Zeitschrift: Botanica Helvetica
Herausgeber: Schweizerische Botanische Gesellschaft
Band: 98 (1988)
Heft: 1

Artikel: The anatomy of adventitious root formation in greenwood cuttings of Populus tremula L.

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DOI: https://doi.org/10.5169/seals-68574

Botanica Helvetica 98/1, 1988

0253-1453/88/010097-06 $ 1.50+0.20/0

The anatomy of adventitious root formation in greenwood cuttings of *Populus tremula* L.

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Manuscript accepted February 24, 1988

Abstract

Plüss, R. and Schmid, A. 1988. The anatomy of adventitious root formation in greenwood cuttings of *Populus tremula* L. Bot. Helv. 98: 97–102.

Adventitious roots in greenwood cuttings of *Populus tremula* originate from the ray initials which swell and start to divide intensively. 90% of the adventitious roots are formed 0–5 mm above the basal cut surface. The adventitious root formation occurs in an anatomically identical way in auxin-treated and untreated plants.

Introduction

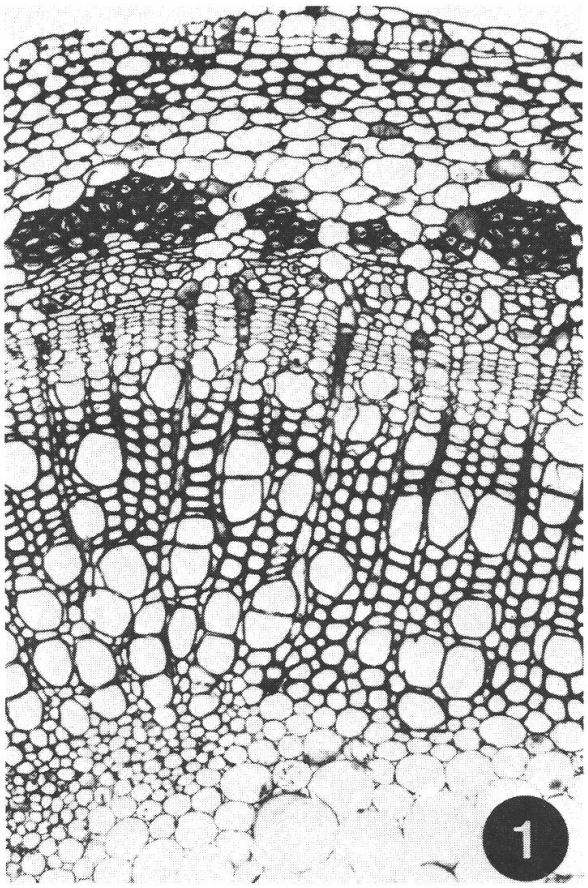
For vegetative propagation of shoots the regeneration of new roots is necessary. Roots formed at an anatomically atypical site were named adventitious roots by De Candolle (1827). They can arise from different tissues in the stem depending on the species (Esau 1977, Fahn 1982, Lovell and White 1986). In *Populus euramericana* adventitious roots originate from latent primordia which have been initiated by cell division of rays in the secondary phloem (Luxova and Lux 1981). Primordia of this type are missing in *P. tremula* which may explain some of the difficulties of vegetative propagation of cuttings. In greenwood cuttings propagation succeeds without special intervention (Marcet 1963), but in woody cuttings it is only successful if the buds are removed and an auxin is applied (Gemperle 1968).

In this paper the origin of the adventitious roots in greenwood cuttings of *P. tremula* with and without indole-3-acetic acid (IAA) application is studied by light microscopy.

Materials and methods

Plant material: Seedlings of *P. tremula* L. were cultivated in the greenhouse, as described by Schmid (1981), under natural daylight in summer and supplemented by a high-pressure sodium lamp (Philips T 400 W) in winter when daylight was poor. After 3–4 months of growth the seedlings were cut 12–15 cm below the apex. After removal of all except the two uppermost leaves, the cuttings were subjected to three different treatments:

