Effect of Container Size and Media Volume on the Growth of Plantlets *in vitro*

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***Ficus lyrata* and a clone of an unnamed *Zantedeschia* were grown in four different sized plastic containers filled with 15, 30 or 60 ml of MS medium. Plant fresh weight accumulation was assessed at 2-week intervals for 14 weeks and the experiment terminated with dry weight determination. Maximum fresh weight of both species developed in the smallest containers with the largest volume of medium.**

INTRODUCTION

Many trials investigating plant growth *in vitro* have focused on the composition of the supporting medium These experiments have given us the basic media formulations, such as the Murashige and Skoog medium (MS) used in many tissue culture laboratories today (Murashige and Skoog, 1962).

Relatively few people have considered how the volume of the medium influences the potential of a culture system to grow and sustain plants. Fadia and Mehta (1975) demonstrated a direct relationship between the volume of the medium and plant growth.

Traditionally, glass culture vessels have been used, but these have been largely superseded by a wide range of disposable plastic containers. As with media volume, few people have considered that the culture vessel characteristics may influence the performance of an *in vitro* plant production system. Adams (1972) noted that strawberry cultures rooted only after transferring to a larger culture vessel. Monette (1983) showed that early growth (up to four weeks) of grape cultures was fastest in small containers, but after six weeks most shoot growth occurred in the largest containers.

A trial was planned to investigate the effect of media volume and container size on the *in vitro* growth of *Ficus lyrata* and a hybrid *Zantedeschia*.

Ficus and *Zantedeschia* were chosen because of their relatively fast growth rates coupled with their differences in culture appearance and shoot proliferation rates. *Zantedeschia* cultures tend to be more apically dominant and produce fewer shoots than *Ficus* cultures.

MATERIALS AND METHODS

Plants were grown on MS medium supplemented with (in mg l^{-1}) glycine, 2; myo-inositol, 100; thiamine-HCl, 0.1; nicotinic acid, 0.l; pyridoxine-HCl, 0.l; benzyl adenine, 3; plus sucrose, 30 g l^{-1} and agar, 8 g l^{-1} .

Four kinds of container were used (Table 1).

Table 1. Characteristics of the four types of experimental container

Container[1]	Height (mm)	Mean diameter (mm)	Volume (cm^3)	Surface area (SA) (cm^2)	SA/ volume (cm^{-1})
A	12.8	83 8	66 6	122 4	1 84
B	29 6	86 6	169 5	196 8	1 17
C	51 0	88.6	289.2	262 0	0 90
D	128 8	115 4	93 5	1076 0	0 55

[1]Container "A" was a disposable (polystyrene) plastic petri dish and the other containers were clear (K-resin) plastic food containers each with a close fitting lid.

For each species ten replicates of the four plastic containers were filled with 15, 30, or 60 ml sterile medium, (except for the 60 ml treatment and container "A" which was not considered practicable.)

Each dish was inoculated with either 10 pieces of *Zantedeschia* (average weight 120 mg) or 8 shoots of *Ficus* (average weight 30 mg) and incubated at 20°C under fluorescent lights (20 µmol m^{-2} s^{-1}) with a 16 h photoperiod.

The plant material in each replicate was weighed aseptically every 2 weeks over a 14-week period. At the same time the pH of the medium was also monitored.

RESULTS AND DISCUSSION

In all four containers the volume of medium had a direct influence on the rate of accumulation and total production of fresh weight by *Ficus* (Figure 1) and *Zantedeschia* (Figure 2). The initial growth rates in each treatment were similar until after the second week. By the fifth week it was apparent that some factor was limiting *Zantedeschia* growth. This coincided with a dramatic reduction in fresh weight accumulation and the first visual signs of culture deterioration. *Ficus* took approximately eight weeks to reach a similar fresh weight maximum. Significant deterioration of the cultures did not occur during the experiment. This suggested that *Ficus* is considerably more tolerant of nutrient depletion than *Zantedeschia.* The reduction in fresh weight of both species later in the experiment would have been due to desiccation

When the container size was compared with different volumes of medium it was clear that, irrespective of the plant material used, the largest fresh weight gain occurred in the smallest containers and the least growth in the largest container.

