The Lack of Rootability of Chestnut Cuttings

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ABSTRACT. Mature chestnut cuttings do not root, preventing their vegetative propagation by this method. There is a general agreement that the lack of rootability of chestnut cuttings is due to the presence of root inhibitors, detectable by the ABA-sensitive *Avena* and *Phaseolus* tests. Direct evidence comes from experiments comparing water extracts from *Salix viminal is,* which is an easy-to-root species, and from *Castanea saliva.* Whereas the willow extracts increased rooting, the chestnut extracts caused significant inhibition. It is suggested that willow extracts contain an IAA synergist and those from chestnut contain rooting inhibitors that neutralize the IAA effect.

Mature chestnut cuttings, stored at —10 C for 2 mo, lost their rooting inhibitors. A gradual decrease in inhibition was observed in cuttings stored in the cold from 2-5 mo. Not only are the inhibitors deactivated, converting them to inactive compounds, but new rooting promoters (conyferil and salicelyl alcohols) are formed that are not present in fresh cuttings of chestnut. Juvenile cuttings, which are easily rooted, lack a significant level of root inhibitors, and they have two cofactors that act together to enhance the rooting effect of $IAA^{-5}M$. When juvenile cuttings become 5-8 mo old, those factors disappear and root inhibitors develop. It seems likely that the cofactors could act to protect IAA from destruction, thus increasing the effective IAA available for root initiation. It is possible that phenolic compounds act synergistically by forming an IAA complex more effective than the IAA alone.

Etiolation enhances rooting of chestnut cuttings, and 100% of cuttings tested will form roots when auxins are applied. Etiolation acts locally, suggesting that light favors the synthesis of the rooting inhibitors. Some ellagic derivatives, such as 3,3',4 tri-0-methylellagic acid and 3,4,4'-tri-O-methylellagic acid, act as markers for the lack of rootability of chestnut cuttings.

Interest in rooting chestnut cuttings became apparent after the fungi *Phytophthora cambivora* (Petri) Buism., *P. cinnamomi* Rands. and *Cryphonectria parasitica* (Murr.) Barr, destroyed most of the chestnut trees in Europe and the United States. Consequently, chestnuts resistant to the these fungi were obtained, but it was not possible to propagate the selections from cuttings. The poor rooting character of these cuttings created a real problem that attracted the attention of people from both management and scientific fields. Chestnuts are impossible to propagate by cutting on a large scale, and other conventional methods have difficulties or restrictions. At present, stooling is the only method used for large-scale vegetative propagation of resistant chestnuts. Sometimes new shoots are girdled, as is done in France (53), or treated with auxins (70, 71), or girdled with an auxin application (15).

The long-term goal is still propagation by cuttings. The poor-rooting character of chestnut cuttings has defeated many attempts in this effort (18, 27, 53, 58, 61, 69).

Positive attempts to root mature chestnut cuttings were reported by some authors (36, 38, 72). However, success was rare and the results were of no practical use. These methods might be useful, however, for studying root formation mechanism in chestnut cuttings. The problem has yet to be resolved. *In vitro* culture is another tool, which correctly handled, may be a real solution for vegetative chestnut propagation after proper hardening of the plantlets.

The mechanism of root formation in woody cuttings continues to be an obscure problem for plant physiologists. Why the cuttings of some woody species root easily and others not at all continues to be a question without a satisfactory answer.

It is accepted that three general factors may be correlated with the control of root formation in woody cuttings:

1. The balance of hormones and cofactors (32);
2. the presence or absence of root-inhibiting substances (2, 7, 14, 51, 59); and,
3. anatomical features such as a ring of sclerenchyma whose absence or incompleteness would make rooting easier (4, 5).