a) the basal end was immersed in distilled water to a depth of 4 cm,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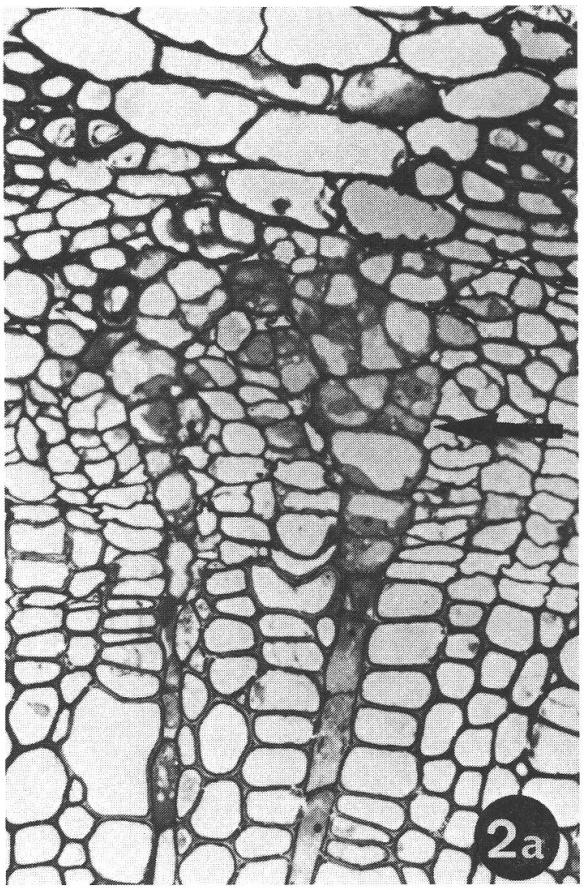
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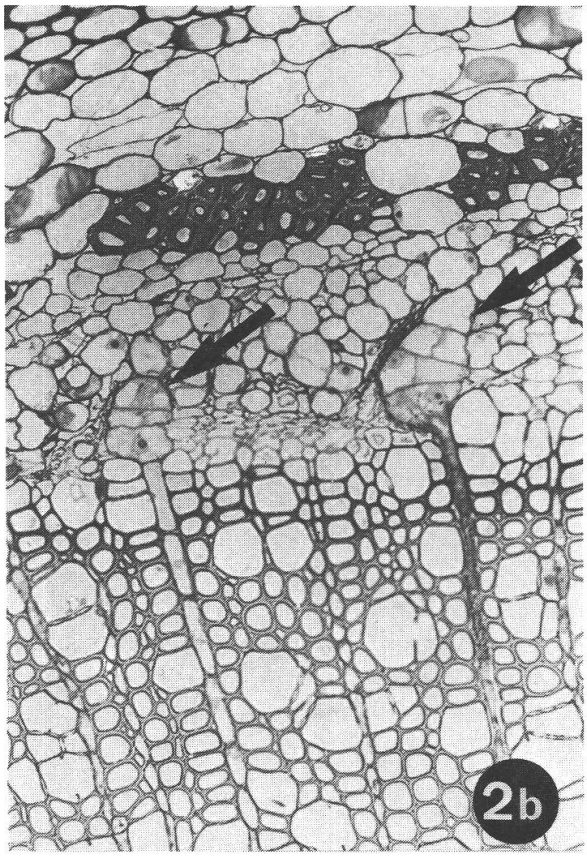
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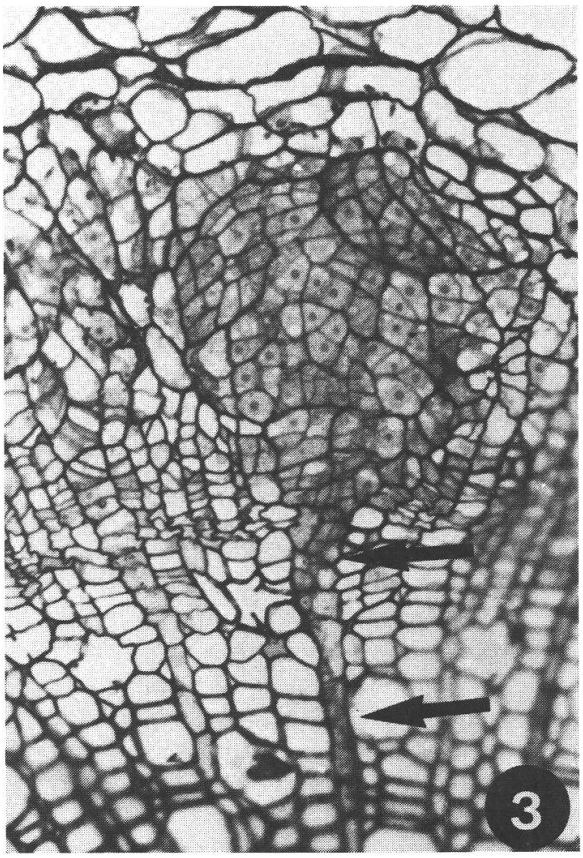
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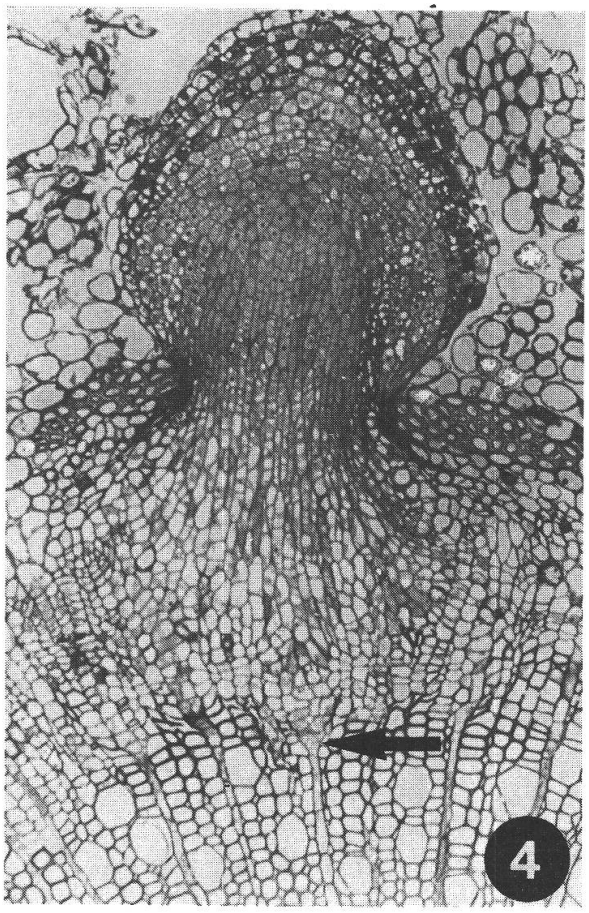

