II. *The Genus* Dreyfusia (*Order Hemiptera, Family Chermesidæ*) *in Britain, and its Relation to the Silver Fir.*

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(PLATES 3–7.)

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GENERAL INTRODUCTION.

The following paper embodies the results of an investigation, carried on during the past two years under the Department of Scientific and Industrial Research, at the Royal Botanic Gardens, Kew, on the biology and host relationships of the silver fir *Chermes*, which belong to the genus *Dreyfusia*.

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The *Chermesidæ*, a family of the sub-order *Aphidoidea* (*Hemiptera-Homoptera*), commonly known as plant lice, attack a large and varied number of plants, piercing their tissues in order to obtain their food.

The family *Chermesidæ*, which confines its attacks to conifers, has, in common with many members of the closely allied families *Aphididæ*, *Pemphigidæ* and *Phylloxeridæ*, a very complicated life cycle. This may involve two hosts, of which one, the so-called primary host, is invariably a species of *Picea* (Spruce), on which gall formation is caused ; while the other, the secondary host, may be any one of the genera *Larix* (Larch), *Pseudotsuga* (Douglas Fir), *Abies* (Silver Fir), *Tsuga* (Hemlock), and *Pinus* (Pine), on which the insects, in most cases, live on the stems and branches without forming a gall. The migration from host to host is effected by winged individuals.

THE GENUS DREYFUSIA BÖRNER.

Historical Summary.

Two species belonging to this genus occur in Britain, *D. nüsslini*, Börn., and *D. piceæ* (Ratz), Börn. These two species remained for many years undistinguished from one another, under the name *D. piceæ*. In 1903, however, NÜSSLIN (16), working upon the biology of *Dreyfusia* in Germany, noticed that this species showed evidence of winged migration from the silver fir to the Norway or common spruce, *Picea excelsa* Link., the results of which were abortive, in that the sexual forms did not develop on that tree, and no gall generation resulted. CHOLODKOVSKY, commenting upon the results of NÜSSLIN's work, expressed the opinion that two species were involved, and separated them by the morphological characters of the "*sistens larvæ*" into two species, *C. piceæ*, Ratz, and *C. funitectus*, Dreyfus. These contributions were followed by MARCHAL's papers in 1906 and 1907, in which he confirmed CHOLODKOVSKY's opinion that two species were involved ; but stated that the species named by CHOLODKOVSKY *C. funitectus* was, contrary to the opinion expressed by NÜSSLIN, capable of passing through a full cycle of five generations, provided that the true primary host tree, the Oriental spruce (*Picea orientalis*, Carr.), was present.

MARCHAL's work was followed in 1908 by BÖRNER's monograph of the Chermesidæ, in which, however, MARCHAL's results were overlooked, and the two species or races in question were still regarded as one, under the old name *C. piceæ*. Shortly after this work appeared, NÜSSLIN (17) published a further paper on the species, in which he once more demonstrated the existence of two races. One of these showed a rudimentary migration to the spruce, and its forms living on the fir seemed to confine themselves, as a rule, to the bark of the young branches, and were designated by him "Jungtriebläuse," whilst the forms belonging to the other race, which he called "Altenrindenläuse," were confined to the bark of the older branches and the main stem. Of these two races he concluded that the latter was derived from the former, and specially adapted for living on the trunks and larger branches of the tree, in the deep shade of the forest, and as such had lost the summer generation, which is specialized for living on the young branches and needles. This prevented the development of the winged generation, which is produced only upon the needles of the fir.

Börner (2), in view of Nüsslin's latest work, took up the question once again in the same year (1908), and, as the result of his study of the species in the forests of the Vosges, he finally decided to separate them into two, for one of which he proposed the new name *Dreyfusia nüsslini*, and for the other the original name *Dreyfusia piceæ.*

Marchal (14) followed Börner's work in 1913 with an exhaustive study of the two species in France, published in his 'Contribution à l'étude de la biologie des Chermes,' and containing their life-histories in full, with references to his previous papers and those of Börner and others. These are cited in the following account of the life-history as observed in Britain:—

The Species of Dreyfusia in Britain.

Both *Dreyfusia nüsslini* and *Dreyfusia piceæ* occur in Britain, and Steven (24), in 1917, in a general paper on the Chermesidæ in Scotland, gave some notes on their life-history, but hitherto no detailed account of their general occurrence and life-history has been published.

