and disease resistance, and hardiness. This programme can take several years before a plant is given a cultivar name and commercially produced.

SIGNIFICANCE OF GELLING AGENTS IN A PRODUCTION TISSUE CULTURE LABORATORY

BEVERLEY J. NAIRN

Tasman Forestry Micropropagation Laboratory
P.O. Box 149, Te Teko, Bay of Plenty

This paper gives a brief account of some experiences with gelling agents and their effect on the survival, multiplication rates, and vitrification in a production crop of micropropagated *Pinus radiata*. No attempt has been made to obtain quantitative data. Vitrification was also influenced by benzylaminoprine (BAP) concentration and other factors (1). The additives to the medium studied were Merck 2186 activated charcoal, Difco Bacto agar, Davis Bacto agar, Coast Biologicals agar (batch 950), Agarose type V, and Gelrite.

The study was undertaken to find the best combination of agar, Gelrite, and BAP to give the greatest multiplication of shoots without vitrification.

MATERIALS AND METHODS

The basic proliferation medium was modified Quoirin Le Poivre (2) with 3% commercial sugar. Gelrite is the trade name for a polysaccharide gellan gum compound produced by the bacterium *Pseudomonas*. The gum produces a mineral dependent, water clear, brittle gel at much lower concentrations than agar, making it very desirable for routine use. Gelrite is supplied by Kelco Division of Merck & Co., Kelco, San Diego, California, U.S.A. Difco Bacto agar is supplied by Difco Laboratories, Detroit, Michigan, U.S.A. Davis Bacto agar is supplied by Davis Gelatine Co., Auckland, N.Z. Coast agar is supplied by Coast Biologicals Ltd, Auckland, N.Z.

Embyros were initiated in plastic petri dishes for the first 12 weeks and were later transferred into clear polystyrene culture pots for subsequent elongation stages. Incubation conditions were 25°C by day, 19°C night temperature with a 16-hour photoperiod of 40μ Einsteins/m^2/sec using cool white fluorescent tubes as the light source.

The seedlot in use was an open pollinated 850 selection from Tasman Forestry's Te Teko seed orchard.

SHOOT DEVELOPMENT AND ELONGATION

Embryos from *Pinus radiata* were extracted from sterilized seed and planted with cotyledons embedded in the medium with 5mg/l BAP and gelled with 0.8% Difco Bacto (DB) agar as described by Aitken-Christie et.al. (3). After the original shoot initiation, elongation took place on QLP medium in the absence of BAP, shoot clumps being divided each 4 to 6 weeks until fully elongated as 3cm shoots. The following observations were of 50,000 shoots, and with the exception of a few clones were seen in greater than 90% of the crop.

Good meristematic tissue was produced and developed into shoot clumps over the next 12 weeks. In the 6 to 12 months following, the shoots failed to elongate and steadily declined in health until die back was prevalent, and elongation was so slow that losses exceeded growth. (Figure 1). While other factors probably

