

Optimizing growth of conifer seedlings *in vitro*

G. Flygh, R. Grönroos and S. von Arnold

Department of Forest Genetics, The Swedish University of Agricultural Sciences, Box 7027, S-750 07 Uppsala, Sweden

The aim of this work was to determine which culture parameters limit growth of conifers under *in vitro* conditions.

Materials and Methods

Seeds of *Pinus sylvestris* and *P. contorta* were used. After surface sterilization the seeds were sown in glass jars. If not otherwise stated, 800 ml glass jars containing a sterile mixture of 300 ml of vermiculite and 200 ml of 25% Ingestad nutrient solution (Ingestad, 1979) were used. The pH was adjusted to 5.9 before autoclaving. Thirty seeds were sown in each jar. The jars were sealed with plastic foil and incubated in a growth cabinet at 20°C under continuous light ($165 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

The effect of aeration on seedling growth (fresh and dry weight and general morphology) was tested by supplying the jars with a contin-

uous flow of air with atmospheric or raised CO_2 concentrations. The CO_2 concentration in the jars was analyzed by gas chromatography after various times.

The influence of vermiculite, autoclaving and seed germination on pH and nitrogen content in the culture medium was tested in a series of experiments. The liquid fraction was separated from the vermiculite before analyses.

Results and Conclusion

Aeration and CO_2 concentrations

The increase in dry weight from d 18 to 46 was 125% when seedlings of *P. contorta* were cultured in aerated jars but only 40% in the control (Figs. 1, 2). Root growth and

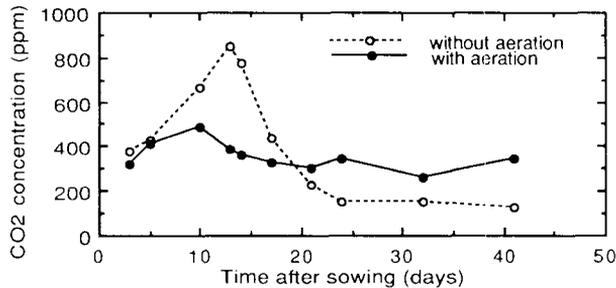


Fig. 1. CO_2 concentration in glass jars with *P. contorta* seedlings with and without continuous aeration. Each point represents the average of 3 jars.

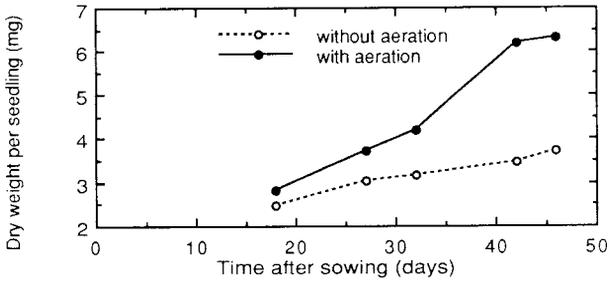


Fig. 2. Dry weight of *P. contorta* seedlings after culture in glass jars with and without continuous aeration. Each point represents the average dry weight per seedling based on about 50 seedlings (= all seedlings in 2 jars). The whole experiment included 20 jars.