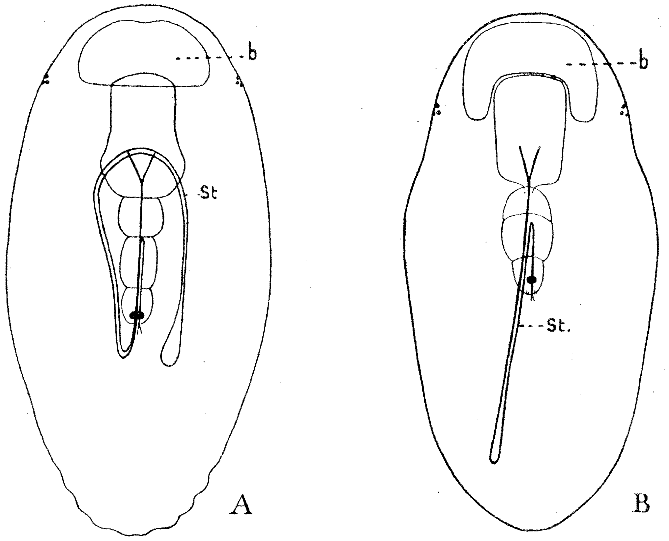
The Life-History at Kew.

Dreyfusia nüsslini.—The life-history of this species was studied at Kew, partly on a young tree of *Abies nordmanniana* growing in the Gardens, and partly on young nursery plants of *A. pectinata,* which were procured from the Forestry Commission's nursery at Inverliever, Argyllshire, early in January. At this time the hibernating *sistens* larvæ are to be found concentrated in great numbers on the young twigs of the fir, close to the buds, and even on the lower portions of the buds themselves in some cases. These young larvæ are about 0·5 mm. long, oval in shape and black in colour, the body being outlined by short strands of white wool, which render the insect rather more conspicuous. The progress of development was studied both in the open and in the greenhouse attached to the Jodrell Laboratory at Kew, and as the times of development varied in the two cases, they may be considered separately. In the case of the plants kept in the greenhouse, development began early in February, and the first stem mothers matured and commenced to lay eggs on February 23. Egg-laying became general about February 28, and the first spring larvæ hatched out on March 14. In the open, no signs of development appeared until February 23, and the first stem mothers were not observed until March 15, after which date oviposition proceeded rapidly, both under cover and in the open (Plate 3, fig. 4).

The first spring larvæ appeared in the open about a month later, on April 16, and were all of the so-called *progrediens* type, light brown in colour, with a weakly chitinized integument, and showed the characteristic short rostral stylets and peculiar shape of brain lobes, first described by MARCHAL (text-fig. 1).

More than 50 per cent. of the first larval brood were of the *progrediens* type, and soon after hatching they sought the needles, where they settled down, passed their stylets through the stomata, and commenced to feed. They began to develop late in April and passed through five stages, separated by four moults. On May 9 they had reached the fourth stage, and the presence of nymphal forms indicating a winged stage, was found. These nymphs appeared in relatively small numbers, but were present both in the greenhouse, and in the open.

By May 11 the nymphs were well grown, about 1 mm. in length, and of a reddish brown colour, with the whole body covered by whitish scales, the areas corresponding to the wax glands being clearly visible on the body segments. The first fully developed winged forms appeared on May 14, and experiments were started to determine the species of tree to which these forms would seek to migrate. The experiments were conducted in cages in which young silver firs were enclosed along with Norway and Oriental spruce, and in addition newly emerged winged forms were liberated in the cages containing the various species which might serve as host trees. At the same time specimens of the winged forms were examined microscopically, in order to help in fixing their identity. The morphological characters employed by BÖRNER and MARCHAL indicated that these winged forms were probably the *sexuparæ* or winged migrants from fir to spruce of *Dreyfusia nüsslini*, and this was borne out by the results of three experiments on June 1, 5 and 6, when 20 of these winged forms were found settling and laying eggs on *Picea orientalis,* and showing the characteristic wax formation on the head which distinguishes this species.



TEXT-FIG. 1.—Young larvæ of *Dreyfusia.* A, Sistens form ; B, progrediens form. Diagrammatic figures to show difference in form of the brain lobes (*b*) and in the length of the stylets (St.).

The Sexual Forms.

These hatched about June 28, 1923, from the eggs laid by the sexuparæ on the Oriental spruce, and were observed up to the third moult, but unfavourable weather about that time resulted in the death of most of the young larvæ, and the production of mature sexual forms definitely failed. The numbers comprising the winged generation were, in all the cases observed, very limited, and experience at Kew showed that this generation may not always occur. The Oriental spruce is, according to MARCHAL, apparently the only host tree on which the sexuparæ can mature and a gall generation result. At Kew this summer winged forms were found settling on the Norway spruce, but in no case did they come to the stage of egg-laying. From this observation it may be assumed that, in regions where the Oriental spruce is not present, the sexuparæ settle upon the Norway or other species of spruce, but cannot successfully complete their life-cycle upon these trees.