In chestnut, all three factors are found to be involved. Cuttings from mature chestnuts fail to root; they contain rooting inhibitors; and, exhibit a continuous and sometimes double ring of sclerenchyma. Cuttings from juvenile chestnuts root easily when auxin-treated and show further normal growth; rooting cofactors that enhance root endurement by IAA are present, no rooting inhibitors are found and the sclerenchyma ring is discontinuous. Etiolated cuttings root very well when treated with auxins; they do not have root inhibitors and the sclerenchyma ring is simple and discontinuous.

THE ADULT CHESTNUT

Soft and hard cuttings, when treated with auxins during October and November, root poorly. However, no positive response for rooting occurred when auxins were applied during months other than October—November (72). With the improvement of the rooting benches, with better temperature control of the bottom and tunnels provided with mist systems, root formation in chestnut cuttings, specifically soft ones, was possible; but, results were poor and not effective for chestnut propagation. It may help to have a better understanding of the background of the root formation mechanism in these cuttings.

Hard cuttings from mature chestnuts were taken in late October and their basal ends, 3-5 cm, were kept without any auxin treatment in running water, from November until early March. About 40% of the cuttings formed roots (75), and the roots were similar to those formed in some easy-to-root species, like willows, when put in water. However, I was not able to repeat this experiment.

Cuttings from mature chestnut do not root and it was found that they contain growth inhibitor(s) detectable by the ABA-sensitive *Avena* straight growth coleoptile test (76, 77). Root inhibitors also were detected by the *Phaseolus vulgarly* L. test, but it is not easy to establish a clear separation between the inhibitors revealed by the two tests. From methanolic extraction and paper chromatography in IAW, the growth inhibitors were found at Rf 0.55-0.80, whereas the rooting inhibitors appeared at Rf 0.20-1.0, practically throughout the chromatogram.

Both classes of inhibitors seem to play a role in determining the rooting capacity of chestnut. In particular, there is evidence that root initiation seems to be controlled by the level of inhibitors present in the cutting. When mature cuttings were left in the open with their basal end in running water they rooted at approximately 40%, as reported previously. In this test, the paper chromatogram from the methanolic extract did not reveal the presence of the growth inhibitor(s) present in the mature cuttings (78); substantial amounts of vanillic and salicylic acid were found, which are growth inhibitors for coleoptiles at levels higher than 150 g/1. Furthermore, an unidentified hydroxyaliphatic acid was found and seems to be a growth inhibitor for chestnut. The long immersion of the cuttings in running water perhaps eluted the aliphatic acid from the cuttings. Similar results were reported in cacao cuttings by Nandorf (45) and by Spiegel (56) who reported that difficult-to-root cuttings rooted easily after leaching.

Rooting inhibitors have been reported by Coyama (7), who found that aqueous extracts from the difficult-to-root *Castanea crenata* Sieb. and Zucc., *Pinus densiflora* Sieb and Zucc., *Myrica rubra* L. and C*yptomeria japonica* (L. f.) D. Don. suppressed the normal rooting capacity of *Salix babylonica* L. another easy-to-root species. Barlow et al. (2) extracted a coleoptile elongation inhibitor from shoots of plum rootstock "Myrobalan B." Paton et al. (46) suggest that in the ontogenic aging of *Eucalyptus grandis* W. Hill seedlings, there is a direct quantitative relationship between decreased rooting capacity and increased levels of three rooting inhibitors, identified by Crow et al. (8) as derivatives of 2,3-dioxabicyclo (4,4,0)-decane.

