SOME ANATOMICAL ASPECTS OF ROOTING QUAKING ASPEN 1/

by

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Abstract

Aspen root suckers and stem cuttings consisting of the current year's growth from trees 1, 2, 3 and 4-10 years old were collected for microscopic examination. Preformed root primordia were not observed in aspen cuttings. Root sucker cuttings developed roots from interfascicular regions of the stele or in association with phloem rays. Cuttings from the 1, 2, 3 and 4-10 year age classes failed to develop root primordia when placed in conditions favorable to rooting of sucker cuttings. Thus it was concluded that reduced rooting in physiologically mature aspen cuttings resulted from

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the inability to form primordia rather than mechanical restriction of roots already formed.

Introduction

Quaking aspen (*Populus tremuloides* Michx .) stem cuttings from juvenile root suckers are easily rooted, but cuttings consisting of the current season's stems from physiologically mature trees are difficult to root. Two factors which could account for reduced rooting are lack of preformed root primordia in mature stems and mechanical obstruction of existing primordia. These anatomical factors were examined in mature stems as well as in rooted juvenile aspen cuttings.

Literature Review

Many living cells in plants are capable of dedifferentiation and subsequent organization into adventitious structures. However, adventitious roots in cuttings usually originate from specific loci within the anatomical configuration of the stem. *Salix fragilis* stems contain preformed root primordia which are associated with phloem rays of leaf gaps (Carlson, 1950). Preformed root primordia arise from the cambial zone in stems of *Pozulus deltoides* and P. x *euramericana,* but the difficult-to-root European aspen (*Populus tremula*) contains no preformed root primordia (Ruggeri, 1966).

Stems of many species contain no preformed root primordia; in such stems roots may develop after the cuttings are excised. Mahlstede and Watson (1950), found that root primordia originate from the cambium of *Vaccinium* cuttings. Roots arise from phloem ray tissue of juvenile *Hedera helix* stem cuttings and from phloem ray and basal callus tissue in mature cuttings of the same species (Girouard, 1967a, 1967b).

Mechanical restriction of root primordia has been implicated as a cause of reduced rooting. Nanda, Anand and Kumar (1969) observed thicker bands of fibrous tissues surrounding the stele in species which root poorly. According to Breakbane (1961), apple and pear varieties which are difficult to root contain more continuous rings of sclerenchymatous tissue than easily rooted varieties. However, mechanical restriction was not proposed as the only cause of reduced rooting since difficult rooting plants failed to produce root primordia. Mature *Hedera helix* stems contained thicker fibrovascular caps in the cortex than juvenile stems, but these structures caused no apparent restriction of root penetration (Girouard, 1967b). Sachs, Loveti and Debie (1964) compared the rooting potential of several olive, cherry and pear cultivars with the density and continuity of sclerenchyma in the cortex. They found no obvious relationship and concluded that rooting ability was dependent on factors controlling differentiation of root primordia rather than mechanical restriction of roots already formed.

Materials and Methods

Cuttings for examination were consistently collected from the current flush of stem growth and age classes were assigned according to the age of the parent tree. Thus an age class is defined as the current year's stem growth from a tree "n" years of age. Cuttings which were produced by burying aspen root segments in moist vermiculite and allowing them to sprout in the greenhouse were designated age class "root sucker" (RS).

Stem segments were fixed in formalin-aceto-alcohol (FAA) directly after collection to investigate the possibility of preformed root primordia in aspen stems. Two cuttings each from the age classes RS, 1, 2, 3 and 4-10[4/] were prepared for microscopic examination by standard techniques. Twenty cuttings each from age classes 1, 2, 3 and 4-10 were examined macroscopically after removing the cortex. Ruggeri (1966) observed root primordia in <u>Populus deltoides</u> stems using this technique. The basal one half inch of two cuttings each from age classes RS, 1,

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[/] Cuttings were collected from trees ranging in age from 4 to 10 years.

2, 3 and 4-10 was sectioned and examined microscopically after the cuttings had been in sand rooting medium for 33 days under ambient greenhouse conditions.

Groups of 40 root sucker cuttings were rooted in distilled water under controlled environmental conditions to investigate the root forming process. The growth chamber was maintained at 68° F and illuminated at 431 fc for a 14 hour photoperiod. Visible roots appeared after 5 days. Ten cuttings were removed at intervals of 3, 5, 7 and 14 days and the basal half inch was fixed in FAA. To further investigate the possibility of preformed root primordia in root sucker stems, half inch basal segments from 20 root suckers were fixed and examined directly after removal from the parent roots.

Results

Preformed root primordia were not observed in any age class when examined either macroscopically or microscopically.

Cuttings from trees of increasing age were characterized by increasing numbers of sclerenchymatous cells in the cortex (fig. 1 A, B, C, D). However, neither sclereids nor phloem fibers formed a closed ring around the stele.

Root primordia were observed only in root sucker cuttings among those which had remained in rooting medium.

Root primordia were observed microscopically in sucker cuttings after 3 days in the control room (fig. 2 A, B), and primordia were well developed after 5 days (fig. 2 C). Many roots had penetrated the epidermis after 7 days (fig. 2 D, F).

