VEGETATIVE PROPAGATION OF CHESTNUT

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ABSTRACT

A review is made of the principal methods used for the vegetative propagation of chestnut (**Castanea** spp.). Results obtained by classical grafting, nursery grafting, topworking and budding are reported, as well as those from special techniques such as nurse seed grafting, juvenile tissue grafting, inverted radicle grafting, and layering. For stooling, the stage of growth of shoots is discussed as well as the effect of maleic hydrazide and the effects of etiolation caused by wrapping shoots with aluminium foil. For cuttings, the effect of juvenility, ageing, auxins and growth inhibitors are examined. The physiology of rooting is discussed, and the content of growth promoters and growth inhibitors in different types of cuttings is reported. A possible relationship is also suggested between the rootability of cuttings, their anatomical features and their content of growth promoters and growth inhibitors.

INTRODUCTION

The selection of chestnuts (*Castanea* spp.) which are resistant to blight or ink diseases, and the necessity of propagating them asexually, created one of the most complicated forestry problems of recent decades and attracted the attention of many people from both management and scientific fields.

GRAFTING

Grafting in chestnut has met with only limited success (Turner, 1964; Shafer, 1966). One of the main causes of failure is incompatibility between stock and scion. Reasons for this are many: lack of winter hardiness of stock-scion, chestnut blight in the graft union, poor grafting techniques, and the use of different strains or species of chestnut for stock and scion. There is probably no one cause of incompatibilty in this genus (McKay and Jaynes, 1969).

Nursery Grafting

The most satisfactory nursery technique is splice grafting, although whip grafting is used by some workers. Woodroof (1967) recommends that the root stock be allowed to leaf out fully before grafting dormant scions. The results of an entire season may be lost if grafting is done too early because buds may be killed by a late spring frost.

Kajiura (1955) reported that grafting chestnut was considered difficult, and only 40-50% success could be obtained. After further studies, however, he showed that 80-90% success could be expected in Japan if the following conditions were observed:

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scions should be collected by the middle of February (late winter) and stored below 5 °C, preferably at 2-3 °C; grafting should be done about mid-April (spring) when stock has sprouted and can be peeled.

Topworking

The veneer crown graft has been reported as quite successful as long as sufficient single branches are left on the stocks to draw the sap (Graves, 1956). The main branches of the root stock tree are sawn off some distance above a fork, and one or more scions are inserted in the cut branches (Nienstaedt and Graves, 1955; Graves, 1956).

Budding

Chestnut budding has generally met with failure for reasons that are not fully understood (Jaynes, 1969). This technique is not widely practised because the wood is fluted or grooved, and the cambium of the bud and stock do not join uniformly (Woodroof, 1967). To improve the percentage of takes Hartmann and Kester (1964) recommended inverting the buds.

Nurse Nut Grafting

This technique was developed by Moore (1963), who thought that seed cotyledons would supply some kind of rooting substance which would induce root formation on the scion grafted onto the nut. However, roots are not formed on the scion, but from the differentiation zone of the root of the seedling. While Moore's idea served to develop a series of new grafting methods (Jaynes, 1964; Park, 1968; Beck, 1970), it has not itself proved to be a commercially successful method (Cummings, 1970).

Nurse Seed Grafting

This technique was developed by Jaynes (1964) as a modification of the method proposed by Moore (1963). Essentially it was similar except that nut stocks were used shortly after germination and before the epicotyl had emerged. Good roots were generally established on 60-80% of the grafts within 21 days after grafting. But heavy losses of grafts occurred during the hardening period. Factors responsible for the losses include transplanting shock from disturbing the roots, too sharp a drop in humidity, or incompatibility between stock and scion. As the nut is more than a nurse for the grafted scion Jaynes and Messner (1967) proposed the name "nut grafting" for the method.

Juvenile Tissue Grafting

Park (1967) reported good success in utilising as scion material the new elongating shoot from either germinated seeds or mature trees, and grafting these onto young seedlings. The principal factor affecting the success of grafting was the degree of development of elongating shoot of both stock and scion.