The Stem Mother and Gall Generations on Picea Orientalis.

Owing to the failure of the sexual generation to develop on the Oriental spruce at Kew, these generations were not observed, nor have the galls of this species on the Oriental spruce been found by the author in the gardens at Kew or elsewhere. STEVEN records the finding of the gall generation in Scotland, but gives no details of its occurrence. It is probable, therefore, that while this generation may occur, and a full life-cycle of *D. nüsslini* in Britain follow, the occurrence is not a usual one.

As in MARCHAL'S, the experiments at Kew this summer have shown that the winged forms in this species are exclusively sexuparæ, and that there are no "*alatæ non migrantes*" which could carry the *Chermes* infection from one silver fir to another in any given area.

The Summer Progrediens Generation on the Silver Fir.

Side by side with the nymphs of the sexuparæ, the summer generation of "progredientes" was observed developing on the needles. The larvæ of these, unlike those of the "sistentes" on the bark, passed through five stages, separated by four moults, and the first stem mothers were observed egg-laying on June 1, and by the end of that month were found egg-laying in large numbers. The first young larvæ commenced to emerge from the eggs late in June, and were of the sistens type, seeking the bark of the young shoots shortly after emerging, settling down thereon, and assuming the typical, hibernating, sistens form.

The Sistens Generations.

On the hatching of the eggs laid by the winter stem mothers in March and April, as has already been stated, the young larvæ could be divided into two groups.

(*a*) *Progredientes*, which sought out the needles and commenced development immediately, and (*b*) *Sistentes*, which migrated to the bark, and, on settling down, assumed the hibernating form. The latter had commenced to separate out as early as March 22, and their numbers were always being added to throughout the summer. Late in June, some of these larvæ were observed to begin feeding once again, and by the middle of July they had begun egg-laying. These corresponded to the "æstivo sistentes" of MARCHAL, and they laid 15–20 eggs each, which commenced to hatch on July 27. The young larvæ settled upon the bark alongside the sistentes of former generations and remained over winter in this condition.

Dreyfusia Piceæ (*Ratz*) BÖRN.

This species has been found prevalent in the gardens at Kew on the following species of Abies :—*A. cilicica*, CARRIÈRE, *A. cephalonica*, LOUDON, *A. Forrestii*, CRAIB, *A. pindrow*, SPACH, *A. grandis*, LINDLEY, *A. nobilis*, LINDLEY, *A. firma*, SIEBOLD, *A. Fraseri*, LINDLEY, *A. faxoniana*, REHDER and WILSON.

A study of the species on young trees, from the nursery stage up to about 10 feet in height, has shown that it is either confined to the older bark and branches of the tree, or is to be found settling on the buds and nodes of young branches, on which it causes gall-like swellings. The galled twig may cease to grow in length, but the galled portions increase markedly in diameter, and finally become knob-like with the dead tip of the shoot protruding beyond. In other cases the twig may continue to grow beyond the swollen point of attack. In this species, contrary to what has been found in the case of *D. nüsslini*, the young branches are almost entirely free from the *Chermes*, the winter larvæ developing either on the buds themselves, or on the thicker bark of the main stem and older branches. (Plate 3, fig. 2.)

CHOLODKOVSKY (5), in his first description of this injury on silver fir, proposed the name *Dreyfusia piceæ var. bouvieri* for this gall-causing form. This name has not been adopted by either BÖRNER or MARCHAL in their work, and, as is shown later, my experiments tend to indicate that the tree species and not the insect species is the factor determining gall formation (Experiment A, p. 53).

The Life-History at Kew.

The winter larvæ (sistens) of *Dreyfusia piceæ* were first examined on January 26 on a small specimen of *A. Forrestii* growing in the open. They were then in the first stage, and showed no sign of development. This, however, began in the greenhouse, early in February and by the 24th of that month the first stem mothers had begun laying eggs, and by March 3 activity was general. Development in the open commenced towards the end of February, and on March 6 stem mothers with eggs were found on *A. grandis* in the Gardens. The first young larvæ appeared on March 20, and these were observed to be progrediens in type. They migrated to the needles shortly after hatching, and settled there. These larvæ were found both indoors and out, but all failed to develop, dying off in most cases after a few days. The reason for this has not so far been ascertained, and since their first appearance, no further sign of a progrediens generation has been seen at Kew. It has accordingly not been possible to compare the British conditions with MARCHAL'S account of the species in France, in which he states that this "progrediens" generation is dimorphic in character, and gives rise to both winged and wingless individuals. The former develop into "alatæ non migrantes," show well-defined morphological differences from the sexuparæ of *D. nüsslini*, and further, seek out as their host plant not a spruce, but another silver fir, on the needles of which they settle, lay eggs, and give rise to a sistens generation. The wingless stem mothers of the "progrediens" generation remain on the original silver fir host and lay a small number of eggs on the needles or in an axillary position on the shoot, where they give rise to a further series of "sistens" larvæ (MARCHAL).

