YIELD PREDICTION IN HEVEA

A Study of Sieve-Tube Structure in relation to Latex Yield

BY

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Introduction

It may be generally conceded that the tapping test provides the most efficient method for the assessment of yield capacity in Hevea. Its application is unaccompanied by any technical difficulties or ambiguity in interpretation, while the results achieved are conclusive. Unfortunately, however, its application is restricted entirely to the period when the latex system is sufficiently developed to permit of effective comparison and a period of seven years or more must elapse before these conditions are satisfied. The inherent defect of the method is the time factor and it will be evident therefore that, until some alternative method is evolved whereby the potential yield of Hevea can be forecast at an earlier stage, progress, not only in the production of new and better clones, but in the possibly more fertile field of genetics in relation to this plant, will be seriously impeded.

The limitations of the test-tapping method have long been appreciated and several attempts have already been made to exploit certain factors present in the young plant. These investigations have been summarised elsewhere (1) and as the results were inconclusive no useful purpose would be achieved by discussing them in further detail.

As indicated above the solution of the problem is entirely dependent on the presence of some measurable character or factor in the young plant. The plant body is essentially a congeries of interacting cell units adjusted to the performance of its varied life functions, of which latex production is but a single expression in the general metabolism. The fact, however, that high yield is in some measure associated with the presence of an increased number of latex rings suggests that this general metabolism may be influenced or even controlled by a specific cell organisation or, in other words, that structure and function are correlated. Such a conception is of value for the purpose in view, for if such a correlation can be demonstrated it may be expected that any increase in the latex-tube complement will of necessity involve some modifications in the accessory structures associated with it. Whatever purpose latex may serve in plant economy it is reasonable to suppose that any increase in its volume connotes an increased rhythm in the general metabolism, accompanied by a more rapid transport of water and solutes from the soil and elaborated constituents from the leaves. If then these increased activities are indeed reflected in a correlated modification of some factor or factors in the transporting system, it remains only to ascertain the degree of correlation in order to provide data from which the potential yield could be predicted.

It must, however, be stressed that the production of latex is entirely a function of living cells, subject to the limitations imposed by numerous known or obscure internal and external influences. It is improbable, therefore, that a system of analysis founded on anatomical data alone can serve to differentiate between the relative merits of trees belonging to a high-yielding category. If, however, the anticipated correlation can be definitely established, the identification of inferior or low-yielding individuals by such a method should impose no insuperable difficulty, and it is in view of the latter contention that the present investigation was undertaken.

To understand clearly the significance of the sieve-tubes in the present investigation, it is desirable to form a mental picture of the channels through which the transport and interchange of food materials is accomplished. Before proceeding, therefore, to the examination of the results achieved, the following brief outline of the origin and development of the vascular system in plants in general, and their organization and relationships in Hevea in particular, may serve this purpose.

General Anatomy

In plants of simple structure, and in embryonic tissues of all higher plants, the transport of food materials is accomplished by the relatively slow process of diffusion from cell to cell. With increasing complexity in organisation this method is supplemented by the development of a vascular system which, extending throughout the entire body of the plant, provides greatly enhanced possibilities for the rapid transport of materials in any direction.

The fundamental unit of the vascular system is the **vascular bundle,** a composite structure of three structurally and functionally different elements. Of these the outer and inner structures are known as the **phloem** and **xylem** respectively, each of which contains functionally appropriate conducting vessels embedded in accessory tissues. Between these two conducting strands lies the third unit or **vascular cambium,** a narrow band of undifferentiated cells which, by their inherent capacity for unlimited division, provide the mechanism for the further growth of the vascular bundle.

In herbaceous plants the vascular bundles are arranged concentrically in such a manner that the stem, viewed in transverse section, is divided by a ring of separate bundles into a central region or **pith** and an outer region or **cortex,** the outer surface of which is protected by a tough skin or **epidermis.** The strips of tissue lying between the bundles and thus connecting the inner and outer regions form the **primary medullary rays.** This primary differentiation of the stem, in which a central pith is enclosed by an interrupted vascular cylinder bounded by a cortical envelope or sheath and with intercommunicating medullary rays, represents in varying degree the final organisation in the stems of all herbaceous plants. In Hevea, as in all arboreal types, however, it is the prelude to a further series of growth phenomena whereby the more complex architecture of the latter type is finally evolved. This second phase in development is initiated by further growth and extension of the cambium. In the primary condition as already described the cambium is confined to the vascular bundle between the phloem and xylem and bounded radially by the strips of tissue—the medullary rays. By the division of a line of the latter cells contiguous to the cambium, new segments of cambial tissue are differentiated which, bridging the medullary gaps, result in the formation of a continuous cambial cylinder. The stem is now completely divided into an outer region of phloem and cortex and an inner region of xylem—or wood, and pith. The establishment of the cambium cylinder is the starting point for all subsequent growth; from its inner face are derived all the woody fibres and vessels which constitute the woody cylinder, while from its outer face are differentiated the various tissue elements of the phloem or 'bast'. Meanwhile in the outer part of the primary cortex a second cambium, the **phellogen,** makes its appearance. Its activities, of a less protean character perhaps than those of its predecessor, result in the formation of an impermeable corky layer on the outside, while at the same time contributing further increments of cortical cells from its inner face. With increasing maturity stone-cells are evolved by heavy lignification of groups of cortical cells to form the main mechanical support of the bark. The stem now consists of two distinctive parts, a cylinder of wood derived entirely from the inner face of the cambium and an outer covering of 'bark' of twofold origin—the inner part or soft bast of vascular cambial origin and an outer part derived from the cork cambium or phellogen. The establishment of these two meristematic layers provides the mechanism for all subsequent growth of the stems.

It will be observed that the once-isolated conducting strands or vascular bundles have now been resolved into concentric and contiguous cylinders, the inner xylem or wood devoted to the conduction of water and soil solutes and an outer phloem or bast for the translocation of elaborated compounds from the leaves. Between them lies the thin line of cambium from which successive increments of wood and bast are added to meet the ever-increasing demands imposed on the vascular system. (Plate 1)

The Phloem

The phloem or bast is the main channel for the transport of plastic food materials elaborated in the leaves. It is a somewhat complex tissue of varying cell types, all having a common cambial origin. The most important constituents are the sieve tubes, which are formed by the longitudinal fusion of rows of elongated cells, with oblique partition walls on which are developed a varying number of perforated areas known as the sieve plates. The tubes are lined with a thin layer of cytoplasm or living substance which, passing through the perforations of the sieve plates, maintain a living connection throughout its entire length. In the active state they are filled with a liquid containing the food substances either in solution or in a fine state of suspension. The sieve tubes are distributed in varying number and pattern in a non-tubular ground tissue of more or less elongated cells—the phloem parenchyma. The function of these cells in conjunction with the medullary-ray system, is concerned largely with the radial transport and storage of food materials received from the sieve tubes and water with soluble mineral food material from the wood. The medullary rays are common to wood and phloem and consist of radially-disposed plates of tissue running from the cambium inwards into the wood and outwards through the phloem thereby bringing into intimate anatomical relationship the contiguous conductive tissues of wood and phloem. (Plates 2, 9 and 10)

Latex System

In latex-producing plants, whether herbaceous or arboreal, the tissue organisation so far described is accompanied by the development of a system of either cells or tubes containing the characteristic substance, latex, the chemical constitution of which varies considerably in the various species of the group. In all cases however the main bulk of the latex consists of substances such as caoutchouc and resins, which appear to be incapable of further metabolism. For this reason the relationship between the latex vessels and the vascular system has not yet been clearly established.