Two compounds correlated with lack of rooting capacity of chestnut cuttings were found by Vieitez et al. (85). Analysis of fresh bark extracts of mature chestnut carried out by HPLC in a MeOH-H20 (60:40, v/v) solvent system, consistently showed two peaks with retention times of 11 and 15 min that were not present or present only in very low concentration in the chromatograms of juvenile or etiolated specimens. The experiments have been repeated over 3 yr with similar results. Isolation of the two compounds was most effectively carried out by cellulose chromatography and final purification on a Chromatotron. The compound at the 15 min retention time was identified as 3,3 ',4-tri-0-methylellagic acid; the second peak, at 11 min retention time, yielded 3,4,4-tri-0-methylellagic acid. Only the compound, 3,3 ',4-tri-0-methylellagic acid, was obtained in sufficient quantity in pure form to allow a test of its biological activity by means of the bean rooting assay. The authors found that 3,3 '4-tri-0-methylellagic acid inhibited the rooting bean cuttings at a concentration of 3.0×10^{-5}M. It caused 32% inhibition compared with rooting of controls grown in distilled water and 40.9% inhibition compared with controls grown in 10^{-5}M IAA. When mixed with an equal quantity of that compound, the root-promoting activity of IAA was reduced by 31.5%. This compound not only reduced the number of roots formed but also affected their arrangement in the hypocotyl, which was less uniform than the symmetric radial array produced by treatment with water or IAA. When that compound was applied, roots emerged mainly from the base of the cuttings.

EFFECT OF ETIOLATION ON CHESTNUT CUTTINGS

Etiolation is defined as the total exclusion of light (43). In 1923, Reid discovered that etiolation accelerates the root formation in cuttings (50). One of the external factors known to influence rooting in cuttings, is the inhibitory action of light. Shaphiro (54) found that in certain species of *Populus,* the development of root primordia already formed on cuttings was interrupted by exposing the cuttings to light. In 1937, Gardner viewed etiolation as a method of great utility as a tool for plant propagation of woody cuttings (20). His method to induce etiolation has remained practically unchanged, although modified in several ways.

Gardner (20) reported that, in some difficult-to-root varieties of apple, cuttings taken from etiolated shoots rooted better than those from non-etiolated controls, and similar results were obtained by Leonova (41). Herman and Hess (29) likewise improved the rooting rates of certain varieties of *Hibiscus Rosa-sinensis* L. by prior etiolation of the cuttings.

Vieitez and Vieitez (80) reported that when basal, medial and apical segments of current year adult chestnut shoots were etiolated with aluminum foil, abundant root formation occurred at the base but not in the other two parts of the shoots. Simultaneous application of exogenous auxin greatly reinforced the effect of etiolation with large numbers of roots being formed wherever the etiolated shoots received auxin treatment. Rooting rates of 94, 100 and 79% were obtained for shoots treated at the base, medial segment and apex, respectively.

Vieitez and Ballester (82) found that the cuttings from adult chestnuts, well-known for their poor rooting character, are changed if previously etiolated. To show this, the authors etiolated shoots in mid-May by wrapping their apical 6-12 cm in aluminum foil, and left others non-etio-

lated. In October, cuttings were taken from both etiolated and non-etiolated shoots and the wrapping removed from the former. Four 20-cutting lots from each group were treated with talcum powder containing 4mg/g IBA + 4mg/g NAA. The treatment was applied to the previously etiolated region or to the corresponding part of the cuttings that had not been etiolated, with the treated area downward. Etiolated and non-etiolated controls were similarly planted in perlite in a rooting chamber with controlled temperature and misting. The rooting results were observed the following April (Table 1).

Table 1. Rooting results from etiolated and non-etiolated controls, with and without auxin.

	Rooting Percentage	
Cuttings	+Aux	–Aux
Non-etiolated Controls	2	0
Etiolated	100	33

Etiolated shoots clearly exhibited greater rooting while the controls once again exhibited their well-known lack of rootability (Table 1). Auxin treatment of the etiolated cuttings increased rooting from 33% to 100% without any loss of viability; all roots produced were physiologically normal. Shading of the parent plants also enhanced rooting of chestnut cuttings.