At Kew, after the appearance of the first progrediens larvæ, which were few in number and died off early, it was found that all the young larvæ which subsequently appeared were of the sistens form. These returned to the buds again, and settled down as "sistentes" remaining undeveloped for a certain period. They resumed activity in some cases later on in the summer, developing into a generation of æstivo-sistentes stem mothers on the buds, laying eggs, and producing a further progeny of sistens forms, which will over-winter on the buds of the silver fir. The cycle of development therefore, in this species at Kew, has, in my experience, been confined to parthenogenetic generations on the silver fir host.

THE RELATION OF DREYFUSIA NÜSSLINI TO THE SILVER FIR.

The foregoing study of the life-history of *D. nüsslini* on the silver fir has shown that this species chooses for its habitat the young shoots of the silver fir, and that on this tree the life cycle alternates between the needles and the bark of the shoots. These peculiar feeding habits of the species have been particularly remarked upon by BOAS (3) in Denmark, who in a recent study of the injury to silver fir by this species, figures affected trees, showing the characteristic dying off of the leading shoots, the needles on which curl up, turn brownish-yellow in colour, and finally drop off. (Plate 3, fig. 1.)

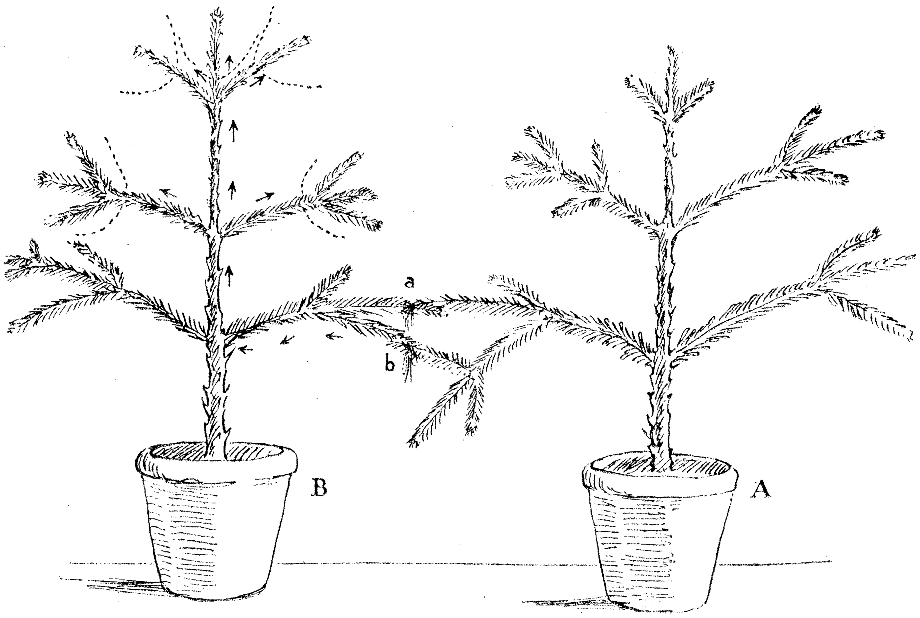
This habit of concentration upon the leading shoots by the Chermes was made the subject of experiment at Kew in April of this year. Two young plants of *Abies pectinata* planted in pots, the one healthy, and the other (A) infested with *Dreyfusia*, were placed side by side, and the tips of some branches of each tied together at the points (*a*, *b*) (text-fig. 2). At the time of the experiment, the young larvæ of *Dreyfusia* were hatching on plant A, and in a few days' time, a stream of these was observed moving across from the infested plant to the healthy one, by way of the two points, *a* and *b*, already mentioned.

These larvæ showed the following habits :—

(1) They exhibited a very marked negative geotropism, migrating towards the topmost branches of the tree.

(2) The "progrediens" forms congregated in great numbers on the needles at the tips of the young branches.

(3) The "sistens" forms congregated in masses on the bark of the branches, in the same region.