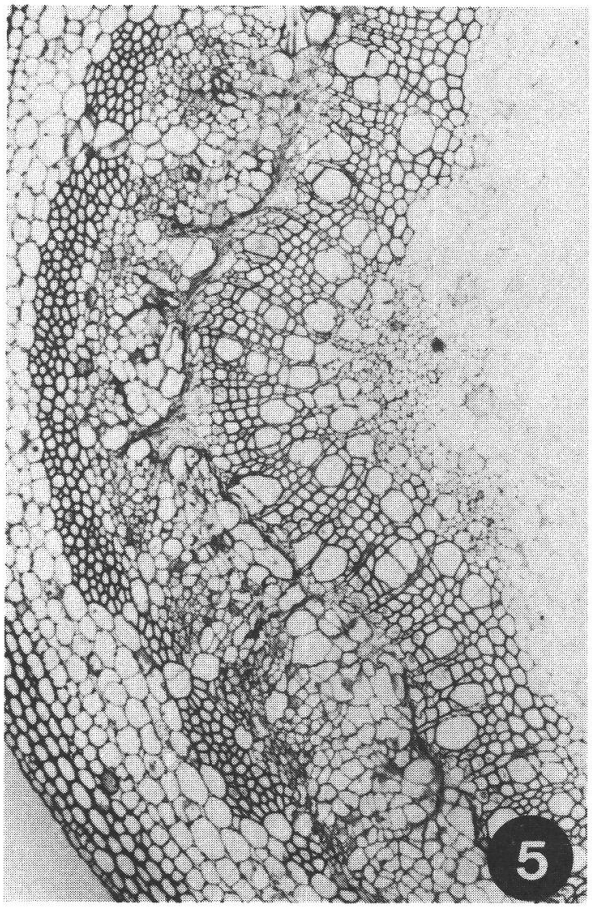

Fig. 1. Cross section, taken about 10 cm below the apex from a 80–90 days-old and 25 cm high seedling; × 130.

Fig. 2a and 2b. Cross section four days after the preparation of the cutting. Some ray initials are greatly enlarged and radial cell divisions have started. Fig. 2a, without IAA-treatment, × 320; Fig. 2b, with IAA-treatment, × 160.

Fig. 3. Cross section, six days after preparing the cutting. The adventitious root primordium and its origin from the ray is clearly visible (arrow); without IAA-treatment, × 90.

Fig. 4. Cross section, eight days after preparing the cutting. The adventitious root penetrates the cortex. The arrow shows the funnel-like enlargement of the ray; without IAA-treatment, × 75.

Fig. 5. Cross section, four days after preparing the cutting. The cambium is strangely deformed; with IAA-treatment, × 85.

b) the basal end was immersed in distilled water to a depth of 4 cm. After 24 h the apex and the remaining leaves were cut off and the stem further cultivated in distilled water,
c) the basal end was immersed in a solution of 285 µM IAA to a depth of 4 cm for 24 h. The apex and the remaining leaves were then cut off and the stem further cultivated in distilled water.