The best grafting stock was when the seedling came to bear about four leaves, and the best grafting scion when the shoot came to bear about four ordinary leaves. It was claimed that juvenile tissue grafting was more successful than nurse seed grafting. In the exposed field bed the optimum depth of planting of grafted plants was 7-8 cm giving a survival percentage of 80% (Park, 1968).

Inverted Radicle Grafting

Inverted radicle grafting is a modification of the nut grafting method (Beck, 1970). The root is cut off 3-5 cm below the hypocotyl, inverted and split down the middle for about 1.5 cm to receive the scion. It is an easy graft; the cotyledons are at the opposite end away from the area when the graft is made and there is no danger of the cotyledons being cut off.

Anatomical studies carried out by Park (1969) showed that the meristematic activity of the embryonic pith of inverted radicle stock was higher than that of the hypocotyl, making union easier. Inverted radicle grafting was more successful than modified nurse seed grafting.

Jaynes and Messner (1967) have pointed out that none of the seed and seedling grafting techniques avoids the general problem of incompatibility, and that transplanting during the growing season as required by many of the methods may diminish survival.

LAYERING

Air-Layering

This technique was unsuccessfully tried by Urquijo (1946), Graves and Nienstaedt (1953), and Nienstaedt and Graves (1955). Shelton (1969) air-layered walnut and this method may be applicable to chestnut. In walnut, auxins assist root formation in air-layers (Vieitez, 1955a). Shreve and Miles (1972) induced root formation in shoots of *Castanea mollissima* treated with 5,000 ppm indole-butyric acid (IBA). Root formation depended largely on the type of twig treated, one-year shoots being the most successful.

Vieitez (1961, 1963 and unpublished) studied the effect of etiolation of shoots on root formation, in order to acquire more information about the rootability of chestnut. By the end of May the basal zones of 75 shoots produced from one clone, usually by stooling, were treated with 4 mg IBA + 4 mg naphthalene-acetic acid (NAA) per g of vaseline and were then wrapped over 15 cm of the shoots with aluminium foil. The treatment was repeated at the medium and top zones of the shoots at one month intervals. At the basal zones, shoots rooted 87%; medium zones 100% and top zones 79% (Figs. 1, 2). The decreased percentage of rooting at the tops is explainable as the twigs were very soft and showed necrotic areas following treatment. Control shoots rooted 41% but only at the basal zone of wrapped shoots.

Layering (by methods other than air-layering) has been tried by Coudrec and Mercel (Solignat, 1964); Nienstaedt and Graves, 1955; Vieitez, 1955b. This method, however, has no advantages over the related stooling method.

STOOLING

This method was first used on a large scale in France with girdling (Schad *et al.,* 1952); later in Spain auxins were used (Vieitez, 1955a), and finally in Portugal (Fernandez, 1972) the method was used with both auxins and girdling.

The rootability of stooled shoots is affected by external factors such as physical properties of soils. Heavy, compact or alkaline soils may reduce or even prevent rooting. To get the best rooting, the auxin concentration must be "adjusted" according to the stage of growth of shoots. Variation in endogenous auxin level could explain the great variability in rooting shown by stooled chestnut (Solignat, 1964).

Effect of Maleic Hydrazide

Shoots with very vigorous growth are difficult to root and so some parent plants became very difficult to propagate by stooling. One of our best selections grows vigorous shoots when stooled, but the shoots root very poorly. The growth rate of shoots was reduced with maleic hydrazide (MH) sprayed on the leaves at rates of 50, 100 and