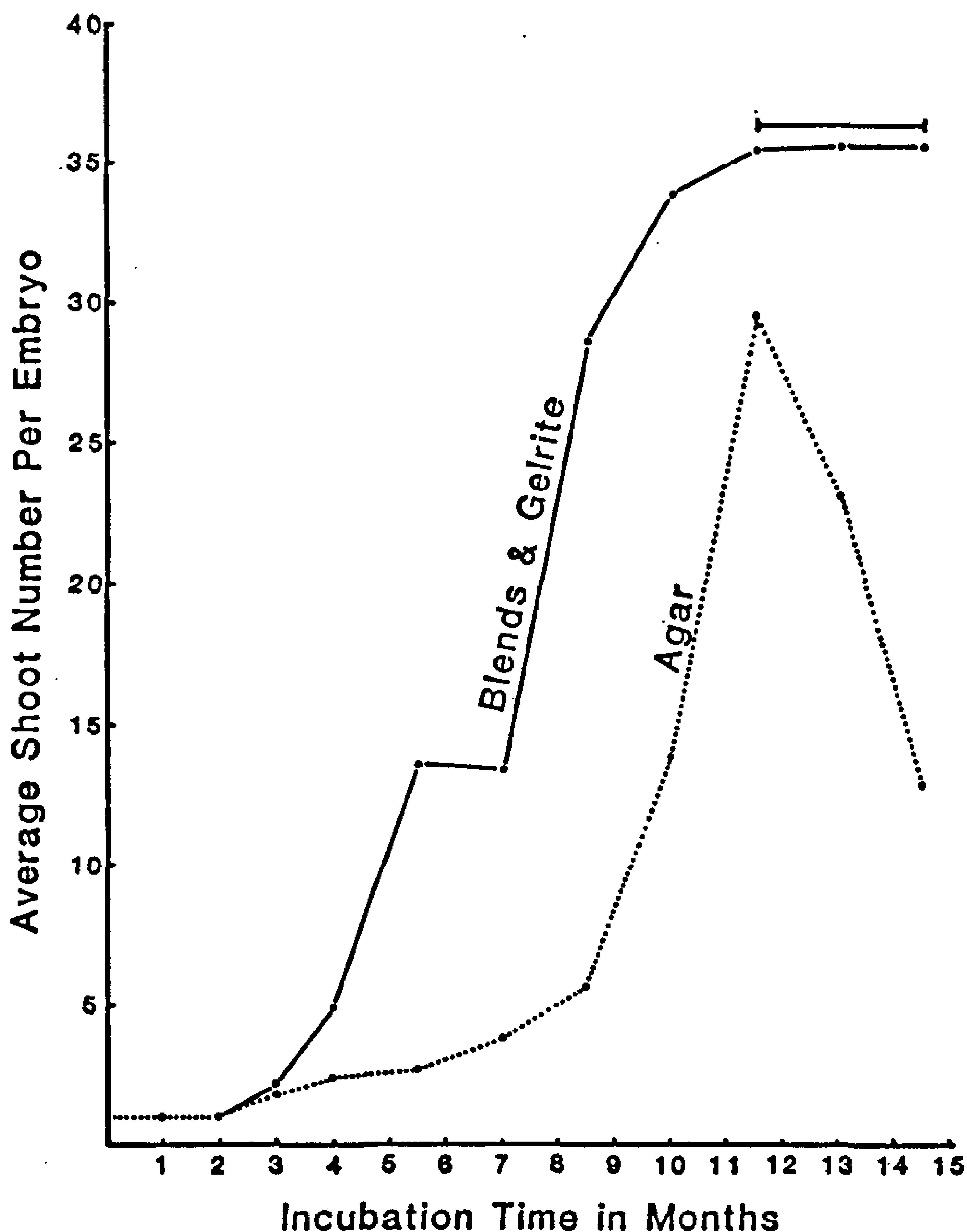

Figure 1. Growth comparison. Agar vs. Gelrite-agar blends.

had an influence, the major cause of decline proved to be continued growth on medium solidified with 0.8% DB agar. Similar medium with 5gms/l Merck activated charcoal exhibited good health and elongation. Continued growth on a charcoal-containing medium was undesirable for reason of reduced light transmission when pots were stacked and bacterial contamination which increased significantly. Medium dehydration was excluded as a major cause.

Trials showed media gelled with 2 gms/l Gelrite producing much improved growth as T. Ichi found with several species (4) (Figure 1). After 4 successive transfers shoots became vitrified. The needles became dark green, translucent, thickened, and brittle. "Vitrification" refers to these visible symptoms only, as no cytology was done. In young shoot clumps the tissue quickly became necrotic, but in the fully elongated shoot the phenomenon was readily reversed to one of normal appearance by the addition of agar. If left unchecked the vitrified shoot ceased growth and died some weeks later.

The re-introduction of high proportions of agar immediately stopped elongation and after 4 to 5 months shoots again deteriorated.

Attempts to prevent vitrification by increasing sucrose levels and Gelrite concentration alone were unsuccessful. A blend of agar and Gelrite provided the only satisfactory result. A range of gels were prepared from 100% down to 2%, from our standard concentrations if used alone, of 2gms/l Gelrite and 8gms/l DB agar. For example, a ratio of 50:50 comprised 1gms/l Gelrite and 4gms/l DB agar. A pattern emerged as the agar concentration dropped, of decreasing death, increasing elongation, and increasing vitrification. (Table 1 and Figure 2). Shoots were graded on a 1 to 3 scale which included the incidence and degree of each factor studied.

Table 1. Effect of Gelrite/Agar Blends Without BAP on Dieback, Elongation and Vitrification.

Gelrite/Agar Ratio				
Gelrite @ 2gms/l	Difco Bacto Agar @ 8gms/l	Die back @ 5 months	Elongation	Vitrification
0	100	+++	(+)	–
50	50	++	(+)	–
75	25	++	(+)	–
90	10	–	+	–
95	5	–	++	(+)
97	3	–	+++	+
98	2	–	+++	+++
100	0	–	+++	+++

KEY:

–	Nil	++	Moderate
(+)	Weak	+++	Marked
+	Slight		

We have been routinely using a 97:3 blend now for 12 months and find it gives us the greatest multiplication. Any vitrified shoots that occur are placed on the 90:10 blend briefly which corrects the phenomenon.