TEXT-FIG. 2.—Experiment upon migration of the young larvæ of *D. nüsslini* from an infected silver fir (A) to a healthy plant (B).

(4) The tips of the branches were always sought in preference to the lower portions, and the upper branches were more markedly sought after than the lower. The undersides of the branches were chosen in preference to the upper and less protected region.

The results of this experiment were interesting as reproducing the peculiar behaviour of the insect already observed under natural conditions, and correlated the presence of the Chermes at the tips of the branches with the death of these, a fact which had long been suspected.

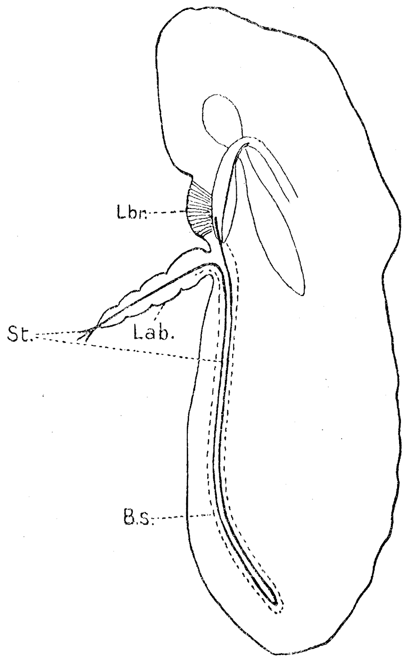
This laboratory experiment and the extensive field data available, relative to the destructive nature of the insect's attack, suggested an investigation into the source of supply from which the insect drew its food, and the physiological effect upon the plant of its attacks. This question has already been studied in the case of other hemipterous forms by a number of workers, and a short review of the literature concerned is given below.

Literature on the Food of Sucking Insects and the Means by which it is Obtained.

Büsgen's (4) work on the *Aphididæ* in 1891 may be regarded as the first contribution to the subject, his researches bearing upon the secretion of "honey dew" by these insects. Petri (18, 19, 20, 21, 22) has studied the question in Phylloxera and several species of *Coccidæ* (Scale insects); Zweigelt (26, 27) has worked on several species of Aphis; and, more recently, Davidson (7) has added to our knowledge by his work on *Aphis rumicis*. In addition to these major contributions, Williams (25), Hargreaves (12), Smith (23), and others have discussed the subject of feeding in Froghoppers (*Cercopidæ*), Whiteflies (*Aleyrodidæ*), and Capsid bugs (*Capsidæ*).

Mouth Parts and Salivary Glands.

The mouth parts and mechanism of suction in the *Hemiptera* have been studied by several writers, notably Mark (13) in 1876, in the Aphids and *Coccidæ* (Scale insects), Awati (1) in the Capsid bug *Lygus pabulinus*, Linn., Grove in *Psylla mali*, and Davidson in *Eriosoma* (*Schizoneura*) *lanigera*, Hous. In the *Chermesidæ* a considerable amount of information has been supplied by the work of Börner and Cholodkovsky, the latter dealing especially with the structure of the salivary glands.

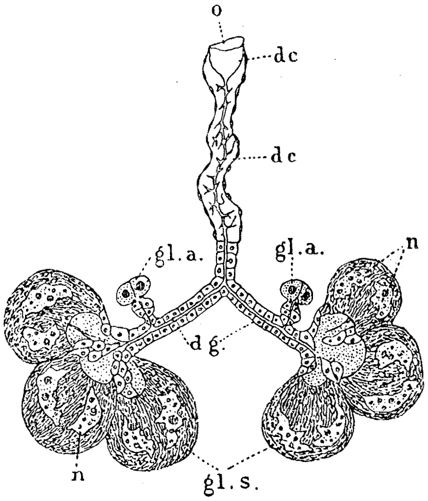


Text-fig. 3.—Diagrammatic figure of the mouth parts of *Dreyfusia* in longitudinal section. Lbr., Labrum and clypeus; St., stylet; B.s., bristle sheath; Lab., labium.

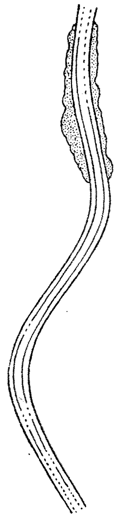
A description of the intimate structure of the Chermesid mouth parts does not form a part of the present paper, only the main features being considered here. These consist of the forehead, which comprises the clypeus and labrum, the labium or proboscis, a pair of mandibles, and a pair of maxillæ (text-fig. 3). As in the *Aphides*, the extremities of the mandibles and maxillæ are pointed, and these elements are closely apposed to one another, forming a continuous tube or piercing organ. In the *Chermesidæ* this tube is very long when extended, being in some cases more than twice the length of the body, while in repose it is retracted within the body in a thin-walled sheath or sack, which the German writers have termed the "Borstensack" or bristle sack, the opening of which lies at the base of the labium (text-fig. 3). It is interesting to note that in the *Cherme-*

sidæ this tube* is much longer in the stem-feeding or so-called " sistens " forms than in the leaf-feeders or " progrediens," and this is probably directly correlated with the different feeding habits of the two forms.

