Experiments with willow cuttings - two suggestions for projects on root growth

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Willow cuttings are well known for their unusual ability to produce adventitious roots. They will grow such roots if merely stood in water for a few days. A 17 cm twig standing 6 cm deep in water produces about 100 mg wet mass of roots in just 7 days. It is also known that willow cuttings will produce adventitious roots if suspended in air saturated with water vapour, although I have been unable to induce this in cuttings personally (see the diagram in Waring and Phillips, p 17 [1]). A variety of experiments are possible with this useful and abundantly available material. This article describes a simple investigation of the effect of orientation to gravity on adventitious root production. At the end of the article a second investigation on the effect of water potential is briefly described.

EXPERIMENT 1 THE EFFECT OF GRAVITY

In this simple but fascinating class experiment some willow cuttings were subjected to a reversal of their orientation to gravity. The cuttings were then carefully observed over a period of a few weeks, comparing them with control cuttings. Students then attempted to explain their observations in terms of the interaction of gravity, the direct stimulation by water and the migration of hormones.

Auxin is synthesized at the apex of a shoot and is transported downwards towards the root. There appears to be a gradient of concentration from shoot to root. The effect of auxin is to 'trigger off the process of root initiation' in cuttings by 'stimulating the formation of root initials'. Appropriate concentrations of cytokinins in the target tissues are also required [1, p107, 164]. If willow cuttings are placed upside down in a beaker of water they produce adventitious roots around the shoot apex, just like those cuttings that are placed in a beaker of water the right way up. This suggests that adventitious roots may be produced at concentrations of auxin that would inhibit normal tree roots. The following questions were presented to the students:

1 What would happen if we subjected **both** ends of a cutting to water simultaneously?
2 What would happen if we compared such cuttings that had been placed in normal and inverted orientations with regard to gravity?

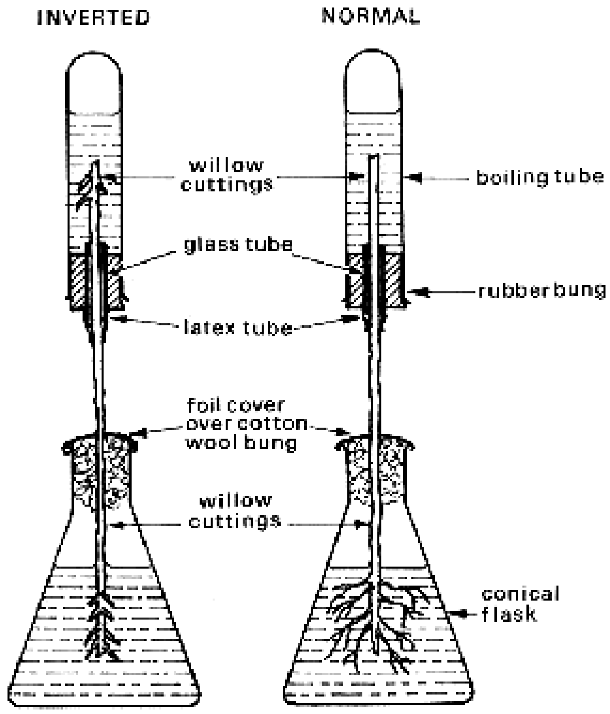

Figure 1

Figure 1 shows an apparatus designed to answer these questions. Thin willow branches (*Salix fragilis*) were collected in the spring (March to April) before the buds broke, and selected to easily fit the internal diameter of the glass tubing set in the rubber bungs. They were thoroughly washed, then cut through at both ends onto a wooden board with a sharp, one-piece scalpel.

(Warning! To avoid any accidents these cuts should be done beforehand by the teacher. A craft knife is a suitable alternative.)

The cut branches were then slid carefully through the glass tubing and latex tubing, whilst taking care not to snap the branches. The latex tubing acted as a gentle seal to prevent water leakage. The apparatus was then assembled. The boiling tubes were held in clamps and a retort stand. The whole apparatus was placed in even daylight illumination at room temperature (about 20 °C). Changes in the cuttings were observed for a period of about 4 weeks and all changes noted and recorded. The shoot-end of cuttings was referred to as the apex and the root-end as the base.

Adventitious roots first developed at the apex of the inverted cuttings, then at the base of the normal cuttings and finally at the base of the inverted cuttings. No roots developed at the apex of the normal cuttings. The roots at the base end of the normal cuttings were long and had secondary branches. Those at both ends of the inverted cuttings were single and had no secondaries. After a period of 24 days the roots on each cutting were counted and weighed. The quantitative distribution of roots is shown in Table 1.