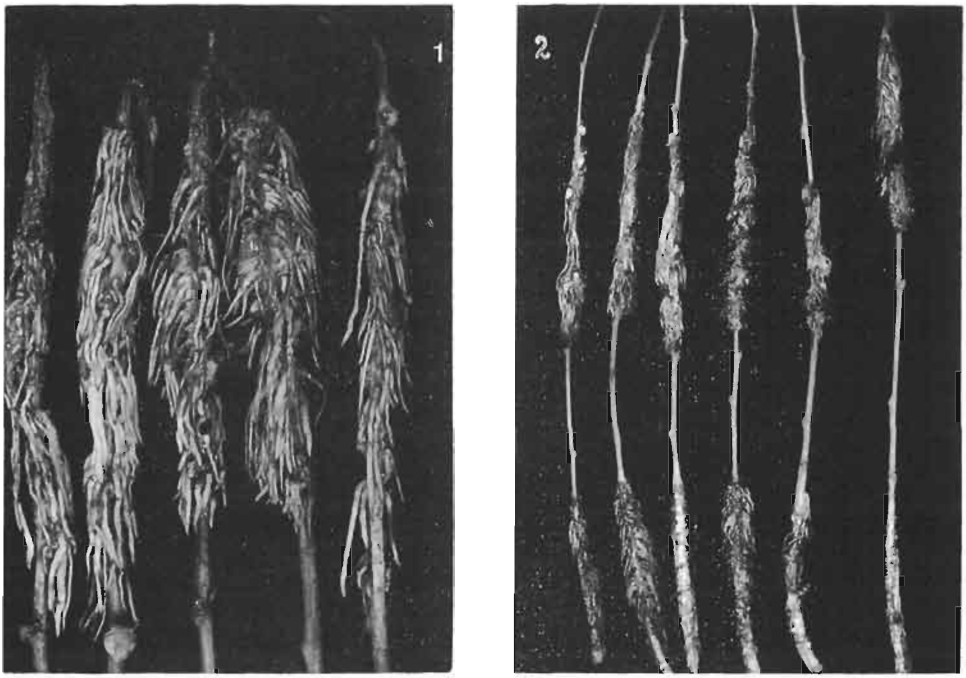


FIG. 1 and FIG. 2—Rooting at different positions on air layers.

200 ppm, just the day after 12 mg IBA plus 0.2 mg 2, 4-D dimethylamine salt was applied to the shoots. The rooting was:— control 0%; auxins alone 9.1%; auxins plus 50, 100 or 200 ppm MH, 13.7%, 15.5% and 20.3% respectively. The rooting percentage was increased as concentration of the growth inhibitor was increased (Vieitez, 1961).

In Portugal, Fernandez (1972) has employed stooling by girdling or applying auxins to the shoots. The rooting percentage was 30.6% for girdling and 17.6% for auxins. Cummings (1970) reports that the automatic girdling system of Gagnon has enough promise to suggest its trials with chestnuts.

CUTTINGS

Cuttings are the long-term goal of chestnut propagators. Failures are reported by Stoutmyer and Close (1946), Urquijo (1952), Schad *et al.* (1952), Vieitez (1952), Graves and Nienstaedt (1953).

However, cuttings may respond positively during October when treated with auxins at the following rates: 4 mg indole-acetic acid (IAA) + 4 mg NAA; 8 mg NAA; 4 mg IBA + 4 mg NAA; 8 mg IBA or 12 mg IBA per gram of talc (Vieitez, 1956). Only a few cuttings rooted, with very tender roots, and they decayed very easily when the moisture content and temperature of the medium was not correct. In contrast to the control, most rooted cuttings did not callus. Callus and root formation are different processes. It is very easy to induce 100% formation of callus in winter by storing the chestnut cuttings in wet peat, sphagnum moss or similar material and keeping them horizontally in the dark. The cuttings' production of callus was markedly polar, with more callus at the apical than the basal end.

Effect of Juvenility

The effect of juvenility on rootability of chestnut cuttings was studied by the writer (Vieitez, 1963) using cuttings from seedlings 3, 4½, 5½ and 6½ months old, treated with 6 mg IBA per gram of talc. Cuttings rooted 20, 54, 60 and 90% respectively. Swelling, which is considered to precede rooting, was 70, 38, 30 and 10% respectively. Roots were fibrous and abundant and after transplanting into soil cuttings grew normally (Fig. 3).

