Anatomical Investigations on Chestnut Adventitious Rooting

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ABSTRACT. Research was carried out to study the effects of girdling and etiolation on the anatomical structure and on adventitious rooting in shoots of `Marigoule' *(Castanea crenata x C. saliva)* stoolbeds. The treatments were: i) "girdling," ii) "etiolation," iii) "girdling and etiolation," and, iv) "control." Girdling was made 2-3 cm above the shoot insertion on the stool, and etiolation consisted of covering the stool with a mound of soil. Samples for the anatomical observations were taken immediately above the girdle [treatments (i) and (iii)), or in a similar position on the nongirdled plants (treatments (ii) and (iv)].

Only the shoots of treatment iii, girdling and etiolation, formed adventitious roots (69.3% rooting). With regard to the anatomical structure, our observations were: 1) etiolation, apart from a larger accumulation of starch grains, did not result in substantial differences in shoot anatomy, when compared to the control; 2) girdling (treatments i and iii) stimulated cell division and enlargement, especially in the cortex, which resulted in a swelling just above the girdling point, and in the production of multiseriate xylem rays, particularly in treatment iii; 3) in the latter treatment, reduced tissue differentiation also was noticed, when compared to treatments ii and iv, and the formation of poorly differentiated cortex sclerenchyma cells, that were not arranged as rings, but rather grouped in irregularly shaped structures; and, 4) root primordia, in the early stages of their formation, were present in the youngest phloem, next to multiseriate xylem rays of shoots of treatment iii.

The research showed that root formation took place over a fairly long time period, and was associated with some anatomical changes occurring in the interested shoot zones. Separation of the effects of etiolation from those of girdling in the shoots treated with both girdling and etiolation was not possible, therefore a synergistic effect of these two treatments on rooting in chestnut was hypothesized.

Chestnut is commonly propagated by budding or grafting on seedlings, although completely asexual propagation techniques would be welcome. Propagation by cuttings so far has given very poor results, at least on a nursery scale. More promising is the technique of stooling, a procedure already used for the commercial propagation of rootstocks and, occasionally for cultivars of several fruit species. In chestnut, the success of this technique also has required the application of growth regulators and, more recently, basal shoot girdling (1, 2, 4, 5, 9, 11, 12, 13, 14). Besides biochemical factors, the poor rooting ability of chestnut also seems to be related to the anatomical characteristics of the shoot; the existence of a correlation between abundant sclerification of the cortex and poor rooting of cuttings has been postulated (11). The sclerenchyma sheaths, that are present in the shoot during early growth, might indeed act as mechanical barriers, although it has been shown that in this species, the newly formed roots cause the disaggregation of these tissues in the course of growth. The resulting roots then may appear, to a certain extent, to be strangled by the sclerenchyma rings, but their emergence does not appear to be hindered in any way (10).

This research was undertaken to study the modifications induced in shoot structure by girdling and etiolation, and the consequent formation of adventitious roots.

MATERIALS AND METHODS

In the spring of 1990, 100 healthy stoolbeds of a clone of `Marigoule' (a *Castanea crenata* Sieb. and Zucc. x *C. saliva* Mill. hybrid) were chosen, and randomly subjected to the following treatments: 1) "girdling and etiolation," 2) "etiolation," 3) "girdling," and, 4) untreated "control." Girdling was made with a plastic-covered metal wire, 2-4 cm from the shoot insertion on the stool. Etiolation of the lower part of the shoots was accompanied the same day (20 June) by covering the stools with a 10-20 cm thick soil mound. Rooting was evaluated at the end of the growing season (30 November) for all treatments.

Anatomical observations were made on sections from samples taken on 23 May, 28 June, 25 July, 9 August, 21 August, 3 September, 25 September and 5 October. The samples were collected each time from shoots with average vigor and consisted of the 4-5 cm portion above the girdle, or in the analogous position in "control" and "etiolation" treatments. The material was fixed in FAA and dehydrated in an ethyl alcohol series, then embedded in a prepolymerized resin, a mixture of butyl- and methylmethacrylate (7:3). Sections were stained with either PAS (Periodic Acid-Shift's reagent) or acid fuchsin, and counterstained with toluidin blue (7).

RESULTS

Rooting. Of all the treatments, only the "girdling and etiolation" shoots produced roots. They could be seen first emerging from the portion immediately above the girdle on 3 September, 75 days after the treatment. The shoots of the treatments that included girdling developed a hyperplasia just above the girdle, that usually was much

larger in the "girdling" treatment than in "girdling and etiolation."