In the young seedling of Hevea the latex vessels form a network in the primary cortex of stem, root and branches, accompanying the vascular strands through the petiole and leaf veins, forming a primary latex-vessel system throughout the young plant. They take their origin not by cambial activity but by the fusion of cell rows of the ground tissue. With the beginning of secondary thickening, following the establishment of the cambial cylinder, a secondary latex system is evolved. In this system the vessels are derived entirely from the outer face of the cambium with the other units of the phloem. Each phloem increment contains its quota of latex vessels and, as growth proceeds, the latex system resolves itself into a series of concentrically arranged cylinders of anastomising vessels embedded in the inner bast. It is from this system that the latex crop is ultimately derived.

Material and Methods

To ascertain the extent to which the anticipated variation in the sieve-tube system of Hevea occurs, a range of bark samples were obtained from a block of 200 six-years-old seedlings at the R. R. I. Experiment Station (Block 2) whose yields varied from 2 lb. per annum in the lowest-yielding class to as much as 25 lb. per annum in the highest. These yields were calculated from the results of test tappings carried out in the months of June and July, 1934, only, although the trees had been test-tapped at intervals of six months for a period of three years. For the present purpose the calculated annual yields are of less significance than the demonstration of the fact that the relative yields had been maintained throughout the test period of three years. Bark samples were also obtained in triplicate from budded trees of 70 established high-yielding clones of varying age categories, and from 200 two-years-old trees of unproved Malayan clones (Block 15). The samples were taken at a uniform height of 50 inches by means of a one-inch diameter cork-borer and placed immediately in a solution of Formol-alcohol for two or three days and then transferred to 70 per cent. alcohol. For the study of the sieve tubes, transverse sections were made by microtome, stained in aniline blue, haematoxylin or bismark brown and subsequently mounted in canada balsam. For the latex tubes, both transverse and longitudinal sections were examined in glycerin after staining in either a 0.2 per cent solution of osmic acid or a strong alcoholic solution of sudan 3. By either of these methods the latex tubes are sharply differentiated from the adjacent tissues. For rapid identification of the sieve tubes thin sections may be obtained with the aid of an ordinary razor and examined in water under a magnification of 100 diameters.

Analysis of the Sieve-Tube Complement

To enable the non-botanical reader to appreciate more readily the results obtained, a series of micro-photographs are presented which display at a glance the characteristics of the sieve tubes as seen in transverse section in trees belonging to definite yield categories. On the basis of the speculations developed in the introduction it is to be expected that variations in the development of the sieve-tube system will be found to occur on the assumption of a potential increase in efficiency as higher yields are attained. It follows therefore that a maximum divergence should be observable if the sieve tubes of the lowest and highest yielders are compared. In Plates 3 and 5 are reproduced 20 examples of the sieve tubes of trees yielding below 2 lb. per annum. Plates 4 and 7 contain similar examples of established clones whose recorded yields vary from 15 to 30 lb. per annum at the tenth year. It will be observed that the sieve tubes of the latter are, in relation to the cells of the ground tissue in which they are embedded, of large size and closely aggregated between the plates of the medullary rays; their mean diameter is 0.04 mm. On the other hand the sieve tubes of the low yielders (Plates 3 and 5) are of such small dimensions that in many instances their identity is lost in the ground tissue. Thus far the results are in complete accord with anticipation. Calculation of the total cross-sectional area of the respective sieve-tube systems indicates that the characteristics of high and low yield appear to be correlated with the extent of development of the sieve tubes. On this interpretation it would be reasonable to expect a co-ordinated response in the latex system itself, for high yield should obviously demand a more highly developed latex organisation. The latex vessels of twenty bark samples of low and high-yielding type were examined, with results in complete agreement with this expectation. Plates 9 & 10 reproduce bark samples from trees Nos. 120 and 512, six-years-old seedlings, yielding 1.7 lb. and 13.2 lb. per annum respectively. The latex tubes are stained orange. Although the low-yielding tree is endowed with an extra ring of latex vessels it will be at once apparent that the total cross-sectional area of the latex-vessel system, and therefore the capacity, is strikingly inferior to that of the high-yielding individual. It would appear indeed, that the respective diameters of the latex tubes are directly proportional to those of the sieve tubes associated with them. The evidence for the persistent and uniform association of large-bore sieve tubes with high yield was further amplified and confirmed by the results obtained from the remaining high-yielding seedlings (Block 2), yielding from 10 to 25 lb. per annum and from the 200 two-years-old "unproved" Malayan clones (Block 15). The absence of yield records in the latter group precludes individual analysis, but it may be reasonably assumed that, as the mother trees were selected on the basis of a five-fold superiority over the mean of the area concerned, the clones will on the whole possess the high-yield factor. Allowance must be made, however, for the inclusion of trees giving abnormal yield through the incidence of root disease. The results conformed to the general expectation, 39 of the seedlings and 90 per cent of the trial clones possessing sieve tubes comparable with those of the established proved clones.

It remains to examine in detail 120 trees of Block 2 with yields extending from the borderline of the low-yielding type to a maximum yield of 9 lb. per annum. The sieve tubes were broadly of the two distinctive types previously associated with low and high yields respectively but their distribution per unit area varied considerably and proved in consequence a valuable means of analysis.

In Table 1 the trees are assembled in groups according to yield and analysed on a basis of the four grades indicated at the head of the table.

TABLE 1. *Comparison of sieve-tube development in 120 trees yielding from 4 lb. to 10 lb. per tree per annum.*

Grade 1 Poor development
„ 2 Moderate
„ 3 Good
„ 4 Very good, i.e. comparable with high yielding clones

Group	Yields in lb. per tree per annum	Grade 1	2	3	4	Total trees
1	4 - 5 lb	15	10	6	—	31
2	5 - 6 „	11	14	9	7	41
3	6 - 7 „		1	11	9	21
4	7 - 10 „	1	1	10	15	27
		27	26	36	31	120

Examination of the disposition of the trees under the four grades reveals a somewhat anomalous departure from the results previously obtained. With three exceptions the trees of groups 3 and 4 conform to the expected large-bore type of sieve tube but the lower-yielding groups 1 and 2 present a diversity of type inconsistent with the general thesis which implies that the sieve tubes should conform to type throughout the groups. The only alternative explanation connotes a random distribution, divorced from physiological concept, but to be effective such a theory must be equally valid for the whole of the material examined which is obviously not the case. The problem must therefore be amenable to a physiological interpretation consistent with the evidence previously obtained.

In the absence of concrete data it is difficult to avoid the conclusion that the trees in groups 1 and 2 may not possess the homogeneous yield characteristics suggested by the test tapping record. If this should prove the case a subsequent increase in yield performance in those trees of grades 3 and 4, possessing the type of sieve tubes associated with high yield, would at once transfer them to groups 3 and 4, a position that would be in accordance with general expectation.

A decision must therefore be postponed until the present yield records are amplified and the general investigation extended.

Discussion

The demonstration of the presence of distinctive types of sieve-tubes in the main stem and branch system of Hevea (Plate 8), combined with an appropriately co-ordinated latex-vessel system (Plates 9 and 10), provides evidence which, in the writer's opinion, can only be interpreted in the light of the hypothesis developed in the introduction to this paper. The assumption that the different yield potentialities of individual trees of Hevea may be closely correlated with definite differences in anatomical constitution is strongly supported.