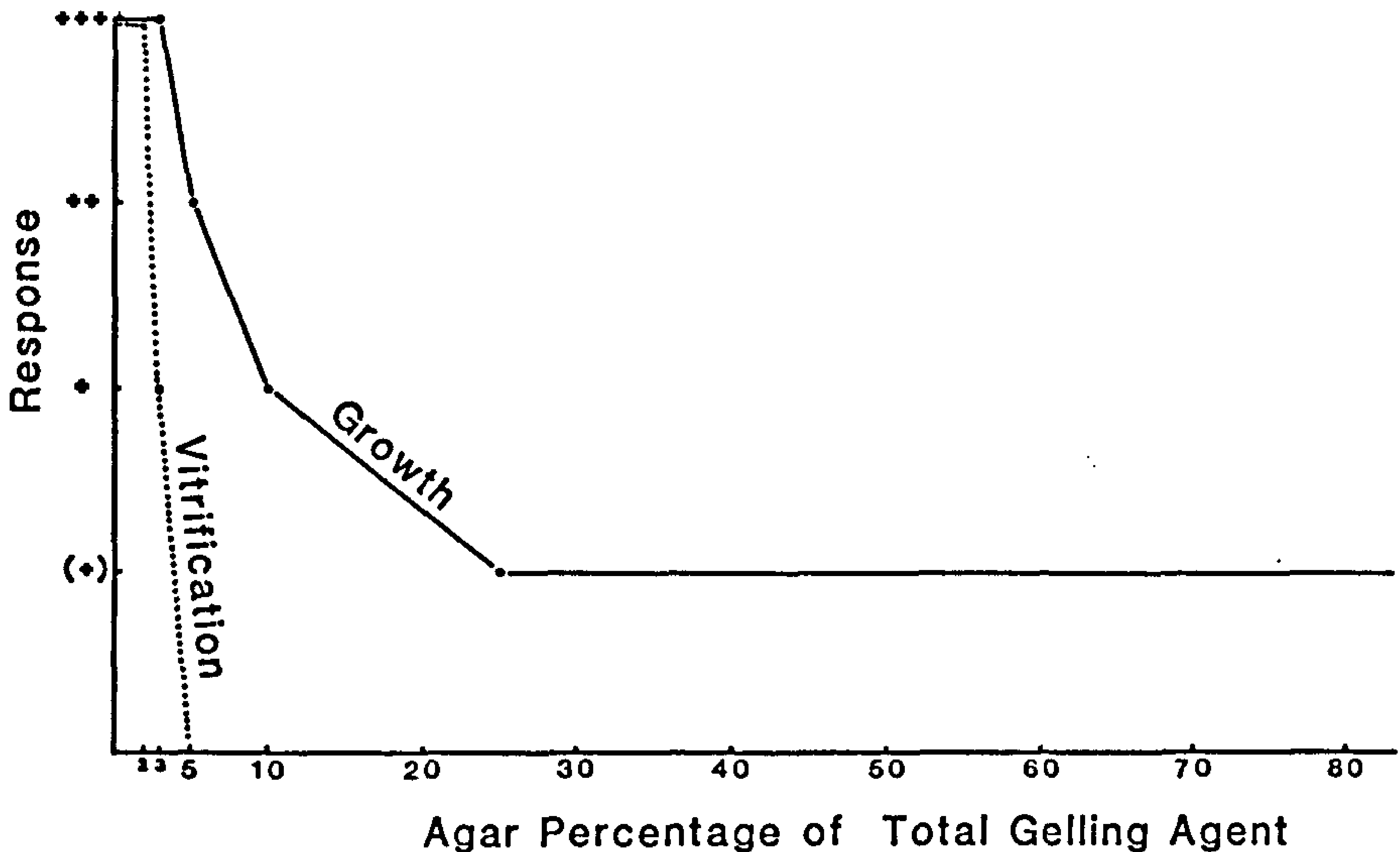

Figure 2. Shoot growth and vitrification as a function of agar ratio.

Table 2. Effects of Gelrite/Agar Blends With BAP on Dieback, Bud Form and Vitrification.

Gelrite/Agar Ratio					
Gelrite @ 2gms/l	DB Agar @ 8 gms/l	BAP mg/l	Die Back @ 4 Weeks	Bud Form	Vitrification
0	100	5	+++	Tight	–
50	50	5	++	Tight	–
50	50	3	++	Tight	–
75	25	5	++	Tight	(+)
90	10	5	–	Tight	+++
90	10	3	–	Tight	+++
90	10	0.5	–	Open	–
90	10	0.1	–	Open	–
90	10	0	–	Open (Low No.)	