On emergence from the " Bristle sack " the stylet lies in the groove of the labium, specially fitted for its reception, at the tip of which it is held by a chitinous ring-shape structure, which may assist in the process of insertion into or withdrawal from the plant. The tip of the labium is also provided with numerous spinules, which **Börner** regards as sensory in function, assisting the insect in its search for a suitable point of entry into the tissues. In this connection also **Dreyfus**, in the course of his observations, noted that the insect never inserted its stylet into the plant until it had explored the selected portion for some time with the tip of the labium, the stylet remaining meanwhile retracted in the bristle sack (**Börner**, ' Studie über die Chermiden,' p. 101).



Text-fig. 4.—Salivary glands of Fundatrix of *Chermes Lapponicus*. (Semi-diagrammatic after **Cholodkovsky**.) gl.s., acini; n., nuclei of the acini; d.g., branches of efferent duct; gl.a., accessory glands; dc., dc., salivary reservoir; o, mouth of efferent duct.



Text-fig. 5.—*D. nüsslini*. Stylet showing two canals.

The stylet is composed of two pairs of chitinous bristles, the internal pair representing the maxillæ, and the external the mandibles. These elements are seen in transverse section to be so constructed that when in close contact with one another (their normal position) they form a single, cylindrical stylet or piercing-organ, but while the internal maxillæ are always closely joined, the external mandibles may separate from one another at the tip, and this often gives the impression that three stylets are present. The internal maxillæ are so formed as to enclose two canals, the existence of which is well shown in text-fig. 5, illustrating a portion of the tube of *D. nüsslini* into which air

* In the present work, this tube or piercing-organ is referred to throughout as " the Stylet."

has passed, clearly marking the course of the canals. The two canals are probably similar in function to those already described by DAVIDSON in the *Aphides*, one being the suction canal along which the plant juices are carried into the pharynx, and so through the œsophagus into the stomach, and the other the salivary canal, which, communicating directly into the efferent salivary duct, conveys the saliva into the plant.

The Salivary Glands.

CHOLODKOVSKY (6), in 1903, described the salivary glands of the fundatrix stem mother of *Chermes lapponicus* on spruce, and his figure is reproduced here as text-fig. 4. He found that the salivary system consisted of one pair of glands, each of which was made up of three rounded acini. From each of these glands there led out a duct, to which was attached an accessory gland (fig. 4, *gl. a.*), the two ducts finally being merged into one, which broadened out towards the mouth and constituted a salivary reservoir. The glands were found in the prothoracic region, the common duct opening into the mouth at the base of the labium. He stated that, while he had noted the presence of salivary glands in the young larvæ, he had not studied their structure, which he considered would not differ in important details from that of the mature form.

STUDIES OF THE SILVER FIR STEM.

The study of the silver fir tissues, both in normal and affected stems, was made by means of sections cut in three planes—transverse, radial and tangential. For the most part it was found that hand sections gave the best results, as in them greater lengths of the stylet could be traced in the tissues, but microtome sections were also made in cases where the origin and extent of spread of the affected tissue was studied. For staining, methyl green and carmine, safranin and light green (in clove oil), and iodine green and acid fuchsin were the double stains used for general work, but a variety of others were also employed which are cited in the particular cases to which they refer.

The plant material used was either fresh or preserved in 70 per cent. alcohol. It was found, when investigating the nature of the stylet-sheath, that preserved material could not be relied upon to show certain reactions as satisfactorily as material cut fresh from the plant.

The Normal Stem.

To facilitate the understanding of what follows, a brief summary of the main regions of tissues in the normal stem of silver fir is now given. The different zones of tissue are indicated by the letters—A, B, C, etc., in the accompanying photograph of a transverse section of the stem from which the description was made (Plate 4, fig. 1). The section is hand cut from a stem of *A. nordmanniana* one year old, and stained with methyl green and carmine. The zones of tissue beginning from the outside are as follows:

A. Epidermal layer of brick shaped cells, which have the external walls thickened.

B. Four to five layers of thin walled cells, constituting the periderm.

C. On the inner side of the periderm there is a continuous layer of collenchyma. It consists of two to three layers of brownish coloured, oval cells, with thickened walls, forming a conspicuous zone round the stem.