It appears, however, that the relationship is not perfect; although it is sufficiently well-defined to permit of the immediate recognition of low-yielding types it is of doubtful value in differentiating between trees of a high-yielding category. This is illustrated by the results summarised in Table 1 which show that large-diameter sieve-tubes may be encountered in trees with a rather wide range of yield. At the same time, attention has been drawn to the fact that the indicated yields, since they are based on the records of early tapping tests, may not be an entirely reliable index of yield capacity and may be subject to considerable modification as the trees increase in age.

Evidence of this is forthcoming from the study of the yield history of some of the well-known proved clones of Hevea. Certain clones, which might quite easily have been discarded on the results of their earliest tests, have reached in subsequent years a very high level of yield. For this reason several years of careful recording are necessary before a true evaluation can be made.

Thus, for example, for the apparently aberrant trees shown in Table 1, Groups 1 and 2, and Grades 3 and 4, a further increase in yield would transfer them to a position in accord with the expected gradient indicated by their anatomical constitution. It is tempting to suggest that young trees which possess the anatomical characters now known to be closely associated with high yield, may show a marked increase in productive capacity with increase in age, whilst those trees whose anatomical constitution is of the type associated with low yield are unlikely to improve materially. It is only by extended observation and careful recording of individual tree yields that the evidence necessary to confirm or refute this suggestion will be forthcoming.

The demonstration of marked differences in size and distribution of latex vessels in the bast of different trees is of particular interest in relation to the numerous investigations in which the aim has been to establish a correlation between yield and number of latex-vessel rings. Although in the present investigation only a proportion of the trees available has been examined in detail, it cannot be doubted that the number of additional latex-vessel rings required in a typical low-yielder to compensate for the superior size and development of the latex-vessel complement of a high-yielder such as No. 512 (Plate 6) is unlikely to be found. Sufficient evidence has been presented to show that trees of the same type as No. 512 have a very definite superiority in yield capacity which may be quite independent of the relative number of latex vessel rows. (Figure 1A and B).

The results so far achieved are of value in directing attention to the necessity of extending the range and scope of the investigation. The early work on the genetics of Hevea indicates a high degree of self-sterility suggesting a large hybrid population and it is therefore particularly desirable to study under carefully-controlled conditions the mode of inheritance of the two contrasted type of sieve-tubes in first generation crosses. This work should be carried out in conjunction with a study of the chromosome complex in Hevea variations which may have a profound influence on the interpretation of the difficulties so far encountered. Evidence obtained from these investigations would probably extend and confirm the value of the facts so far established in their bearing upon the important problem of yield prediction.

Figure 1

A

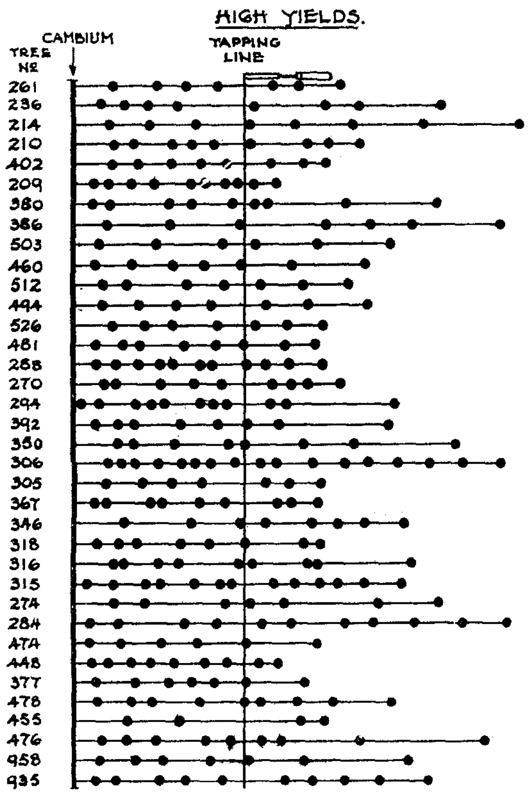


Figure 1

B

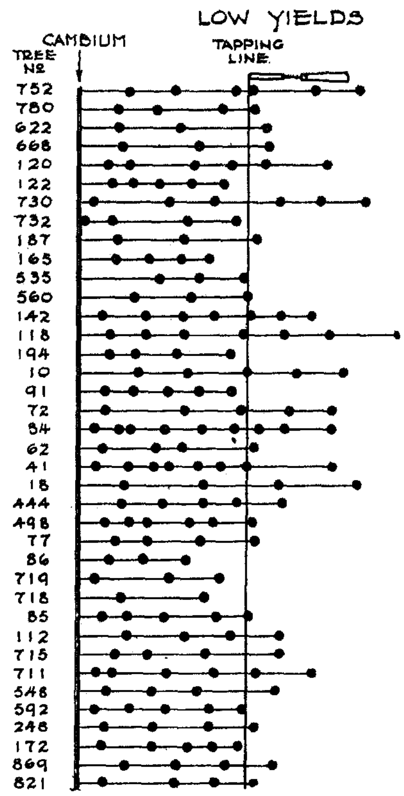


Diagram illustrating the distribution of latex-vessel rings in the bark of 4-years-old trees showing the total number of rings and the number opened when the bark is tapped to within 1 mm. of the cambium—

A. Condition in bark of high-yielding trees

B. Condition in bark of low-yielding trees (× 35)

(Each dot indicates the position of a latex-vessel ring)

Summary

1. An investigation of the anatomical structure of the sieve-tube latex-vessel complex in the bast of *Hevea brasiliensis* is described.

2. Two extreme types of sieve-tube have been shown to occur.

3. Sieve-tubes of small diameter are associated with narrow-bore latex vessels in trees with low latex-yielding capacity. Sieve-tubes of large diameter are associated with latex vessels of large bore, and all trees of high-yielding capacity which have been studied in the present investigation have been found to possess sieve-tubes and latex vessels of this type.

4. The presence of a constant type of sieve-tube in all parts of the tree (Plate 8) and the demonstration of the advanced development of the sieve-tubes in the bark of one-year-old stems permits of immediate qualitative analysis of a mixed population of young seedlings and suggests a means whereby low-yielding individuals may be eliminated at a very early stage.

Acknowledgment

The writer is indebted to his colleagues in the Botanical Division for the provision of the carefully-recorded material used in the course of this investigation. The valuable assistance of Mr. M. I. Nair in the collection of material and collation of yield records is recorded with appreciation. For assistance in the preparation of this paper for publication the writer is indebted to Mr. C. E. T. Mann.

Literature Cited

(1) SUMMERS F. *The Improvement of Yield in Hevea Brasiliensis*

Plates

1. A. Transverse section of a 1-year-old stem of a budding of a high-yielding clone showing the sieve-tubes of large bore characteristic of this clone (× 180)

   B. Transverse section of the bark of a 6-years-old tree showing

   (1) Cambium zone
   (2) Sieve-tubes and latex vessels
   (3) Stone-cell region—cortex (× 30)

2\. A. Radial longitudinal section through the inner bark (bast) showing two sieve-tubes with sieve-plates in surface view (× 550)

B. Isolated sieve-tube showing sieve-plate in profile (× 250)

C. Tangential longitudinal section of inner bark showing

(1) Medullary rays in transverse section
(2) Sieve-tubes
(3) Sieve-plates in profile (× 120)

3\. Transverse sections of the bark of 6-years-old seedlings. Low-yielding trees (× 100)

4\. Transverse sections of the bark of buddings of high yielding clones (× 100)
(Compare sizes of sieve-tubes in Plates 3 and 4)

5\. Transverse sections of the bark of 6-years-old seedlings. Low-yielding trees (× 100)

6\. Transverse sections of the bark of high-yielding seedlings 6 years of age (× 100)
(Compare sizes of sieve-tubes in Plates 5 and 6)

7\. Transverse sections of the bark of budded trees of established high-yielding clones (× 100)

8\. Transverse sections showing sieve-tube development in bark of different ages from seedling trees.

