the base of the hill just as it gets full momentum up it will get up and over without loosing too much speed. If it reaches the hill after some slowing down, for whatever reason, it will lose this momentum, have to drop to a lower gear or gears and will take a long time to get back to speed — if it ever does.

LITERATURE CITED

Barghchi, M. 1987. Shot tip necrosis in micropropagation of woody plants and its control-*Pistacia vera* L. Non-published paper presented to the 3rd Australian IAPTC Conference. His address is Plant Physiology Division, DSIR, Private Bag, Palmerston North, New Zealand.

Grout, B.W.W. and **S. Millam.** 1985. Photosynthetic development of micropropagated strawberry plantlets following transplanting. Ann. Bot. 55:129-131.

Haramaki, C. 1971. Tissue culture of *Gloxinia*. Comb. Proc. Intl. Plant Prop. Soc. 21:442-448.