D. It is followed immediately by the ordinary cortical tissue, which consists of a rather broad band of parenchymatous cells, somewhat loosely connected, with large intercellular spaces. The cortex is characterised by a ring of resin canals (R) which vary in number and size. At varying depths in the cortex, the leaf trace bundles occur, each with its endodermal layer surrounding the vascular tissue within. The relation of the *Chermes* attack to these will be discussed later.

E. On the inner side of the cortical area, small masses of crushed tissue indicate the first formed elements of the phloem or bast, and immediately adjoining this crushed tissue are the later formed phloem elements showing clear radial arrangement and made up of sieve tubes, and phloem parenchyma. At intervals in the phloem tissue and passing radially through it can be seen the medullary rays, which consist of radially elongated brick-shaped cells and are continued through the xylem or wood region, to the periphery of the pith.

F. Just within the phloem elements is the layer of cambial cells, on the inner side of which lies the xylem or wood ring. The xylem (*x.y.*) shows the same well-marked radial arrangement of its elements as the phloem, and is composed of thick-walled tracheids together with the radially placed medullary rays mentioned above. In double stained sections the xylem shows up as a complete ring of lignified tissue, presenting a uniform appearance except where interrupted by the large-celled primary rays. It encloses the central parenchymatous pith (P).

The Affected Stem.

Appearance of Affected Tissues—Transverse Section.—The affected stem differs from the normal in that it shows conspicuous patches of abnormal tissue accompanied in the majority of cases by reduction in the amount of xylem present. These differences can be clearly seen by comparing the normal section, Plate 4, fig. 1, with figs. 2 and 3 of the same plate. The first of the two latter figures shows two areas of abnormal tissue, designated respectively A and B, lying in the phloem region, B also penetrating through a medullary ray into the region of the pith. In the midst of this abnormal tissue which is typical of stems attacked by *D. nüsslini* the stylet of the *Chermes* can be seen, and a highly magnified photograph representing a portion of it is shown in Plate 4, fig. 4. The tissue surrounding the stylet shows the following characteristics :—

The cells are considerably enlarged, and their walls are markedly thickened, the cell nuclei are also of large size, and the cell contents are very dense. In fig. 2 the stem has been attacked at two points only, A and B, but fig. 3 shows the transverse section of a stem which has been attacked at several points all round the stem.

(1) For some distance on one side of the stem, xylem tracheids are non-existent, except for small patches lying here and there in the abnormal parenchymatous tissue.

(2) On two-thirds of the wood ring, the xylem appears in isolated groups of tracheids, varying in size, and the cambial region has been much broken up.

(3) Where the abnormal tissue surrounds a leaf trace bundle some of the xylem cells have been replaced by abnormal tissue, as in the xylem ring of the stem.

(4) The black spots occurring here and there in the section are mostly depositions of tannin, which is also associated with the cells adjacent to the point of entry of the stylet into the stem.

The foregoing features of the abnormal tissue found in silver fir attacked by *Dreyfusia* compare in many respects with what has been found in the case of other plants attacked by hemipterous insects. Thus, thickening of the cell wall has already been noted by PETRI in vine attacked by *Phylloxera*, and by ZWEIGELT in rose-stems attacked by aphids. DAVIDSON also comments upon the enlargement of the nucleus in the tissue of *Rumex* attacked by *A. rumicis*.

I have found that in the silver fir, the cells lying along the course of the stylet frequently show their nuclei situated near the wall along which the stylet passed. This feature ZWEIGELT pointed out in his work, and it is probably due in part to the response of the plant cells to the irritation caused by the insects' saliva resulting in a gathering of protoplasm at that point, and the attraction of the cell nucleus towards the part of the cell, presumably most affected by the presence of the stylet.

Longitudinal Section.

Studies of affected stems cut in two longitudinal planes have shown the following main points :—

(1) Considerable portions of the xylem are replaced by abnormal parenchymatous tissue, the affected regions spreading in both directions in the stem (Plate 6, fig. 1 ; Plate 7, fig. 1). In radial section the tracheids are in some cases entirely absent for some distance, but more usually the interruption of the xylem is partial, scattered patches of tracheids remaining. Those tracheids present usually retain their normal direction in the midst of the abnormal cells (Plate 6, fig. 2), but in

some cases they are distorted owing to the pressure of the surrounding tissue (Plate 6, fig. 3). The degree of suppression of the xylem tissue increases towards the terminal buds of affected shoots, and in very severe cases the xylem region is reduced to a few scattered patches of tracheids at this point.