A. Bark of high-yielding tree (Tree No. 512. Block 2)
B. Bark of low-yielding tree (Tree No. 142. Block 2)

(i) Shoot a few months old
(ii) Bark one year old
(iii) Bark two years old
(iv) Bark three years old (All × 100)

9\. Transverse section of bast of 6-years-old seedling of low yield capacity showing size and distribution of the latex vessels (× 200)

10\. Transverse section of bast of 6-years-old seedling of high yield capacity showing size and distribution of latex vessels (× 200)
(The latex vessels are shown coloured in plates 9 and 10).

Text Figure

Figure 1. Diagram illustrating the distribution of latex-vessel rings in the bark of 4-years-old trees showing the total number of rings and the number opened when the bark is tapped to within 1 mm. of the cambium. (Each 'dot' indicates the position of a latex-vessel ring).

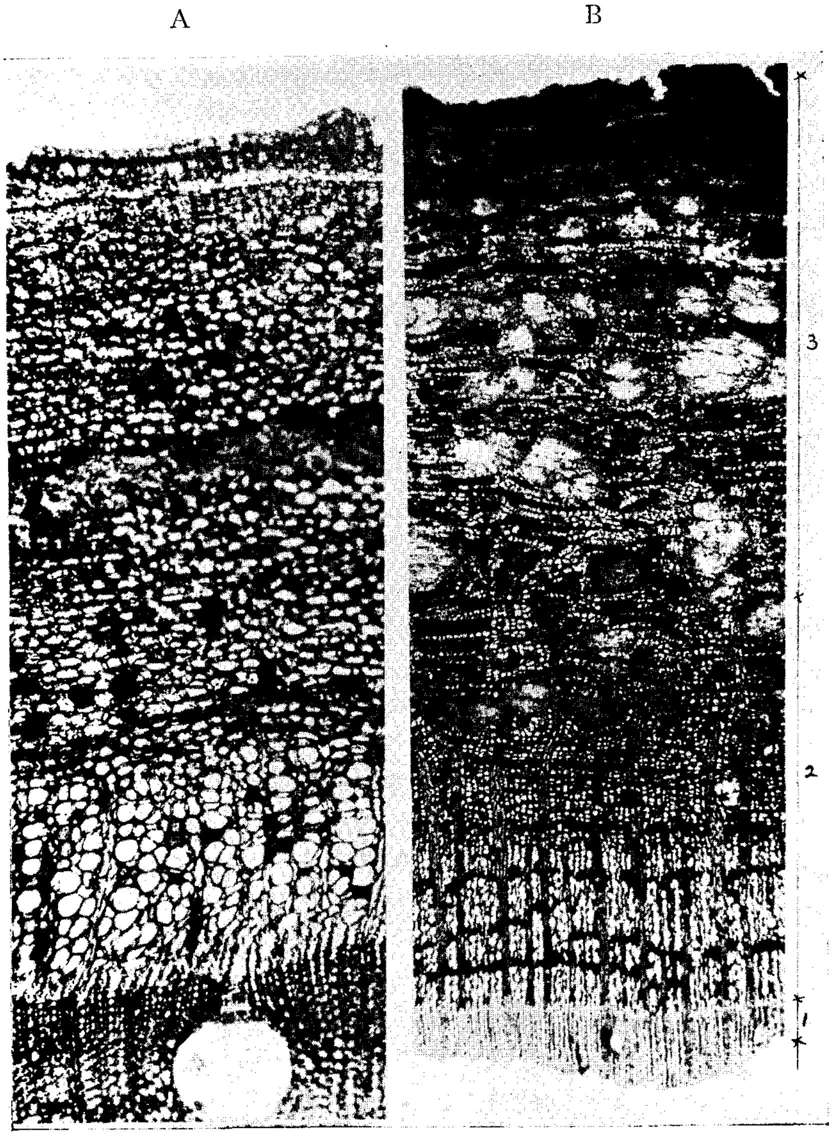
The diagrams have been constructed from camera-lucida drawings made at a magnification of $\times$ 35.

A. Condition in bark of high-yielding trees.

B. Condition in bark of low-yielding trees.

Kuala Lumpur,
17th June, 1935.

Plate 1



A. Transverse section of a 1-year-old stem of a budding of a high-yielding clone showing the sieve-tubes of large bore characteristic of this clone. (× 180)

B. Transverse section of the bark of a 6-years-old tree showing (1) cambium zone, (2) sieve-tubes and latex vessels, (3) stone-cell region—cortex. (× 30)

A

Radial longitudinal section through the inner bark (bast) showing two sieve-tubes with sieve plates in surface view (× 550)