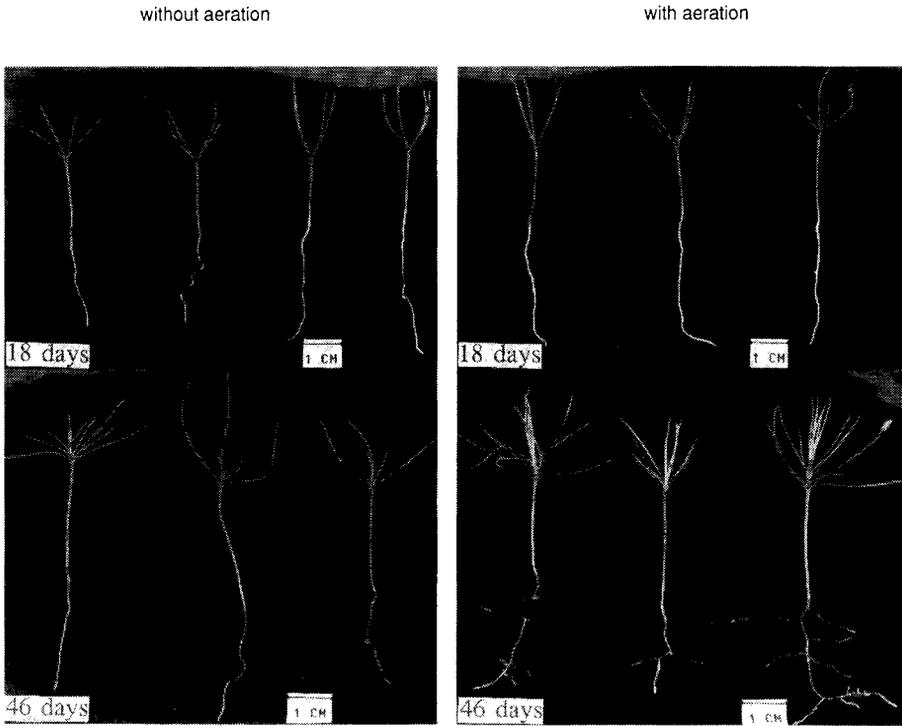


Fig. 3. Seedlings of *P. contorta* cultured *in vitro* with and without aeration.

branching were stimulated by aeration (Fig. 3). A similar stimulation of growth by aeration was obtained with *P. sylvestris*. In another series of experiments, the importance of CO_2 concentrations in the air flow

was tested with *P. sylvestris* (Fig. 4). The results indicated that the CO_2 concentration in the air flow was not critical in the range of 360–7000 ppm. Aeration of the culture vessel stimulated growth *in vitro*.

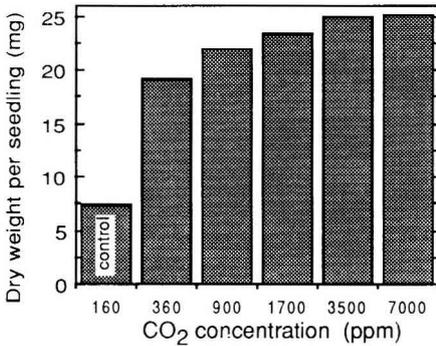


Fig. 4. Dry weight of *P. sylvestris* seedlings cultured for 24 d with various CO₂ concentrations. The dry weight present in each column is an average of 30 seedlings. Control seedlings were cultured without continuous aeration.

Nitrogen content

Vermiculite had a drastic effect upon ammonium content and pH in the culture medium (Tables I and II). Therefore, it was not possible to perform detailed studies of nitrogen uptake and pH changes during growth in vermiculite. After the seedlings had been cultured for 45 d, the medium still contained ammonium and nitrate. No differences were observed in nitrogen concentration whether or not the jars had been aerated, although the seedlings grew much better in aerated jars. Hence, nitrogen was not the limiting factor for growth.

Table I. pH changes in the culture medium with vermiculite.

Initial pH	4.0	5.9	7.0
pH after autoclaving	4.0	5.9	7.0
pH after 1 h with vermiculite	7.8	7.8	7.8
pH after 45 d with vermiculite		6.5–7.5	

The pH was adjusted to 4.0, 5.9 or 7.0 before autoclaving. Seeds of *P.*

Table II. Nitrogen content of the culture medium after germination.

Liquid medium	N-NH ₄ (mg/l)	N-NO ₃ (mg/l)
After autoclaving	29.2	34.2
1 d after mixture with vermiculite	7.9	36.8
12 d after sowing	6.4	28.4
19 d after sowing	5.2	31.0
45 d after sowing	4.3	33.5

30 seeds of *P. contorta* were cultured in each jar containing 160 ml of nutrient medium mixed with 360 ml of vermiculite.

References

Ingestad T. (1979) Mineral nutrient requirements of *Pinus sylvestris* and *Picea abies* seedlings. *Physiol. Plant.* 45, 373-380