Anatomical Features. *Control.* By May, in the untreated shoots, two sclerenchyma rings already were visible in the cortex. The outer and older ring were already well differentiated and continuous, while the inner one was discontinuous. During the vegetative season, continuous sclerification took place both in the xylem and in the phloem. In the xylem, a secondary thickening occurred in some cell walls (reaction wood) (3), that was unevenly distributed over the xylem. In the phloem, up to four sclerenchyma sheaths were formed in addition to the pericycle sheath (Figure 1). The only pericycle sheath was rich in sclereids, and showed an uninterrupted ring. Xylem rays were strictly uniseriate. Phellogen activity became apparent in October. Druses and prismatic crystals of calcium oxalate were present in the most recent phloem in the proximity of differentiating fiber bundles.

Etiolation. Unexpectedly, very few differences were found in the etiolated shoots, when compared to the control shoots. The most important feature appeared to be a more pronounced activity of deposition of insoluble substances, such as druses in the phloem and polyphenols and starch in the medullary rays, especially toward the end of summer and the beginning of autumn.

Girdling. The effects of this treatment on shoot anatomy became visible at the end of July and beginning of August (35-40 days after girdling). In the recently produced xylem, the rays became multiseriate while the pericycle started swelling. The latter event was due to two different proliferative processes that take place in two different locations of the cortex. One occurs among the cells bordering the pericycle ring, both internally and externally, producing large, thin-walled cells, with large druses and ample intercellular spaces. The other originates among the outer pericycle cells where the phellogen would eventually form, to produce smaller cells, often containing tannin deposits, with smaller intercellular spaces and no druses. There usually were two sclerenchyma rings and during the rest of the season no additional sclerenchyma was produced, with the exception of poorly differentiated, irregularly arranged, short fiber bundles. The outer ring (pericycle ring) was shattered in most samples by the cellular outgrowth. Xylem activity following girdling was characterized by abundant production of parenchyma cells and by production of few vessels, often tangentially arranged with reference to the shoot axis. The cells of the multiseriate xylem rays contained polyphenolic inclusions, a feature that was not observed in the first two treatments. By October (day 103), the pericycle appeared to be completely crushed by the outer cortex's proliferative activity. Polyphenols were abundant in both the phloem and the pericycle, and most tissues appeared necrotic (Figure 2). At this time the anomalies described for the xylem were even more marked. The described effects of girdling, however, were only visible in the bulge tissues. The shoot gradually resumes its normal anatomy 2-3 cm above the girdle in all the examined tissues.

Girdling and etiolation. Some anatomical changes noticed in the samples of the "girdling" treatment also could be found in this treatment. We will therefore confine our comments to the most peculiar anatomical events, with special reference to those leading to adventitious root formation. On 28 June, 8 days after the treatments, the shoot structure did not yet appear affected, although the first signs of a reaction were visible at this time. In the cambium, the ray initials started appearing in couples, and some dividing cells were seen next to the pericycle ring. One month later (25 July) xylem cells formation appeared to be even more affected, and a large amount of parenchyma cells was formed by the cambium. The rays were often bi- and triseriate, and comprised of very large cells. In the pericycle, mitotic activity continued, and appeared to be extended to the subepidermic cells. In the area between cambium and sclerenchyma, the activity of cell division was intense, but no other rings were formed. In their place, groups of poorly lignified fibers, irregularly distributed and sometimes very large were produced by the cambium (Figure 3), as were abundant, crystal-rich, parenchyma cells. In many samples, the cambial zone became difficult to detect in the later half of August when cambial activity in the phloem/xylem transition zone became chaotic, and different cell types were often produced without recognizable order. Adventitious root formation at this time was already advanced. Initials in the early stages of meristematic division (Figure 4) and primordia at different stages of growth (Figures 5, 6 and 7) were present. Observation of the smallest groups of initials located their origin among the recently formed and poorly differentiated phloem cells. These meristematic foci usually coincided with phloem rays, so that the first initials were considered to be undifferentiated phloem ray initials. The root primordia, which initially were made of only a few cells, could be distinguished from the surrounding tissues for their smaller size and thinner walls. They were bordered by druse cells that eventually elongated and crossed the cortex tissues. Cells of the root primordia continue to divide and differentiate, and were soon arranged in organized tissues, before root emergence (Figure 8).

DISCUSSION

Unlike etiolation, the histological alterations induced by girdling were evident in the shoot portion immediately above the girdle. The most important modifications that started only a few days after the treatment, are: interruption of the rhythmic production of sclerenchyma rings, and their substitution with less differentiated structures; passage of the medullary rays from uniseriate to multisedate; and, exceptional increase in the hyperplastic cortex of the parenchyma tissues. The site of adventitious root formation, was the same as that reported by other researchers for etiolated plum shoots (6), and for etiolated and hormone-sprayed chestnut shoots (8).