The HPLC chromatograms of control, etiolated and shaded shoots, the initial parts of all three (retention time 0-8 min), exhibit a large number of peaks corresponding to polar compounds, mainly phenols and simple acids such as catechin, caffeic, gallic, p-hydroxybenzoic and vanillic. However, the final segment differs in that the well-defined peaks, which correspond to derivatives of ellagic acid (3,3',4-tri-0-methylellagic acid and 3,4,4'-tri-0-methylellagic acid), are absent in the chromatograms corresponding to etiolated and shaded cuttings. Both etiolation and shading, therefore, seem to prevent the formation of these compounds, which are correlated with the lack of rootability of chestnut cuttings.

Etiolation increased the proportion of stem tissues occupied by undifferentiated parenchyma that is thought to be an intermediate in the initiation of adventitious roots (28). There are many correlations for the presence of sclerenchymatous tissue with the loss of root ability. They suggested that the parent plants be grown under conditions that do not favor the formation of sclerenchymatous tissue, like exclusion of light from the developing shoots.

Etiolation delays lignification of secondary walls and prevents the closure of the sclerenchyma ring (1, 5, 26, 29, 39). Many authors consider that the degree of lignification and the closure of the ring are of prime importance for the rooting capacity of cuttings (4, 21). In consonance with this theory, etiolation facilitates the rooting of chestnut cuttings by delaying the transformation of parenchymatic cells into sclerides (80).

The fact that etiolation acts locally to create zones in the shoot in which the internal conditions allow us rooting, suggests that light favors the synthesis of rooting inhibitors. Kawase and Matsui (40) reported that etiolation did not affect the level of endogenous anxin in hypocotyls of *P. vulgaris.*

Herman and Hess (29) have found no significant correlation between etiolation and rooting cofactors content. Bassuk et al. (3) suggested that the activity of rooting cofactors was attributed to formation of phenol-auxin conjugates, which are more active in stimulating rooting than auxin alone. Phenolics have been implicated as auxin protectors, inhibitors and stimulators of the IAA-0 system, as well as precursors in the synthesis of lignin and suberin, substances implicated as possible anatomical constraints to adventitious rooting (4, 29, 54, 87).

Both etiolation and shading prevent the formation of the ellagic acid derivative that are rooting inhibitors. The formation of these substances therefore seems to require light (82).

The presence of markers for the lack of rootability in chestnut cuttings, 3,3',4-tri-0-methylellagic acid and 3,4',4-tri-0-methylellagic acid, were studied by Vieitez, Ballester and Vieitez (84) in buds and leaves of chestnut trees. At the resting stage and swelling of the buds, high concentrations of these compounds were found; but they dramatically decreased or disappeared at the early stage of the leaf formation. In fully mature leaves and during the growing season until the fall, the concentration of ellagic derivatives was high. It seems probably that their formation occurs in the leaves.

EFFECT OF COLD STORAGE ON CUTTINGS

Cold storage of chestnut cuttings at 4 C affected their content of the two ellagic compounds (84). Lignification of the sclerenchyma ring cells of chestnut cuttings does not seem not to be affected by cold storage, as reported by Diaz et al. (11). He found that the percentage of lignin and the degree of the sclerenchyma lignification was similar in both cold-stored and ambient control cuttings. The endogenous growth promotor and inhibitor content in the cuttings was affected by storage at —10 C for 8 mo, as reported Vazquez and Gesto (65) and determined by the *Avena* coleoptile straight-growth test. After cold storage, they found that the inhibitors present in extracts from freshly collected cuttings disappeared and its effect on the stimulation of rooting by IAA was nullified, but the level of growth stimulation was increased. They suggested that the inhibitors are degraded at low temperature. A decrease or loss of inhibitors by low temperature also has been reported for other species (55).