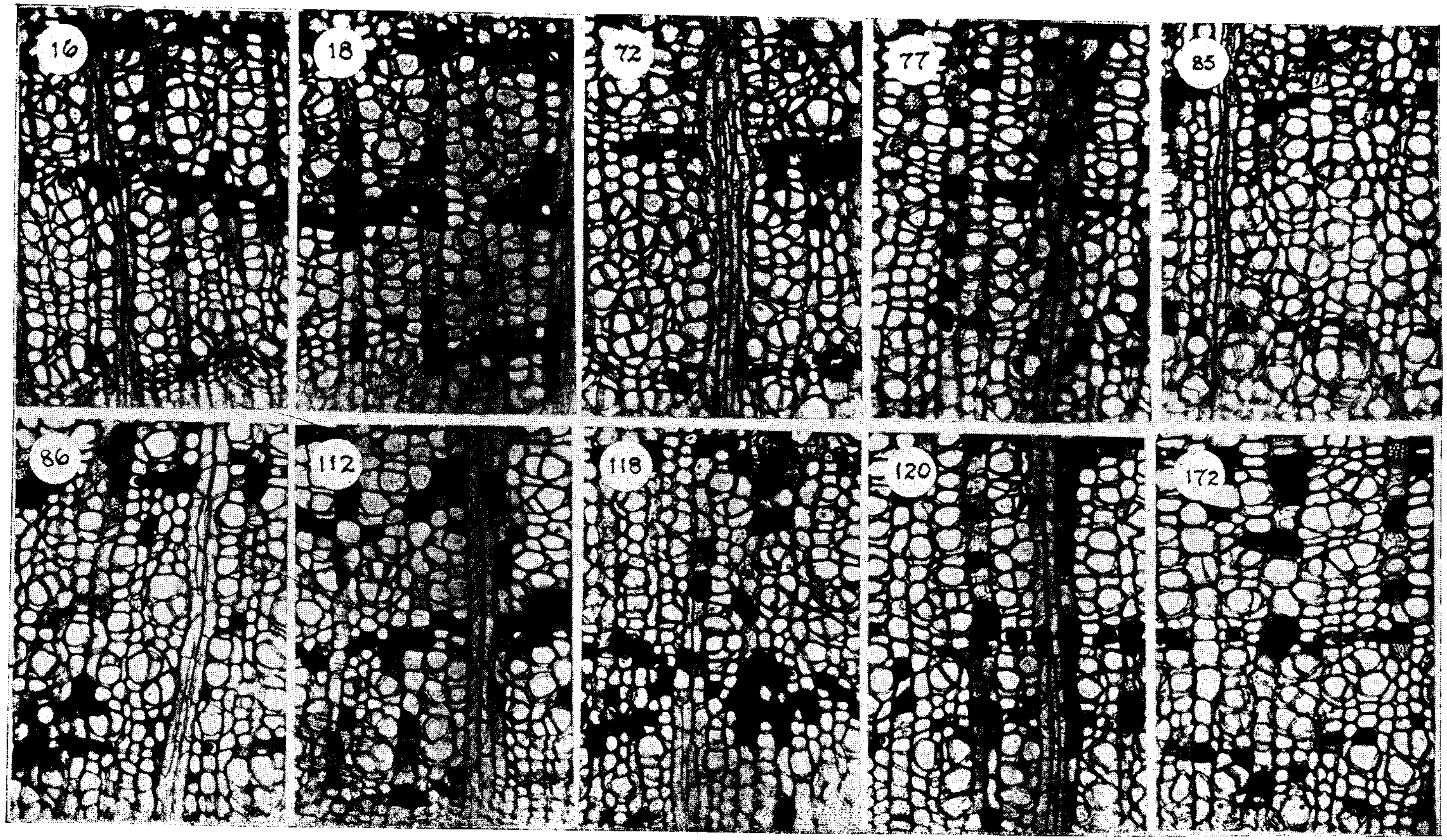
B

Isolated sieve-tube showing sieve-plate in profile (× 250)

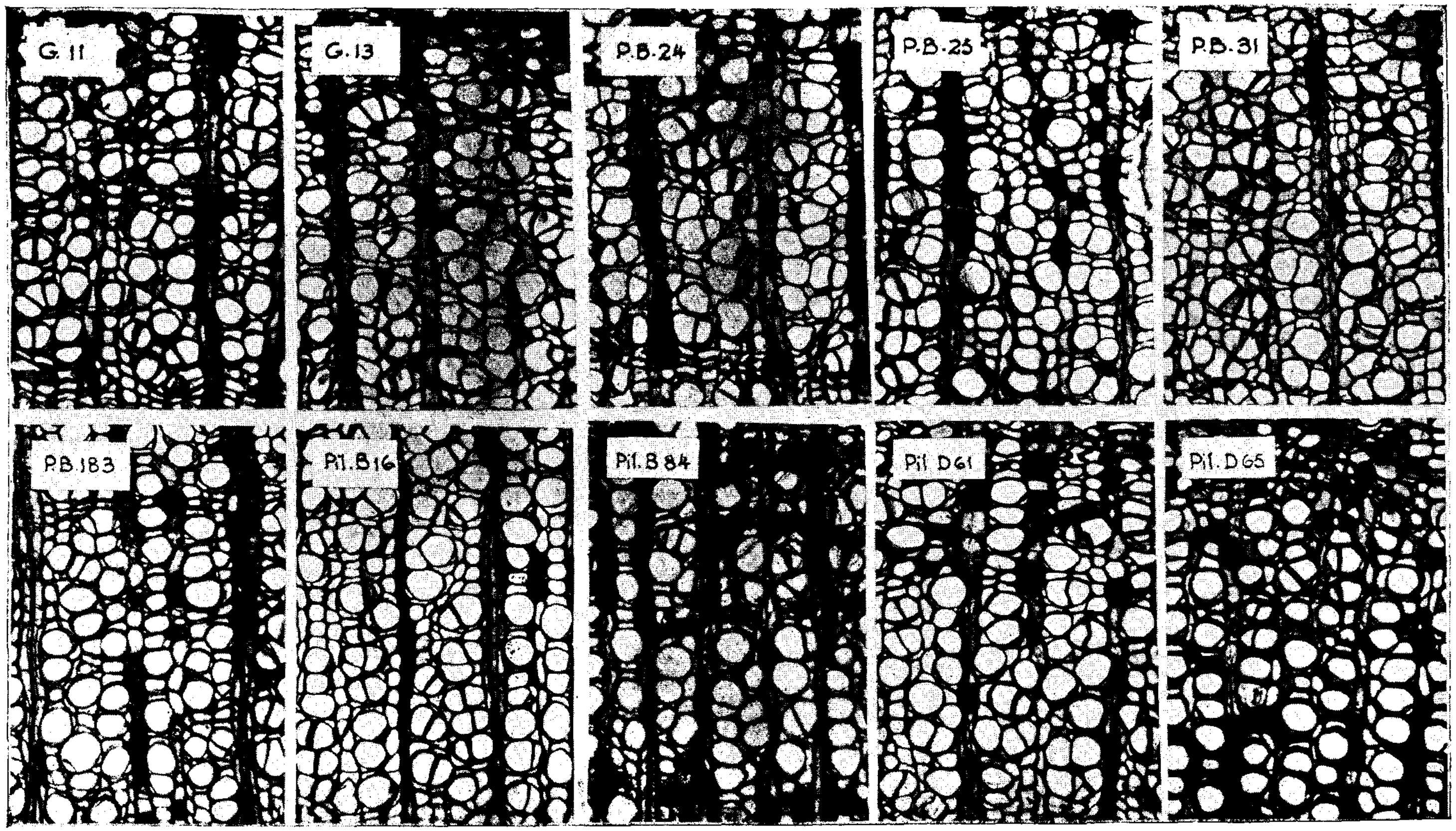
C

Tangential longitudinal section of inner bark showing (1) medullary rays in transverse section, (2) sieve-tubes, (3) sieve-plates in profile (× 120)