Apical growth of aspen stems results in the formation of dictyosteles with vascular bundles encircling the pith (fig. 2 B). Activity of the cambium rapidly closes the interfascicular regions resulting in a continuous siphonostele (fig. 1 A, B, C, D; 2 A, C). Root sucker cuttings with either stelar configuration were capable of rooting (fig. 2 A, B, C, D). Adventitious roots develop in the interfascicular regions of stems in the primary state of growth (fig. 2 B) and in association with ray cells after cambial activity has occluded the interfascicular regions (fig. 2 A, C, D). Root primordia differentiate from the cambium or cambial derivatives toward the phloem in these interfascicular or ray areas (fig. 2 A, E).

Conclusions

Preformed root primordia are not an important source of adventitious roots in either juvenile or mature quaking aspen cuttings.

Frequency of sclereids in current year stems increased with **tree age.** The more **mature age** classes failed to produce root primordia when subjected to conditions favorable to rooting of juvenile root **suckers.** It was concluded that the inability to form primordia rather than mechanical restriction of roots by thick-walled cells is the cause of reduced rooting.

Differentiation of root primordia occurred **in inter**fascicular areas of aspen stems in the primary state of growth and associated with rays after secondary growth had occurred. Roots developed from the cambium or primary cambial derivatives in the interfascicular or ray regions.

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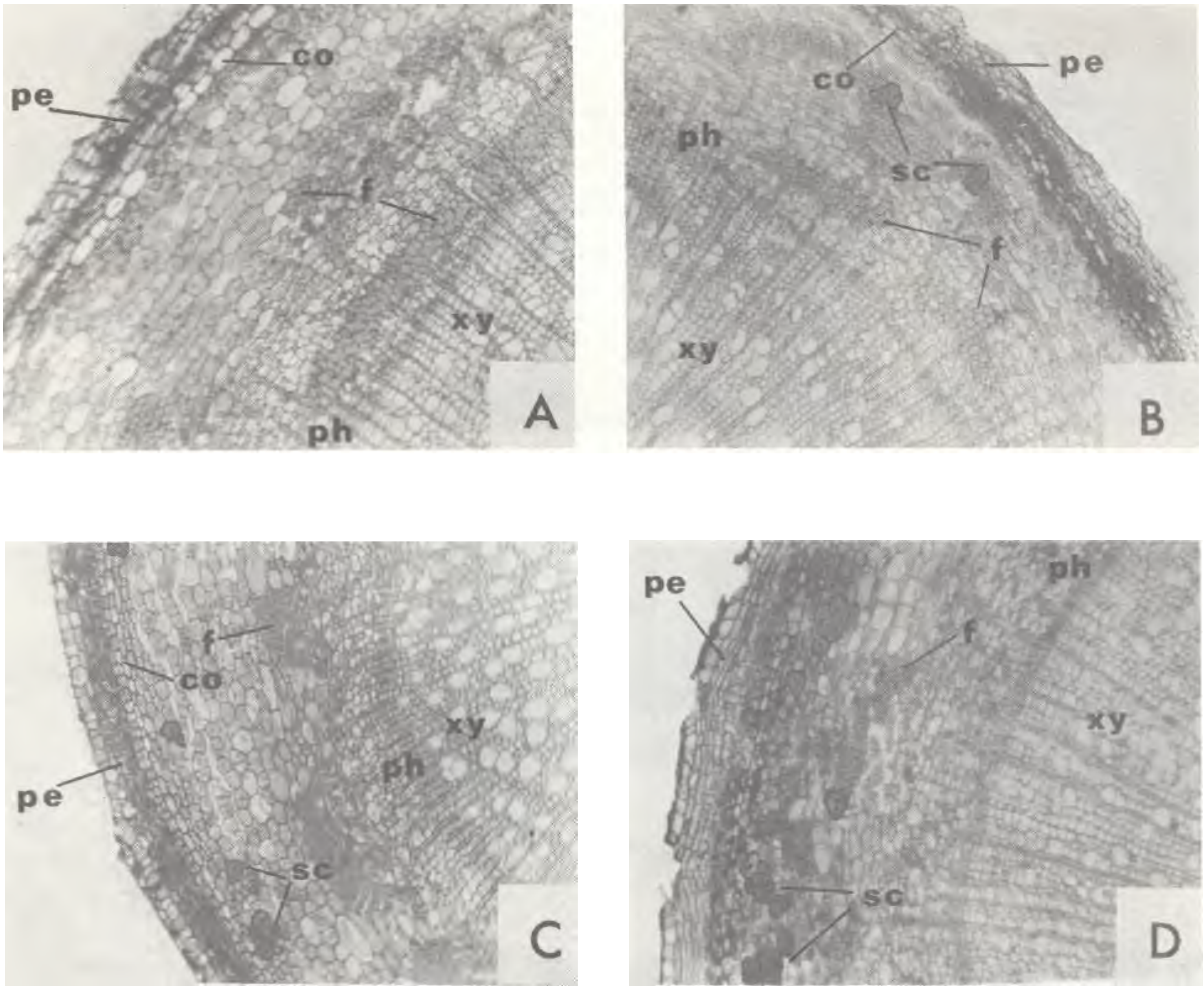

Figure 1. Cross sections of current year aspen stems from trees of varying age classes

(A) stem from the one year age class with well developed periderm (X 50). (B) two year age class stem with collenchymatous layer apparent (X 50). (C) stem from the three year age class with sclereids apparent in the cortex (X SO). (D) stem from the 4-10 year age class illustrating the abundance of sclerenchymatous cells in the cortex characteristic of this age class (X 50).