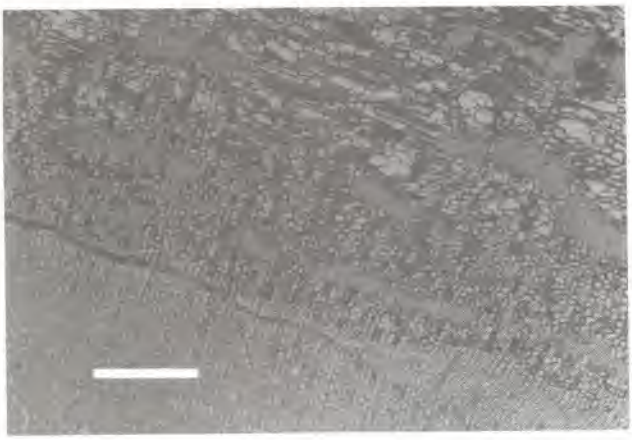

Figure 1. Control, 25 September. Transverse section of the cortex; five sclerenchyma rings are visible. Bar = 200 μm.

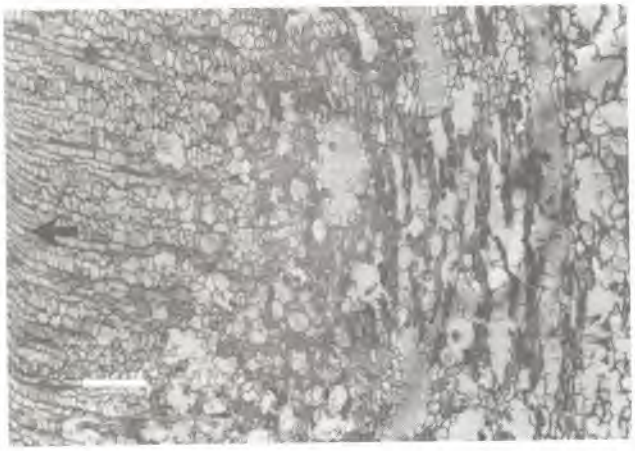

Figure 2. Girdling, 25 September. Typical parenchyma produced by the cambium (arrow) after the girdle. Bar = 100 μm.

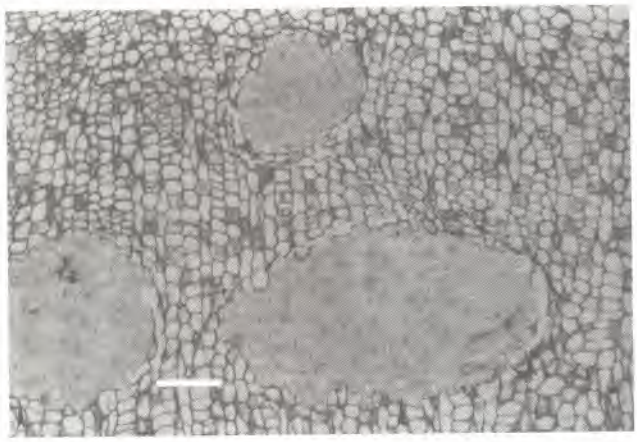

Figure 3. Girdling and etiolation, 21 August. These groups of poorly differentiated fibers appear at random within the phloem parenchyma; the most peripheric cells contain prysmatic calcium oxalate crystals (arrows). Bar = 100 μm.

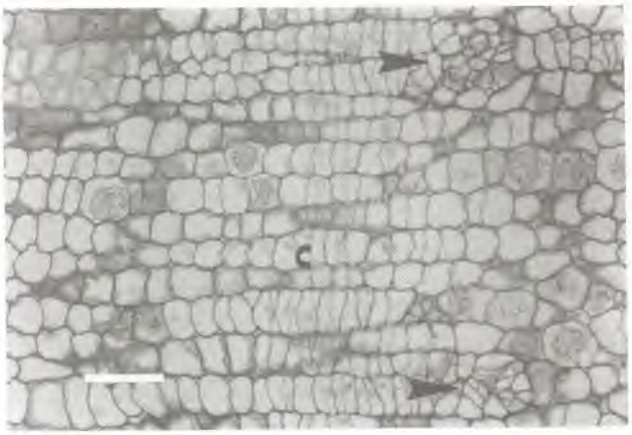

Figure 4. Girdling and etiolation, 5 August. In at least two points (arrows) differentiating phloem cells have resumed mitotic activity to form meristematic foci. Druses also are present in the xylem parenchyma. Cambium = C; bar = 50 μm.