PLATE 2

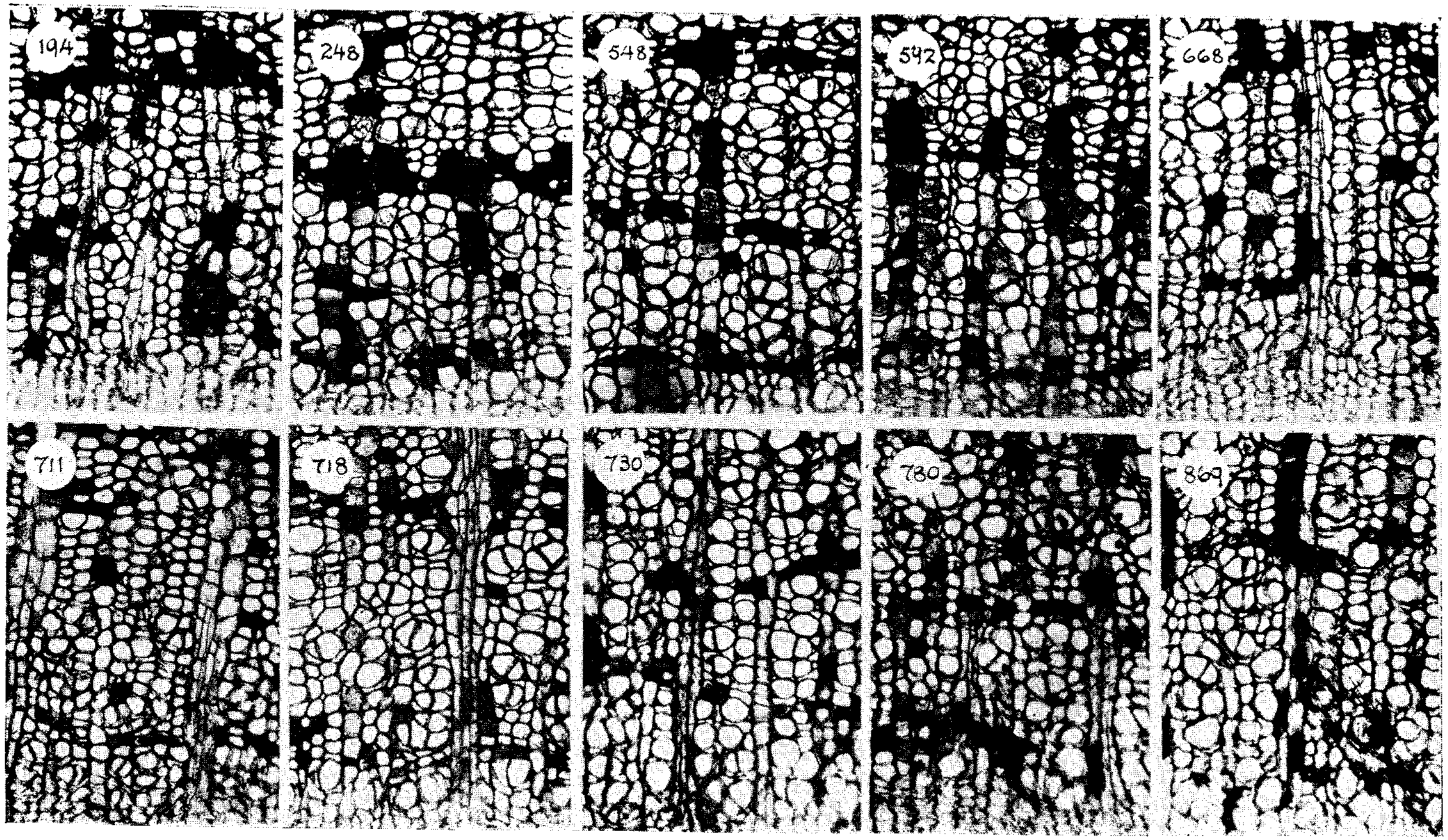


Transverse sections of the bark of 6-years-old seedlings; low-yielding trees. (× 100)



Transverse sections of the bark of buddings of high-yielding clones. (× 100)

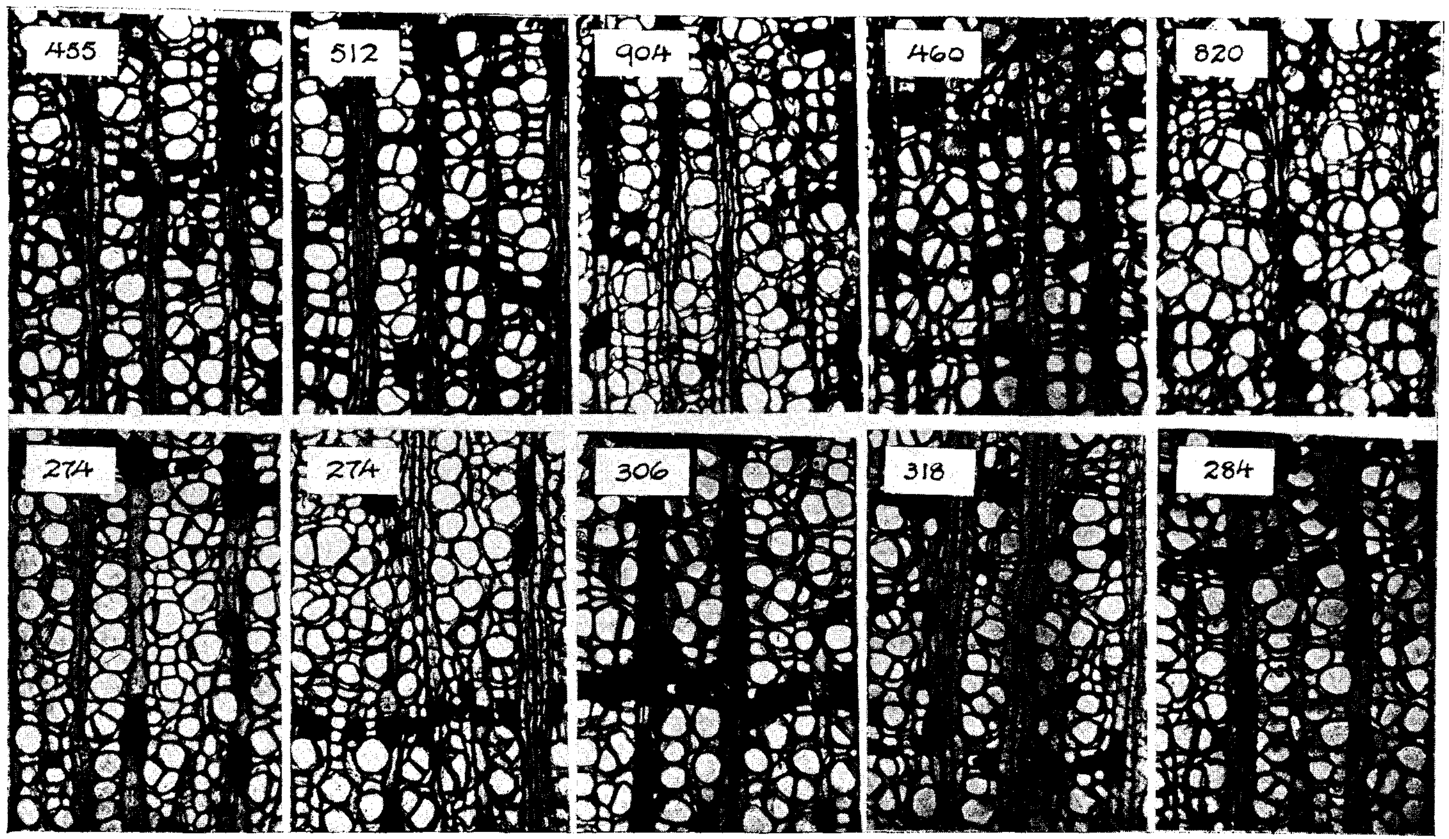
(Compare the sizes of the sieve-tubes in Plates 3 and 4)



Transverse sections of the bark of 6-years-old seedlings; low-yielding trees. (× 100)