Changes in the rooting inhibitory effect of chestnut extracts during cold storage of cuttings were reported by Gesto, Vazquez and Vieitez (25). A gradual decrease of the inhibitory effect was evident when cuttings were stored at —10 C for more than 2 mo. After 5 mo of storage,

no counteraction of the IAA effect on rooting was observed. It was suggested that the inhibitor(s) could be converted to inactive compounds during the cold storage or that the formation of rooting promoters also were produced. The cold stored cuttings contained vanillyl, salicyl and coniferyl alcohols that were not detected in the extracts from freshly collected cuttings. Vanillyl and salicyl alcohols are promoters of rooting (63). Cold storage altered the balance between inhibitors and promoters and these findings are in accordance with those obtained with seeds (86). Diaz et al., (12) found increased rooting capacity of chestnut cuttings, as measured by the *P. vulgaris* test, after storage at 4 C for 4 mo.

JUVENILITY AND ROOTING OF CHESTNUT CUTTINGS

According to Leopold (42), "physiologically, the juvenile state can be described as a period when the plant is capable of exponential increase in size, when the plant develop characteristic morphological forms (leaves, stems, horns, etc.)." It should be emphasized that the juvenile period is not a period in which the plant is necessarily devoid of the ability to flower.

Physiological expressions also are involved in juvenility and one of the most important is that related to rootability of cuttings. For some woody plants that root poorly, cuttings taken in the juvenile stage often root more easily than those taken from mature plants. This was known by plant propagators before the concept of juvenility became generally accepted. This favorable effect on root formation has been reported by some authors (19, 31, 74). According to Hess (33), the higher rootability of juvenile cuttings of *Hedera helix* L. as compared to the mature form, might be understood because an increased amount of the rooting cofactors. Hess was not successful in identifying any of the cofactors.

Raviv et al. (48), have isolated and identified from avocado tissues, four rooting promoters with an acetylene moiety; 1,2,4-trihydroxy-n-heptadecyl is the most active. These accumulate faster in the base of avocado juvenile cuttings during rooting than in mature cuttings and difficult-to-root cuttings. Raviv and Reuveni (49) have reported that these non-auxinic promoters are able to induce rooting of cuttings from juvenile and mature (1-yr-old) avocado plants.

Gesto, Vazquez and Vieitez (23) previously reported a lack of endogenous growth inhibitors in young plants of chestnut as compared to adult plants. Moreover, in a rooting test, the water extracts from adult plants counteracted the stimulating effect of IAA on rooting. Cuttings taking from 3-6-mo-old plants treated with some auxins, rooted, whereas those from adult chestnut did not root. To explore the relationship between the inhibitors and the rooting ability of chestnut, the presence and level of inhibitors that counteract the IAA activity on rooting was tested in plants ranging from 3-12 mo old. For this purpose, the chromatograms of the ether fraction from an aqueous extract of the plants were bioassayed in presence of IAA using a *Phaseolus* rooting test. Simultaneously, rooting capacity of cuttings from plants of 3-9 mo was evaluated. The cuttings were treated with a mixture of NAA and IBA and left in a mist propagation frame for 30 days.

The rooting test revealed the presence of a compound(s) that counteracted the effect of IAA on rooting in plants older than 5 mo. It was located at Rf 0.2-0.4 in the chromatograms developed in isopropanol:ammonia: water (10:1:1, v/v). Its activity was only manifested in presence of IAA as it had no effect on rooting by itself. The highest level of this inhibitor was found in cuttings from 9-mo-old plants, collected in January, when they are in a dormant condition. It was not detected in older plants after bud break and leaf expansion.

Results also showed the presence of substances that acted synergistically with IAA in the rooting of bean cuttings. They were named cofactor Cl and cofactor C2 on the basis of their position on the chromatograms. The highest synergistic effect was found in the youngest plants. Both cofactors disappeared in 7-mo-old plants when they entered the dormant condition. After the winter-resting period, in plants older than 9 mo, the synergistic effect was only observed in the zone of cofactor Cl.