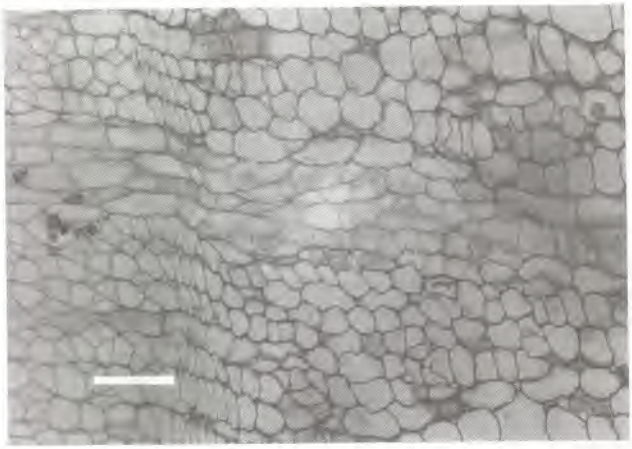

Figure 5. Girdling and etiolation, 21 August. Early cell arrangement in a young primordium, positioned next to a large xylem ray. Bar = 50 μm.

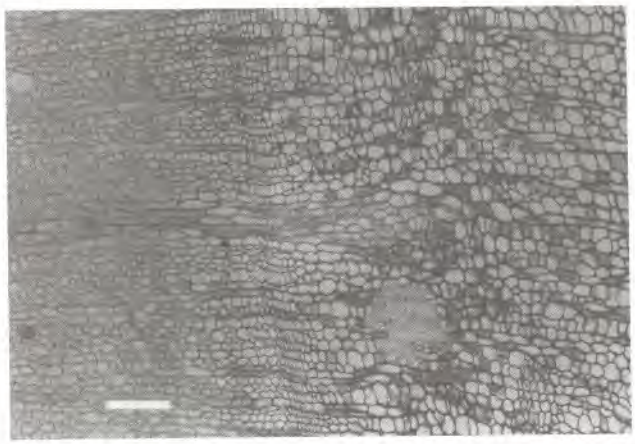

Figure 6. Girdling and etiolation, 21 August. Another developing root primordium; its border cells appear rich in druses. Bar = 100 μm.

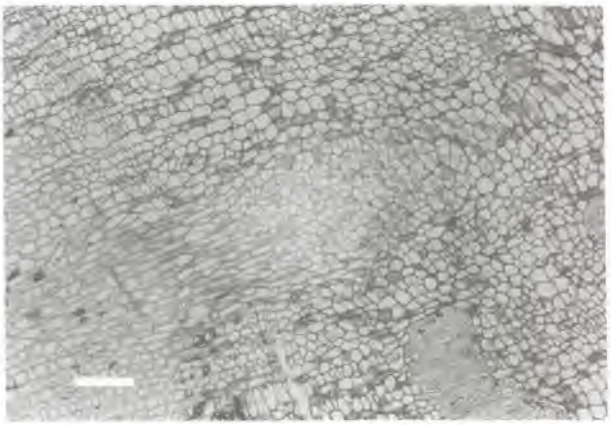

Figure 7. Girdling and etiolation, 21 August. An older and larger root primordium; differentiating vessels with new orientation are already visible in the xylem. Bar = 100 μm.

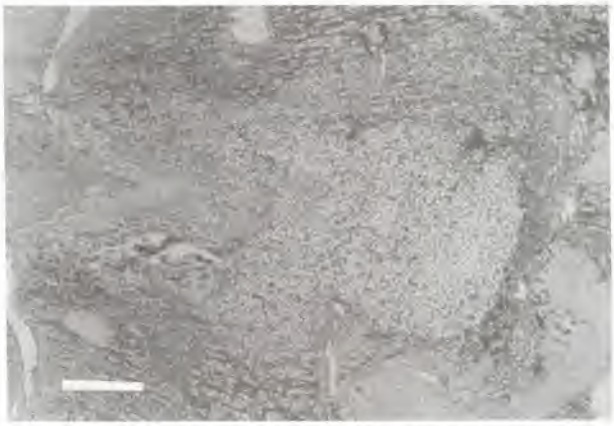

Figure 8. Girdling and etiolation, 5 October. Root primordium emergence. The sclerenchyma is completely shattered and most of the remaining cortex is necrotic and detached from the shoot. Bar = 500 μm.

Therefore, it appears that the anatomical conditions characterized by abundant parenchyma and storage tissues, and scarcity of sclerenchyma, may favor the formation of new roots on chestnut shoots. Although such conclusions are confirmed in the literature, these features, in our material, also were present in shoots that did form root primordia ("girdling"), and therefore cannot explain alone the occurrence of the event. For satisfactory rooting activity, girdling must be followed by etiolation, a treatment that did not show any relevant anatomical effect on our material if applied alone. In the "girdling and etiolation" treatment, therefore, biochemical effects must be added to the anatomical ones. Thus, all effects cannot be studied with the microscope alone. An interesting approach to the problem would be to study the role of starch and of some secondary products, such as polyphenols and calcium oxalate, on root-forming tissues.

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