PLATE 5

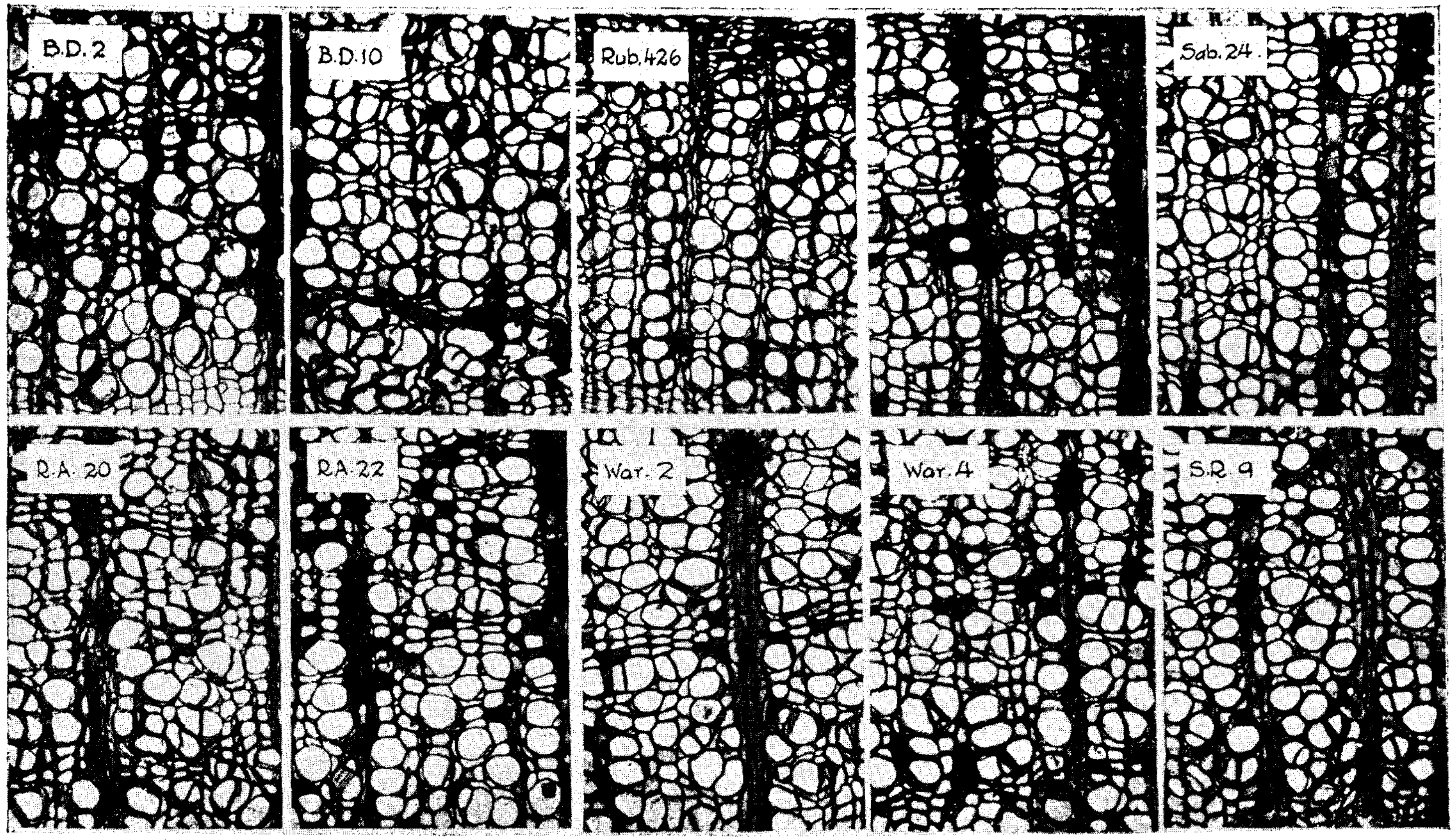
PLATE 6



Transverse sections of the bark of 6-years-old high-yielding seedlings. (× 100)

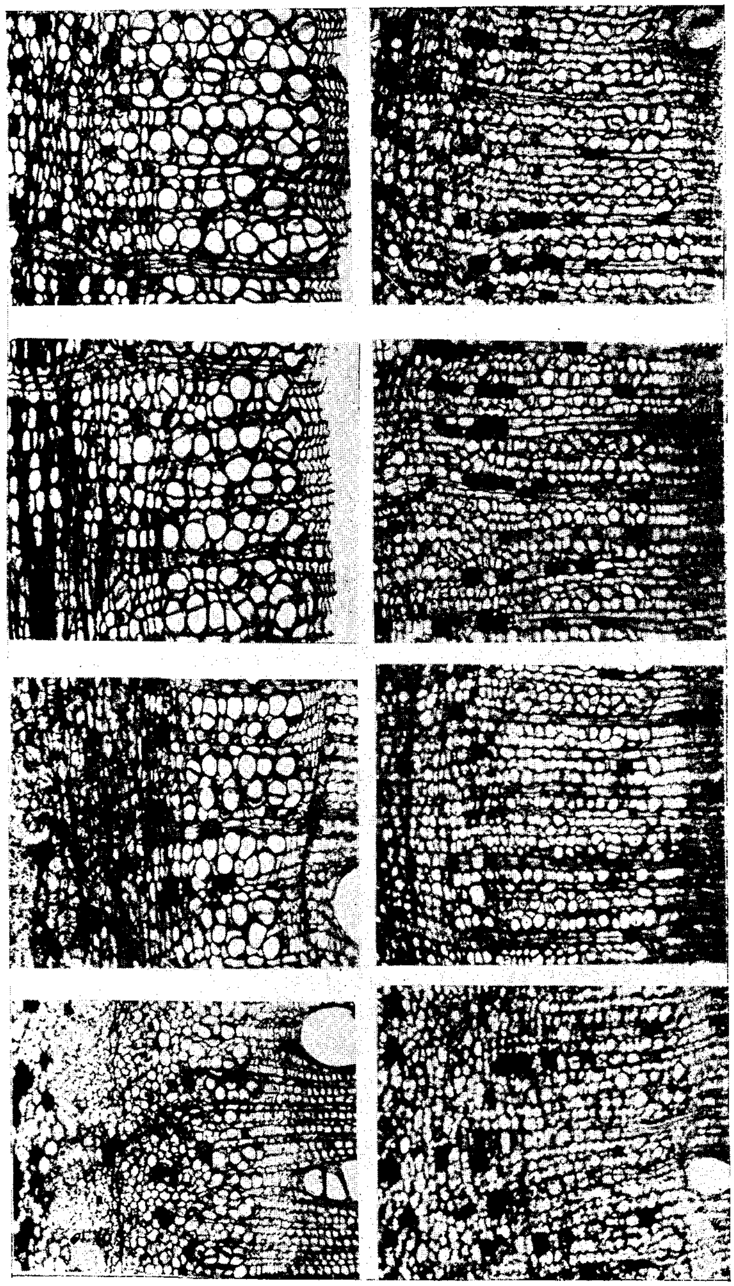
(Compare the sizes of the sieve-tubes in Plates 5 and 6)

PLATE 7



Transverse sections of the bark of budded trees of established high-yielding clones. (× 100)