Juvenility appears to be one of the most important factors in rooting cuttings. According to Doorenbos (13), "the juvenile phase in woody plants is characterized by a greater readiness to form adventitious roots." It seems that the internal conditions of these cuttings are optimally conducive to adventitious root formation. Chestnut does not root in the adult phase but does in the juvenile phase. Thus, juvenility is desirable for vegetative propagation and the feasibility of this depends on the availability and maintenance of juvenile material. The juvenile phase of growth can be extended by growing seedlings under conditions of low light intensity. Cuttings that are easily rooted can be taken from shoots sprouting from stubs after trees have been cut, an area that should produce juvenile shoots. Research on endogenous rooting inhibitors and cofactors in juvenile plants will hopefully reveal why the juvenile phase of growth is favorable to rooting and why exogenously supplied auxin is more effective under these conditions. To this end, a study was undertaken on the rooting effect of the extracts of cuttings from plants in the juvenile phase of growth (24).

The cutting donors were: 3-mo-old plants growing in a greenhouse; 5-mo-old plants growing outdoors under natural light or under light reduced by 75%; 8-mo-old suckers from stubs of young plants growing in pots in the greenhouse; and, 8-mo-old suckers from stubs of old plants growing outdoors. The rooting activity of the extracts was tested as before. The ether fraction from an aqueous extract was chromatographed and the chromatograms bioassayed in presence of IAA by using a *Phaseolus* rooting test. The rooting capacity of the cuttings was simultaneously evaluated. The cuttings were treated with a mixture of NAA and IBA and left in a mist propagation frame for 30 days.

The histograms of the extracts from cuttings that showed high rootability did not show the inhibitor that counteracts the effect of IAA on rooting. Histograms from 5-mo-old plants grown under natural light, and whose rootability was only 7%, exhibit a pattern very similar to that of the adult plants that do not root. Moreover, the histograms from cuttings with high rootability showed the presence of compounds that act synergistically with IAA on rooting. This effect was very high in the cuttings from 3-mo-old plants whose rootability was 80%. These results seem to show that the juvenile phase of growth in chestnut is characterized by the absence of the inhibitor that counteracts the effect of IAA on rooting and the presence of rooting cofactors.

Vazquez and Gesto (65) found that the juvenile condition in chestnut (easy rooting), is associated with high levels of endogenous rooting promoters and the lack of compounds that counteract the effect of IAA on rooting. Conversely, the transition to the adult condition seems to occur when the rooting promoter levels fall and inhibitors reach detectable concentrations, as shown in the bean rooting test.

The ease of root formation in the juvenile condition has been reported by some authors, for trees such as *Malus domestica* L. (20, 52, 57, 88), *Ficus pumila* L. (6) *Pinus radiata* D. Don (16, 17, 44, 60) and *Eucalyptus* spp. (9, 34, 46, 47). We have studied the effect of juvenility on root ability of chestnut cuttings (74) and have found that cuttings from juvenile plants root easily. Cuttings from juvenile plants 3-, 4-, and 5-mo old during June, August and September, treated with 0.6% IBA on talc, rooted 20, 54 and 90%, respectively. The roots were fibrous and abundant and after transplanting into soil, the cuttings grew normally. An attempt was made to determine the growth substance and inhibitor content and the changes that might occur in these substances during the juvenile-mature phase change (81). Hardwood cuttings from mature chestnut and cuttings from juvenile plants were extracted with methanol and fractioned into acid, alkaline and neutral fractions. The biological activity of compounds separated on the chromatograms was determined by *theAvena* coleoptile straight growth test.