KEY:

–	Nil	++	Moderate
(+)	Weak	+++	Marked
+	Slight		

SHOOT MULTIPLICATION WITH BAP

When cytokinin is added to the medium to stimulate axillary bud production, the tendency to vitrification becomes much higher, so a similar "titration" was carried out with cytokinin as an extra factor. (Table 2).

DISCUSSION AND FUTURE PROSPECTS

The incidence of vitrification in radiata pine tissue was controlled predominantly by the agar fraction of the gelling agents. Pasqualleto (5) describes similar results on the role of gelling agents for micropropagation of apple. In radiata pine 3 gms/l Gelrite alone resulted in vitrified shoots and the loss of growth, and because of this observation and the good growth on charcoal medium gelled totally with agar, the "anti-vitrifying" effects of agar appeared to be not only to do with matrix water potential, but a separately active agent present. The growth inhibition may be a combination of matrix potential and phytotoxic ingredients.

To elaborate this we tried several tests:

1. We pre-weighed 8 grams of DB agar and "washed" it in distilled water; after discarding the supernatant we then prepared media with the agar as usual. Vitrification was marked in shoot cultures. This is compatible with Boxus findings where the supernatant of hydrolysed agar prevented vitrification even in liquid medium (6).

2. Agarose type V was substituted for agar with the same gross vitrification resulting as washed agar. The gel strength here was comparable using 3 gms/l Agarose.

3. Davis Bacto grade agar gave identical end point results as Difco Bacto with a poorer shoot form resulting (with short spiralling needles at the crown). Charcoal corrected this malady.

4. Coast Biologicals (batch 950) agar gave a vitrification/elongation end point at a ratio of 75 Gelrite 25 agar with an open long-needled yellowish crown. This agar gave a better result as good growth was maintained without vitrification for 6 months.

The significance of gelling agents for radiata pine in a production laboratory has been shown. Further studies are required to elaborate the nature of these active fractions found in agar for plant species which are particularly sensitive to vitrification and toxicity.

LITERATURE CITED

1. Debergh, P., Y. Harbaoui, and R. Lemeur, 1981. Mass propagation of globe artichoke. (*Cynara scolymus*). Evaluation of different hypothesis to overcome vitrification with special reference to water potential *Physiol Plant* 53:181–187.

2. Aitken-Christie, J., A. P. Singh, and H. Davies. 1987. Multiplication of meristematic tissue—a new tissue culture system for radiata pine. In: Genetic Manipulation of Woody Plants. Edited by J. Hanover and D. Keathley. Plenum Press, New York. p. 413–432.

3. Aitken-Christie, J., C. J. Jones and S. Bond. 1985. Wet and waxy shoots in radiata pine micropropagation. *Acta Hort.* 166:93–100.
4. T. Ichi. Effects of gelling agents (Agricel Report 5:11, 1985, 7:46, 1986, and 8:23 1987).
5. Pasqualetto P., R. H. Zimmerman, and I. Fordham, 1986. Gelling agent and growth regulator effects on shoot vitrification of 'Gala' apple *in vitro*. *Jour. Amer. Soc. Hort. Sci.* III (b): 976–980.
6. Boxus, P. and M. Plaques, 1986. Propagation of woody plants in a culture medium containing hydrolyzed agar to prevent vitrification. Belgium Patent No. 904661.

STERILIZATION OF NERINES USING THE TWIN SCALING TECHNIQUE

MICHAEL MACDONALD

Massey University
Palmerston North

INTRODUCTION

Nerine, a genus belonging to the Amaryllidaceae family, is becoming an important ornamental bulbous plant in New Zealand. Already there have been large plantings of *Nerine* in New Zealand. *Nerine* species have a high potential as export cut flowers. However the natural multiplication rate of *Nerines* is fairly low. Although seed propagation can increase plantlet production by 1,000-fold, such a method does not maintain hybrid traits important for commercial crop production (5).

Large bulbs may only produce only a few daughter bulbs each year. To raise this multiplication rate growers have used a method known as "twin-scaling". This technique involves dividing the bulb into small portions, each consisting of a section of the basal plate. Grootaarts, et al. (2), showed that *Nerine bowdenii* bulblet regeneration always occurred at places where scales contained basal-plate tissue. This technique can be used *in vitro*, for most bulbous species including *Nerine* and *Narcissus* (1). Pierik and Ippel (4) developed the first *in vitro* twin-scaling technique for *Nerine bowdenii* and *Nerine sarniensis*. Bulblet regeneration from twin-scales *in vitro* is dependent on the size and age of the explant material as well as the position in relation to inner and outer regions of the bulb. Twin-scales taken from the outer region of *Nerine* bulbs, seem to regenerate bulblets better than those from the inner bulb (2).

Tissue culture techniques have been used commercially for *Nerine*. Various explant sources such as flower stems, twin-scales,