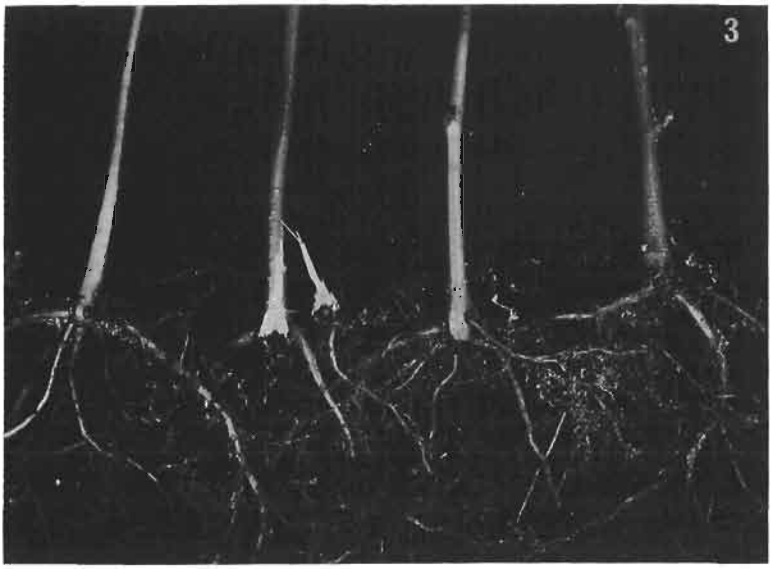


FIG. 3—An abundant and fibrous root system on a cutting following transplanting.

Effect of Setting Date

This has been examined for chestnut cuttings by Moore (1963) who took auxin-treated leaf cuttings at 10 day intervals beginning April 28 and ending July 28. He found that June was the best time; 88% of his cuttings rooted in that month.

Effect of Etiolation

Etiolation may assist rooting in stooled shoots (Solignat, 1964; Shreve and Miles, 1972).

Shoots etiolated with aluminium foil (Vieitez, 1961, 1963 and unpublished) and used as cuttings in November, after applying auxins, rooted as follows:

	Basal zone	Medium	Apical
Etiolated	83.9%	35.0%	3.4%
Non-etiolated	47.8%	23.3%	5.5%

The table shows that etiolated cuttings had an increased rooting capacity. Control shoots showed a decreasing gradient of rootability from the base to the apex. As rooting decreased, the differences in the percentage of rooting between etiolated and normal cuttings was less apparent.

PHYSIOLOGY OF ROOTING

Why some woody cuttings root easily and others are unrootable involves a very difficult problem; three hypotheses can possibly explain it:

a) Presence or absence of some growth inhibitors (Barlow *et al.,* 1961; Coyama, 1962). Coyama obtained water extracts from difficult-to-root plants such as *Castanea crenata.* These extracts contained unidentified growth inhibitors, which stopped root formation in plants such as *Salix babylonica.*

b) Lack of a balanced hormonal level or certain growth cofactors (Hess, 1963). From easy and difficult-to-root cuttings Hess extracted a series of four active root initiation substances which he named respectively cofactors 1, 2, 3 and 4. Their activity cannot be replaced with growth or nutritive substances.

c) The presence or absence of anatomical characteristics such as a continuous ring of sclerenchyma formed by one, two or more fibre layers. Discontinuity or absence of such a layer would make rooting easier (Beakbane, 1961).

Content of Growth Substances and Growth Inhibitors

Growth substances have been studied by Vieitez *et al.* (1965) in cuttings from young chestnut seedlings (about 3-6 months old), in soft and hard wood cuttings from old chestnuts, and in hard wood cuttings kept in running water in the open from November until March, a situation in which root formation occurs (Fig. 4).

FIG. 4—Soft and hard wood cuttings rooted in running water.

Growth promoters and inhibitors were found in the acidic fraction extracted from the basal part of each type of cutting employed (Figs. 5-7). Growth promoting activity occurred at Rf 0.35-0.45, practically at the same Rf as IAA. Ehrlich's reagent gave a blue spot in this area similar to that from IAA but the u.v. spectrum was not the same, A small zone of inhibition was detected at Rf 0.66-0.76 in seedlings, but at Rf 0.40-0.80 in older plants (Fig. 6). A biohistogram from the basal part of the washed cuttings in running water had no inhibition zones (Fig. 7) but growth-promoting activity was present although IAA was not detected.