Growth promoters and inhibitors were found in the acid fraction extracted from the basal part of each type of cutting (77). A non-significant inhibition was found in juvenile cuttings but a markedly significant inhibitory zone occurred in mature cuttings. Active zones with the same Rf as IAA are present significantly in chromatograms from the easy-to-root juvenile cuttings. Chromatograms of extracts from the mature cuttings had larger zones of inhibitors that occurred at Rf 0.43-0.90. It is difficult to say how the rooting of chestnut cuttings is governed by growth promoters, growth inhibitors, or by a balance between both types of substances. Some results support the idea of a prominent role for growth inhibitors explain the lack of rootability of the mature chestnut cuttings. The presence of the ellagic derivatives, 3,3 ',4 and 3,4,4'-tri-O-methylellagic acid that were isolated from these cuttings and that are root inhibitors for the bean test (85), support the idea that the inhibitors play a main role in the difficult-to-root mature chestnut cuttings. The two derivatives of ellagic acid are present at low concentrations in the HPLC chromatograms from juvenile cuttings and, as we have reported previously, both compounds are present significantly in the mature cuttings (84).

ANATOMICAL CHANGES DURING ROOTING

A correlation between extensive sclerification and poor rooting of cuttings has been reported by several authors, suggesting that the sclerenchyma bands in cuttings constitute a mechanical barrier for emerging advent titious root primordia (4, 5, 26, 80). Chestnut cuttings have a sclerenchyma sheath bordering the phloem, the formation of which seems to be correlated with the lack of rootability of cuttings from mature trees.

Transverse sections of juvenile cuttings from 1-3-mo-old chestnut seedlings have extraxylary sclerenchyma formed by fiber strands separated by small groups of parenchymatous cells. As shoots increase in thickness by secondary growth, the fiber bands become more separated by parenchyma cells formed between them (81).

Living protoplasts are found in the fibers whose secondary walls are thin and poorly lignified. When the seed. lings are 4-5 mo old, sclerids become apparent by secondary sclerosis of ordinary parenchyma cells situated between the fibers bands. Besides the sclerenchymatous sheath bordering the phloem, differentiation starts in the secondary phloem of tangeal plates of fibers arranged in radial series of cambial derivatives. As previously stated, cuttings at this stage are still very easy-to-root.

At the phase-change and when cuttings lose their easy rootability, the transverse sections of cuttings showed that extraxylary sclerenchyma forms an inner and outer ring, the former discontinuous and the latter practically con. tinuous. Fibers and sclerids have multilayered and entremely thick secondary walls; the sclerids are more lignified than the fibers. The anatomical changes during the *in vitro* chestnut regeneration were studied by Vieitez and Vieitez (67). Systematic analysis of transverse microscope sections from root development cultures between day 0 and day 23 revealed a sequence of four anatomically distinguishable structures, leading to meristemoid root primordium with its own vascular system, and advent'. tious roots. Significant cellular changes occurred as early as 2 days after initiation of the culture on the IBA medium. At this time, root initial cells can be observed in the phloem parenchyma and vascular rays to be dividing, giving rise to cells of smaller size with larger nuclei and dense cytoplasm, as they have reverted to the meristem atic state.

From the fourth day, more patent anatomical changes are observed. The number of dividing cells rise, so that the masses of meristematic cells form in the phloem region near the cambium. This cell proliferation becomes en-

tremely pronounced and could be identified as "meristemoids." They consisted of meristematic-like aggregations of smaller, isodiametric cells that are non-vacuolated with densely staining nuclei and cytoplasm. The meristemoids proceed to become individualized (day 6) and polarization of the divisions give rise to the typical pointed shape of the root primordium (day 8). At this stage they are generally located at the level of the sclerenchyma ring advancing through the shoot tissue. By day 9-10, many primordia have crossed the schlerenchyma ring and their distal ends have entered the cortex. Displacement of groups of sclerenchyma fibers is occasionally observed as a result of the pressure exerted by the root primordia. By day 11-12, most of the primordia are developing their own vascular system and the link-up between the main vascular system and that of primordia begins to be affected. The vascular bridge develops from tissue adjacent to the proximal end of the young root; vascular connections are virtually completed before roots emerge by 12-14 days.

During the development of adventitious roots, gallic acid and the tannins castalin, castalagin and hamamelitannin were identified. But, an unidentified compound was present at day 0 and disappeared 2 days later (A.M. Vieitez, personal communication).

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