The data for both container and medium volume were combined to reveal the interrelationships between these parameters and plant growth (Figure 3). By week 4 in the *Zantedeschia* cultures differences could be seen between the treatments that took a further 8 weeks to develop in *Ficus.*

The rate of water loss from all the culture vessels was related directly to the surface area of the medium and was proportional to the surface area of the culture vessels. It was not influenced by the volume of medium or by opening during culture assessment. The greatest water loss occurred from the largest containers.

The pH of the media changed over time irrespective of the volume of medium or

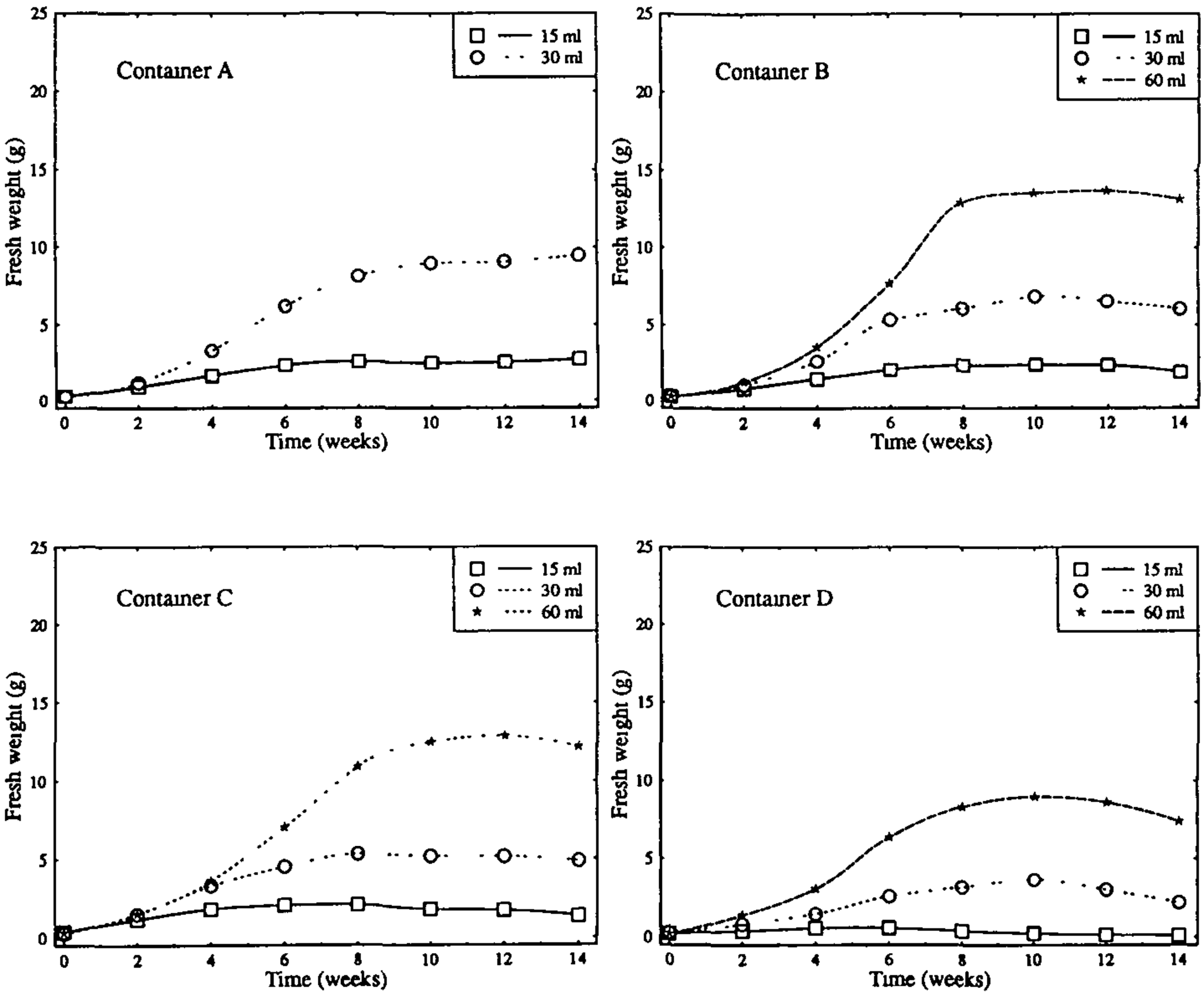

Figure 1. Time course of in vitro growth of *Ficus lyrata* as influenced by container and media volume

the type of container. In *Ficus* cultures the pH had decreased by 1.5 units after 4 weeks, but had increased by nearly 3 units after 12 weeks. In contrast, the *Zantedeschia* medium pH increased by 1 5 units after 6 weeks and then decreased by 0.5 units over the next 6 weeks. The different response by each species was probably a reflection of their preference for ammonium or nitrate nitrogen. It was somewhat surprising that the buffering capacity of the media did not improve when the volume of medium was increased.