Table 1 Results of class group investigations for adventitious root production after 24 days. Mean values per pair of cuttings (n = 5)

Cutting		Mean number of roots	Mean wet mass (mg)
Normal	apex	0	0
	base	13.4	364.82
Inverted	base	7.6	83.64
	apex	3.2	26.18

After a further 4 days the normal cuttings were removed from the apparatus and cut transversely through the mid point.

(Warning! For safety reasons this is best done by the teacher.)

The shoot apex ends were then inverted and stood in beakers of water. Within one week the shoot apex ends had developed adventitious roots. Also leaves developed from the buds above the water.

It would seem from the results with the cuttings in normal orientation that contact with water alone is insufficient to stimulate root production. The results with the inverted cuttings indicate that the polar migration of auxin from developing laterals (leaf buds) is affected by gravity. Thus the adventitious root production at the base of the inverted cutting is much slower than that of the normal cutting, amounting to less than a quarter of the wet mass in 24 days.

Table 2 A class group's results for the effect of water potential on adventitious root production of *Salix* cuttings

Concentration of sucrose (mol dm^{-3})	Water potential (kPa at 20 °C)	Wet mass roots (mg)
0	0	83
0.1	-300	71
0.2	-600	57
0.3	-900	16
0.4	-1120	16
0.5	-1450	4
0.6	-1800	3

Under such circumstances however adventitious roots are produced at both ends.

The response of the cuttings is clearly of considerable importance for the success of natural vegetative propagation of *Salix* in those cases where a twig has been broken free from a tree, for example by a river in spate. Under such circumstances adventitious roots will develop at the root-end alone so long as the twig happens to have normal orientation, even though the whole twig may be immersed in water at the edge of a river or lake. In the case of a twig happening to have an inverted orientation the apex is capable of developing adventitious roots albeit at a slower rate, thus ensuring the likelihood of eventual successful propagation.

EXPERIMENT 2 THE EFFECT OF WATER POTENTIAL ON ADVENTITIOUS ROOT PRODUCTION

For this investigation a series of six concentrations of sucrose solution in de-ionized water were prepared in sufficient quantity so that it was possible for willow cuttings to stand in beakers containing the solution at a depth of 6 cm. The willow cuttings had been selected from the same tree, at the same height and had a similar size and appearance. Each branch was cut to a length of 17 cm taking safety precautions as described in Experiment 1. Each cutting was placed into the solution in a beaker so that its orientation to gravity was normal, shoot-end uppermost. Control beakers were also prepared that contained cuttings in de-ionized water alone.

The tops of the beakers were sealed with clingfilm to prevent evaporation and the position of the surface meniscus of each solution marked on the beaker with a permanent pen.

The cuttings were left in even daylight conditions at approximately 20 °C for 7 days after which the shoots were removed from the solutions. The adventitious roots were removed from the cuttings, blotted dry with soft paper tissue and weighed. The results of a typical experiment are shown in Table 2.

Students observe other differences in those parts of the cuttings in the air above the solutions. Between zero and 0.3 mol dm^{-3} sucrose there are noticeably greater number of buds swelling and opening than at the higher concentrations of sucrose. Often cuttings that are standing in beakers containing the midrange of the sucrose concentrations begin to develop catkins. The ecological significance of this investigation is that water potentials more negative than -1500 kPa occur in sandy soils below a water content of about 10%, and in clay soils below about 20% [2, p 99]. Adventitious root production will be slower under such conditions and this helps to explain the successful regeneration of willow close to the river's edge where the water potential of the soil is likely to be greatest.

Further investigations for class work are planned for the near future. Initial pilot investigations seem to suggest that the inhibiting effect of water potential upon the rate of adventitious root production can be countered by the addition of indoleacetic acid (IAA) at concentrations in the range 10 to 20 ppm IAA in 0.4 mol dm^{-3} sucrose solution. Over a 3-week period the effect of IAA is to increase adventitious root production by more than 50% above the control cuttings in 0.4 mol dm^{-3} sucrose alone.

REFERENCES

1 Wareing, PF and IDJ Phillips, *Growth and Differentiation in Plants*, (3rd edn), (Pergamon, 1981).

2 Sutcliffe, J, *Plants and Water (Studies in Biology No 14)*, (Arnold, 1979).

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