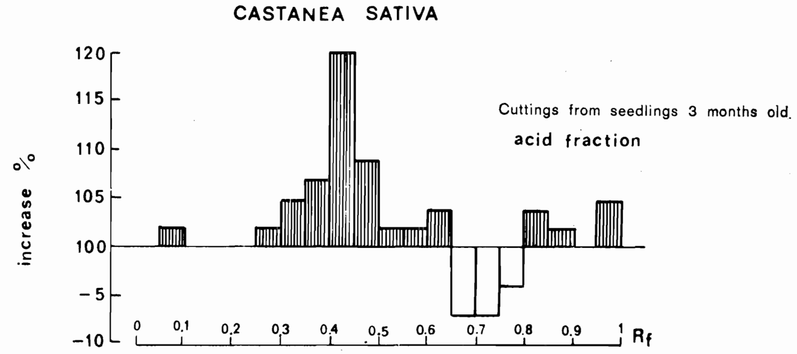
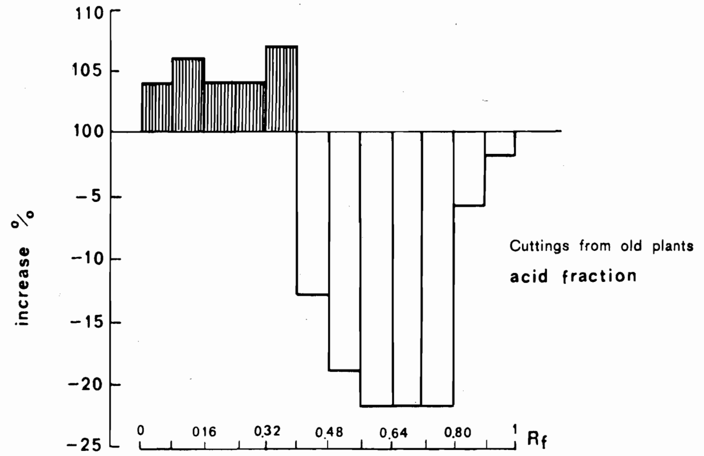


fig.5



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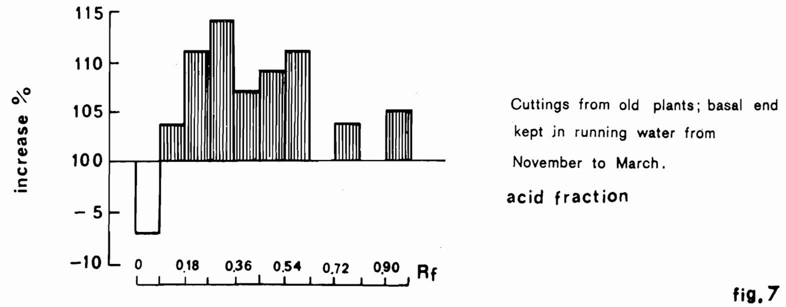

fig.7

FIG. 5—Hormone histogram from seedling cuttings.

FIG. 6—Hormone histogram from cuttings made from old plants.

FIG. 7—Hormone histogram from cuttings made from old plants but kept in running water.

From the results obtained with the different types of cuttings studied some facts emerged: the active zones which theoretically correspond to the IAA Rf are present in all the chromatograms, but a higher activity was present in those cuttings able to root than in those which did not root. The highest activity was found in cuttings from seedlings which root easily. IAA was not detected in such zones of growth activity; this hormone is difficult to identify. Chromatograms of cuttings from old chestnuts, which do not root, had small active zones but on the other hand large zones of inhibitors (Fig. 6). Unfortunately it was not possible to repeat the experiment of rooting chestnut cuttings by washing their bases in running water.