CONCLUSIONS

Plant growth *in vitro* was dependent on both the volume of medium used and on the type of culture vessel Increasing the media volume consistently increased plant growth. Increasing the container size usually decreased plant growth. Reduction

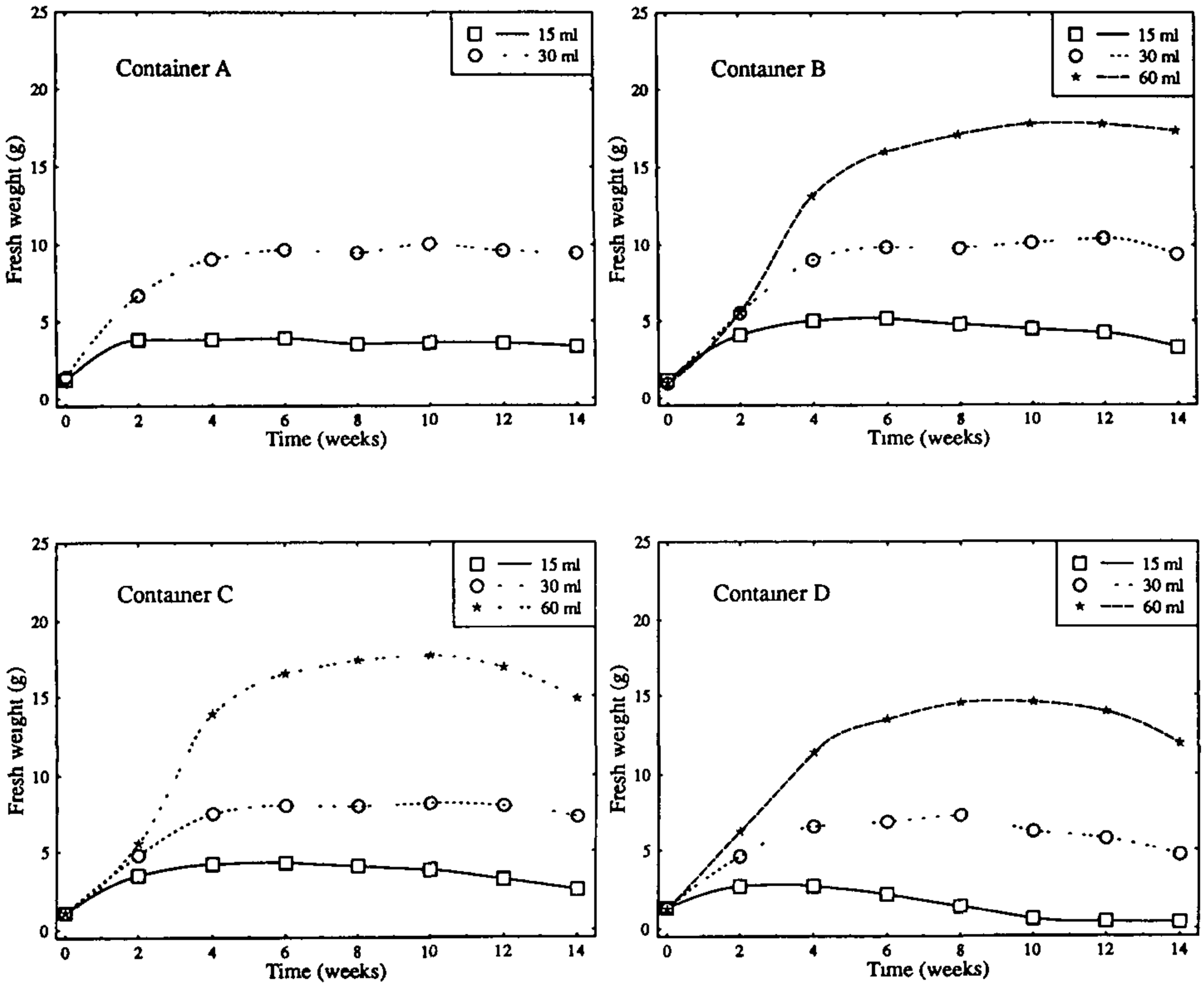

Figure 2. Time course of in vitro growth of *Zantedeschia* as influenced by container and media volume.

in plant growth over time was correlated with changes in media pH and was probably related to nutrient depletion. Maximum growth rates are likely to be achieved with frequent subculturing in small containers with a large volume of medium.