All cuttings were cultivated in a phytotron with a day/night rhythm of 16 h light, 25 °C and 70% rel. humidity and 8 h of darkness, 17 °C and 80% rel. humidity. After 12 days all visible adventitious roots were counted.

Preparation of material for light microscopy: 0, 1, 2, 4 and 8 days after preparing the cuttings, stem segments (2–3 mm) were cut off at the basal end, and both in the middle of the second and fourth internode. The segments were fixed in 2,5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4) followed by 1% OsO_4 in 0.05 M phosphate buffer, dehydrated in acetone and embedded in epoxy resin (Spurr 1969). From the embedded tissue, 1–8 µm thick sections were cut on a Sorvall MT2-microtome and coloured with methylene blue-azure-II-safranin (Warmke and Lee 1976).

Results

With the three different treatments (a, b, c) described in "materials and methods", the following results were obtained (Tab. 1). An average of 5.3 adventitious roots were formed if the apex and the two uppermost leaves remained on the stem (a). If the cutting was deprived of the apex and leaves, 1.1 adventitious roots were formed (b). However, if 285 µM IAA was applied for 24 h and then the apex and the leaves were cut off, 6.8 adventitious roots were formed (c). An average of 90% of the adventitious roots appeared 0–5 mm above the basal cut surface independent of the treatment.

The microscopical investigations of the stem segments from 0–5 mm above the basal cut surface showed that during the first two days after the preparation of the cuttings, no anatomical change was detectable. From day four on, the first changes were visible in certain ray initials which rapidly grew to form, 3–4 days later, an adventitious root seen breaking through the cortex.

Fig. 1 shows the initial state from the middle region of a 25 cm high plant immediately after the preparation of the cutting. The pith consists of two types of cells: a) big cells with thin walls, some containing air, b) small cells rich in plasma and chloroplasts. In the xylem the vessels are dispersed between wood fibres and wood parenchyma cells. The rays are uni-seriate and possess very plasma-rich ray initials. Groups of sclerenchymatic fibre bundles are located external to the phloem.

Four days after the preparation of the cuttings (with or without IAA-application) some ray initials greatly enlarged and divided (Fig. 2a and 2b).

Six days after the preparation of the cuttings (with or without IAA-application) a few hundred plasma-rich cells formed an adventitious root primordium (Fig. 3). The

Tab. 1. Influence of indolylacetic acid on the adventitious root formation in cuttings of *P. tremula.*

Treatment: see materials and methods	Average number of adventitious roots per cutting	Number of cuttings
a)	5.3	31
b)	1.1	28
c)	6.8	61

origin of these cells in ray initials is clearly visible. Neither the other cambium cells nor the cortical parenchyma participate in the formation of the primordia.

Eight days after the preparation of the cuttings (with or without IAA-application) the adventitious roots penetrate the cortex (Fig. 4). Root cap, apical meristem and central cylinder are well developed. The origin from the ray initials is still visible from the funnel-like enlargement of the ray. The sclerenchymatic fibre bundles of the stem form an obstacle through which the adventitious root must squeeze.

The microscopic investigation of sections from the second and fourth basal internode showed no sign of adventitious root formation.

In some sections a strange deformed cambium can be seen (Fig. 5). This deformity exists to varying degrees in the first and second basal internodes as early as 24 h after the preparation of the cuttings, independently of treatment with IAA.

Discussion

The adventitious roots originate from the ray initials which enlarge and start to divide intensively as was shown for the latent primordia of *P. euramericana* (Luxova and Lux 1981). In contrast to *P. euramericana,* neither the other cambial cells nor the adjacent parenchyma cells participate in the formation of the primordia in *P. tremula.* This kind of development resembles more that of *Hedera helix* (Girouard 1967). Here too, the adventitious root has to squeeze through two bundles of sclerenchymatic fibres. It is interesting to note that the formation of the adventitious roots was anatomically identical both in the untreated and IAA-treated cuttings. This corresponds with observations made in *Ficus pumila* (Davies et al. 1982) and in the bean hypocotyl (Friedman et al. 1979) where auxin application had no influence on the origin of the adventitious roots.

The authors would like to thank Miss F. Müller for her preliminary studies and Prof. H. Meier for valuable discussions.

Zusammenfassung

In Grünstecklingen von *Populus tremula* entstehen die Adventivwurzeln aus Markstrahlinitialen, welche stark anschwellen und sich zu teilen beginnen. 90% der Adventivwurzeln entstehen 0–5 mm oberhalb der basalen Schnittstelle. Die Adventivwurzelbildung verläuft in den mit Indolylessigsäure behandelten Pflanzen anatomisch gleich wie in den unbehandelten Pflanzen.

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