Legend:

ph - phloem f - phloem fibers
xy - xylem co - collenchyma
pe - periderm s - sclereids

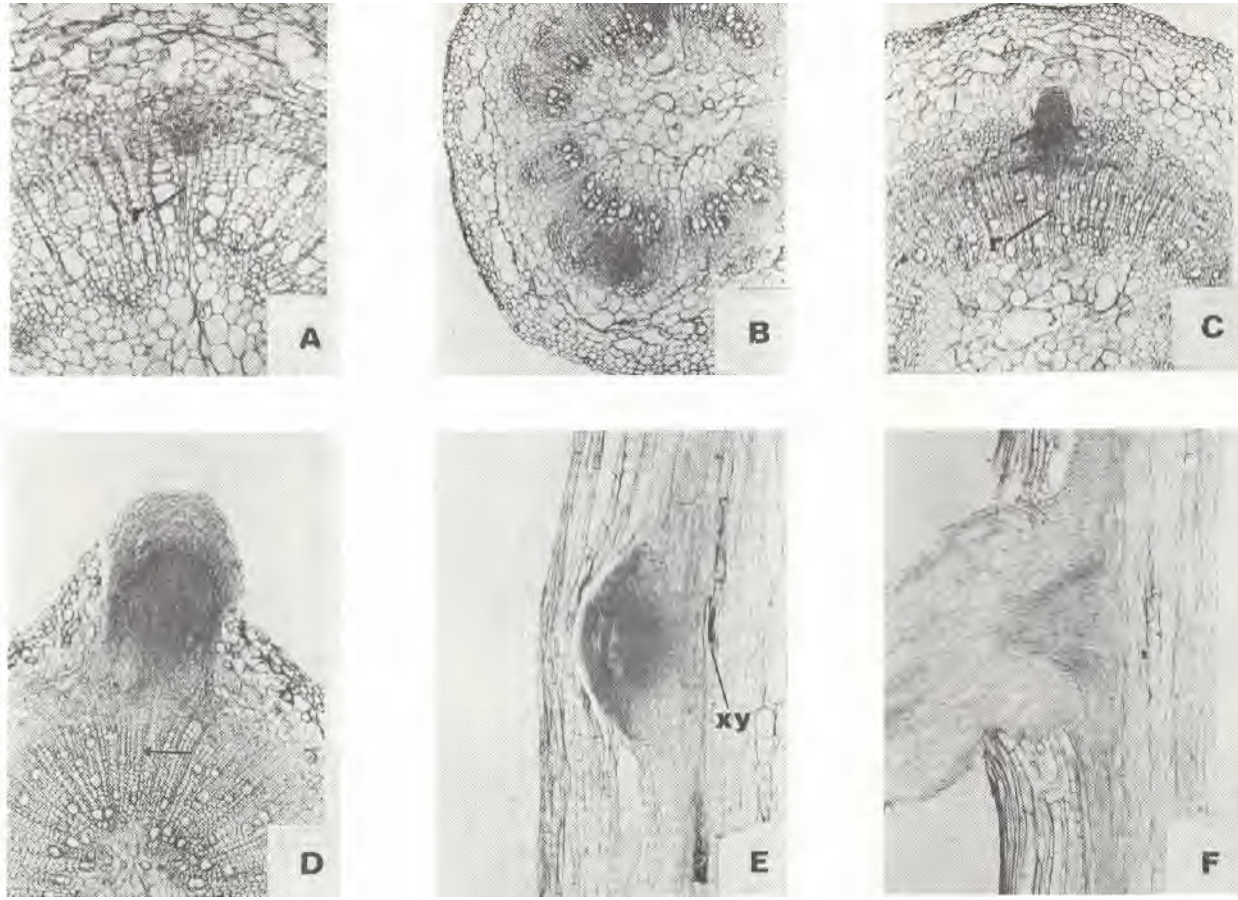

Figure 2. Root formation in sucker cuttings rooted in distilled water

(A) cutting after three days indicating association of root primordium (cells with dense cytoplasm) with ray (X 75). (B) cutting after three days showing primordia differentiating in interfascicular regions (X 60). (C) stem after five days illustrating association between root primordium and ray (X 60). (D) stem after seven days--arrow indicates ray from which the primordium probably originated (X 60). (E) longitudinal section of root sucker cutting after seven days showing the primordium in juxtaposition with the cambial zone (X 60). (F) longitudinal section of a root sucker cutting after seven days with a fully developed root (X 60).

Legend:

r-ray
xy-xylem

Figure 2