The growth-promoting zones in the biohistogram may be explained by the small content of vanillic and p-hydroxybenzoic acid mixed with salicylic and other hydroxybenzoic acids which at a high level are inhibitors. Moreover, vanillic, p-hydroxybenzoic, m-hydroxybenzoic and salicylic acids were obtained from alkaline hydrolysis of the plant extracts. Most of these probably come from cleavage of glycosides (Vieitez *et al.,* 1967).

It is difficult to say how the rooting of chestnut cuttings is governed by growth promoters, growth inhibitors, or by the balance between both types of substances. Some results support the idea of a prominent role for the growth inhibitors. Areses and Vieitez (1970) have studied the monthly variation of growth promoters (Fig. 8) and

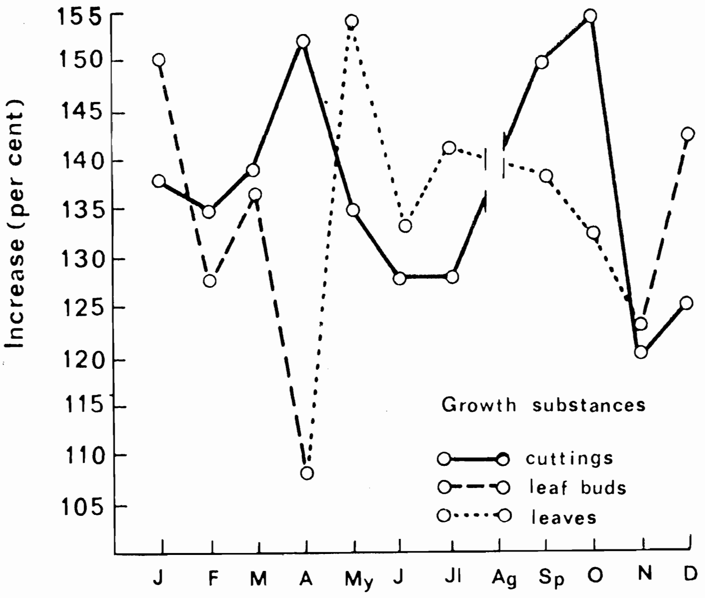

FIG. 8—Monthly variation in growth promoters.

inhibitors in cuttings of European chestnut. In the acid fraction inhibitors were detected throughout the year showing two maxima in July and December (Fig. 9). But chestnut has sclerenchyma rings which certainly are different for the several kinds of cuttings reported and there seems to be some correlation between this anatomical feature and

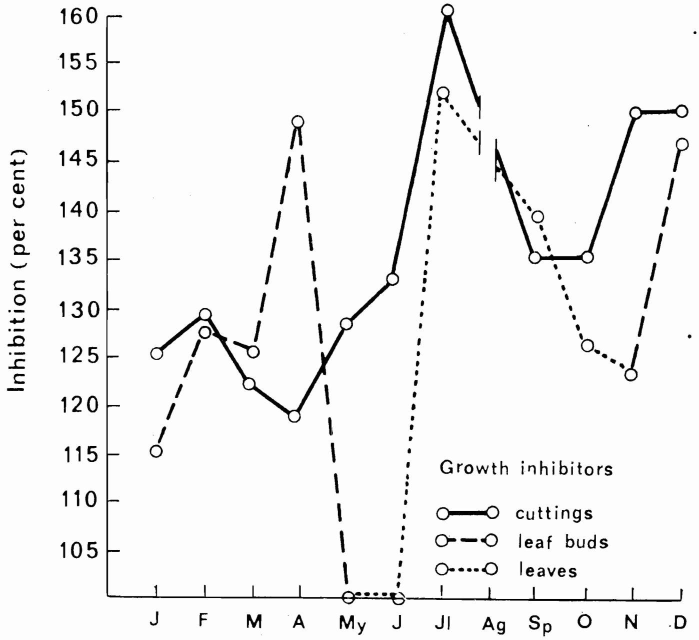

FIG. 9—Monthly variation in growth inhibitors.

rootability. However, a more complete study is necessary before any conclusion can be drawn. At the present time the evolution of the rings of sclerenchymatic tissue (Figs. 10, 11, 12, 13) is being studied in cuttings and shoots from chestnuts at different stages of growth, and those in which the rooting response is well known.