PLATE 8



A | B

(iv) — (iv) Bark three years old

(iii) — (iii) Bark two years old

(ii) — (ii) Bark one year old

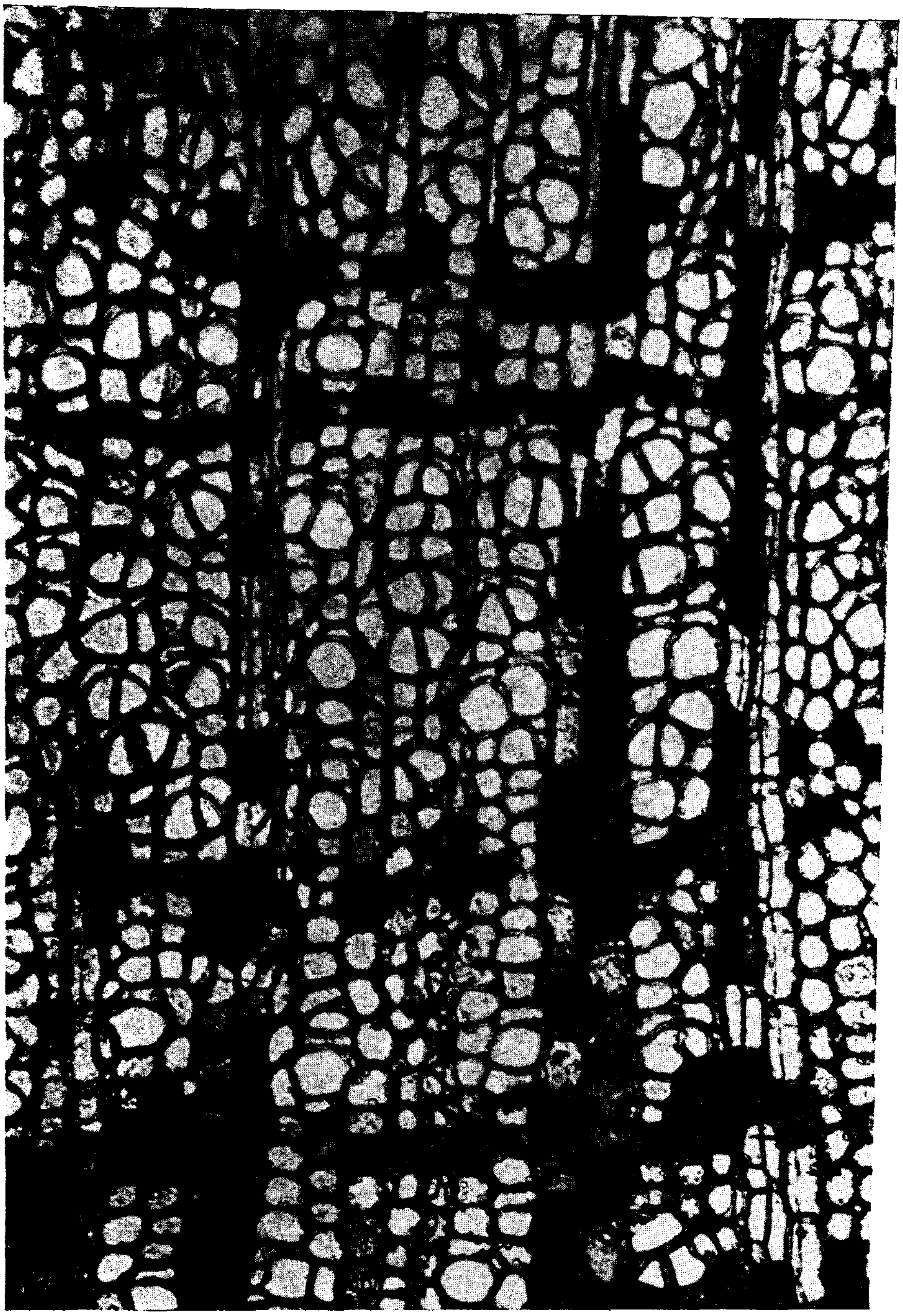
(i) — (i) Shoot a few months old

Transverse sections showing sieve-tube development in bark of different ages from seedling trees—

A. Bark of high-yielding tree (Tree no. 512 in Block 2 of R.R.I. Experiment Station).

B. Bark of low-yielding tree (Tree no. 142 in Block 2 of R.R.I. Experiment Station). (× 100)

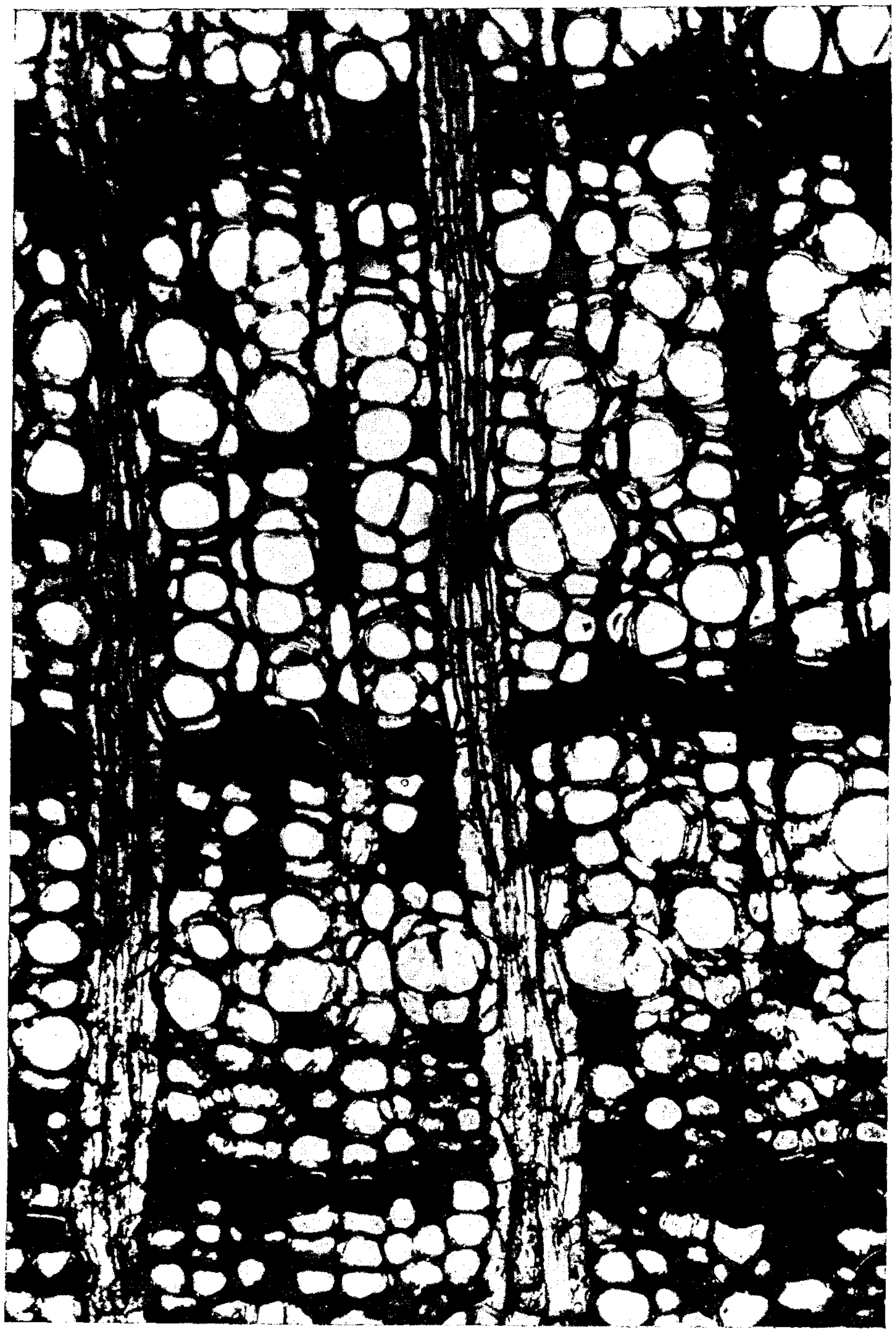
PLATE 9



Transverse section of bast of 6-years-old seedling of low yielding capacity showing size and distribution of the latex vessels. (× 200)

(The latex vessels are shown coloured)

PLATE 10



Transverse section of bast of 6-years-old seedling of high yielding capacity showing size and distribution of the latex vessels. (× 200)

(The latex vessels are shown coloured)