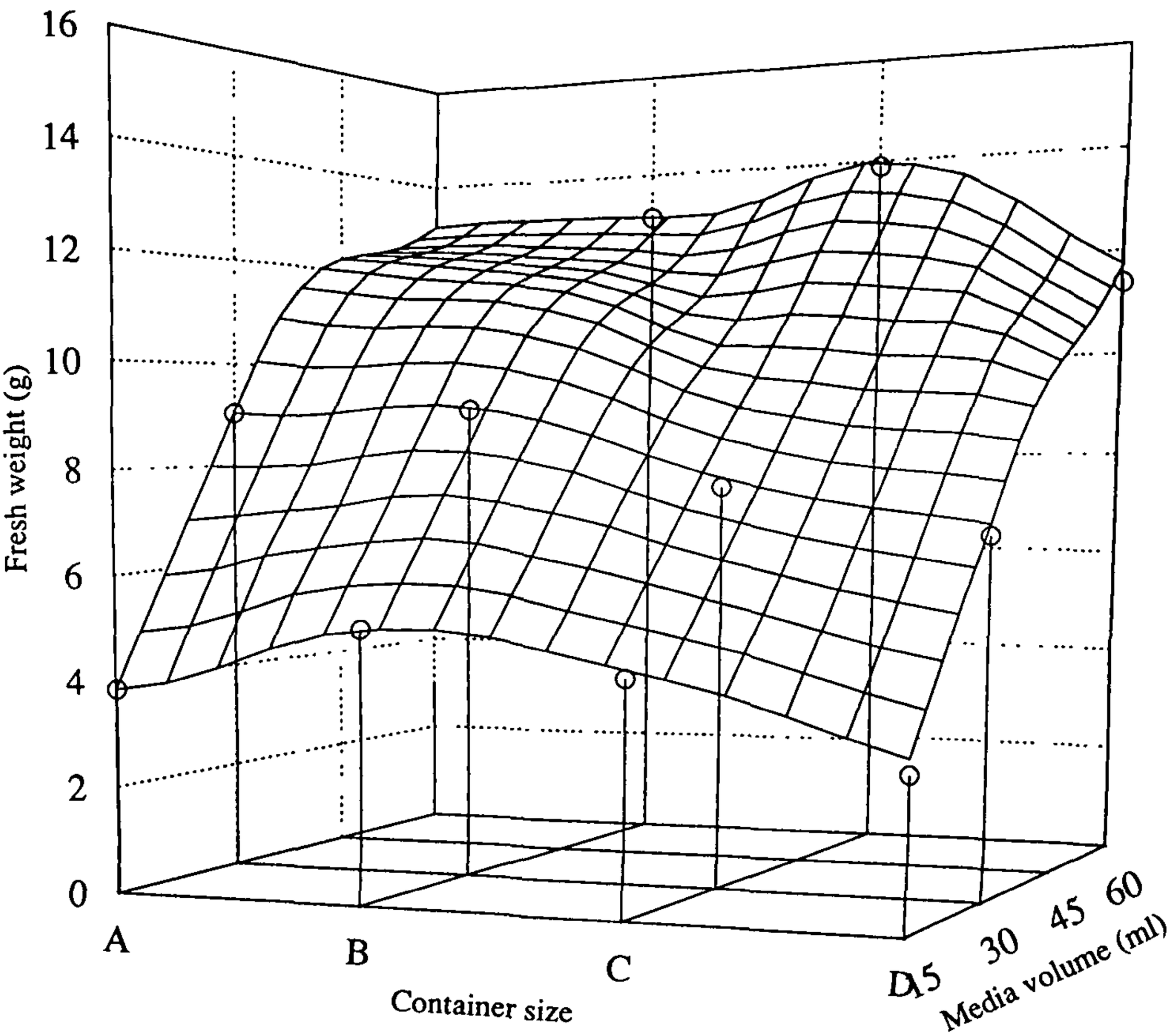

Figure 3. The effect of culture media volume and container size on vegetative growth(shoot) of *Zantedeschia in vitro* after 4 weeks

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