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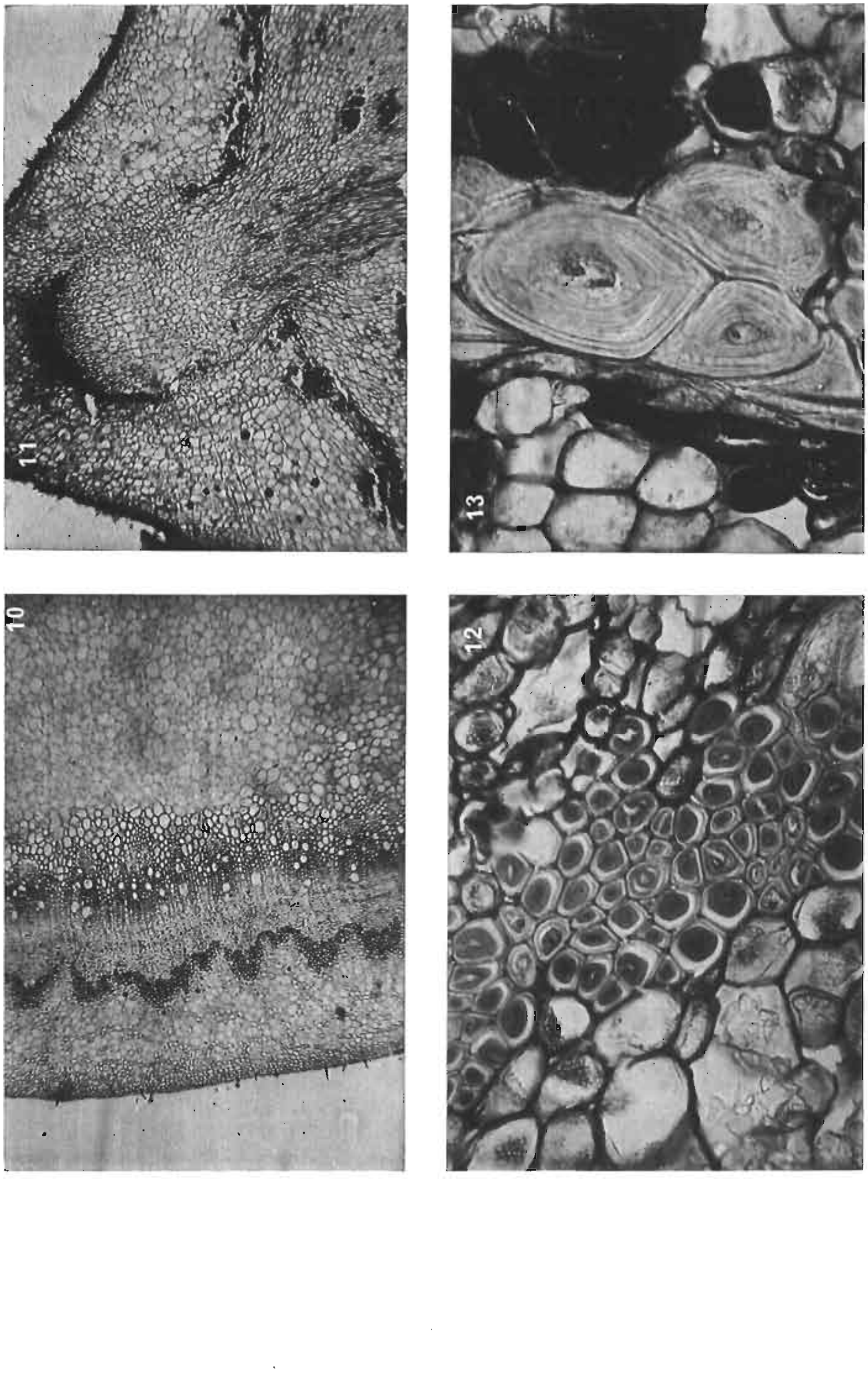

FIGS. 10-13—The structure of the rings of sclerenchymatic tissue.

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