(2) The xylem of the leaf-trace bundles shows similar reduction in amount.

(3) In many cases, where the attack has commenced some distance below the terminal bud, in hitherto uninjured stems, the insect's stylet penetrates as far as the cambium region, the cells of which cease to function as normal cambium at that point. Cambial cells in the affected region appear to retain their meristematic activity for some time after attack, and can be seen dividing in more or less disconnected groups.

(4) The abnormal tissue, consisting of living parenchymatous cells with numerous walls at right angles to the longitudinal direction of the xylem tracheids, may be expected to present a barrier to the adequate transference of water up the stem. This was tested experimentally and is dealt with later.

The Penetration of the Stem.

Penetration of the stem is effected in all the cases I have examined, at the junction between the two epidermal cells, the stylet passing down the middle lamella of the cell wall. This mode of entry into the stem provides a contrast with that of the progrediens generation on the needles, where the stylet passes through a stoma (see p. 32), and this difference is of some biological interest.

I am inclined to the view expressed by DAVIDSON ('Ann. App. Biol.,' vol. 10, No. 1, p. 39) that the mandibles, being capable of independent movement to and fro, probably aid the insect in the penetration of the tissue, being assisted in this by the protraction and retraction of the labium. The co-operation of the labium, in my opinion, owing to the extreme length and flexibility of the stylet, can only be effective by means of the gripping mechanism near its extremity, which can hold the stylet in place and thus aid penetration by giving basal support to a short length of stylet between the insect and the plant.*

In *Dreyfusia nüsslini* I have invariably found that the intercellular course of the stylet is maintained throughout, and its extreme flexibility is frequently in evidence in the tortuous course followed by it in its many ramifications through the tissue.

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* Dr. A. D. IMMS, in his 'General Text Book of Entomology' (1925), p. 332, cites GROVE on *Psylla* in this connection as follows :—"This author suggests that, by means of blood pressure, the apex of the labium becomes distended, and consequently grips the stylet tightly after the manner of a pair of forceps. The projecting portions of the stylets would be forced a short distance into the plant tissues. This being accomplished, the internal pressure would be slackened, which would result in the grip being released and the rostrum would become slightly shorter, so that its apex would have travelled a short distance upwards along the stylets. The pressure being renewed, the grip is re-established and the stylets forced in a step further, and so on until the required amount of penetration may be accomplished."

No evidence has been obtained of any intracellular penetration by the tip of the stylet at any point in its course, and it is therefore assumed that suction takes place entirely through the cell membranes. This is also the opinion of ZWEIGELT in the case of certain aphids, who considers that the cell sap is chiefly obtained by the insects through intercellular suction, and assumes that osmotic forces may be to some extent concerned.

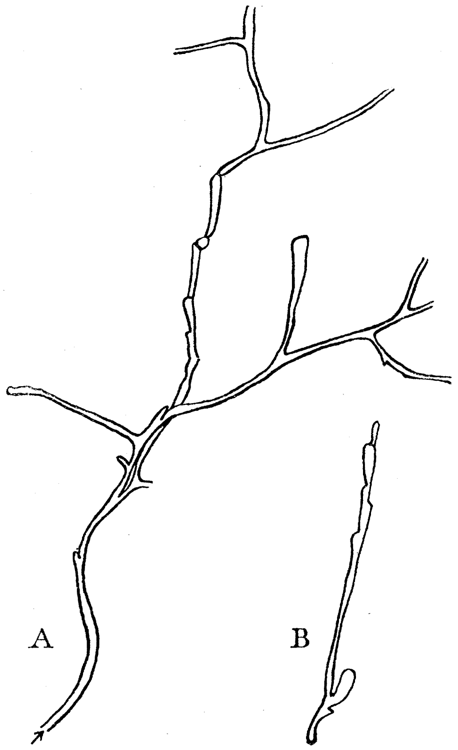
I have found that the stylet may travel in any direction throughout the plant tissues, and very complex stylet tracks were seen. These were well brought out by the iodine and sulphuric acid reaction.

The lignified tracheids of the xylem were not affected by the insect, and the stylet turned away from these when they were encountered (text-fig. 7). This is contrary to the experience of DAVIDSON in *Aphis rumicis*, on Rumex, where lignified xylem vessels were penetrated.

The first effects of attack by *Dreyfusia nüsslini* were often observed in the vicinity of a medullary ray, and in many cases the stylet-sheaths were found in the walls of medullary ray cells, while in tangential sections the abnormal tissue was frequently seen in and surrounding medullary ray tissue.

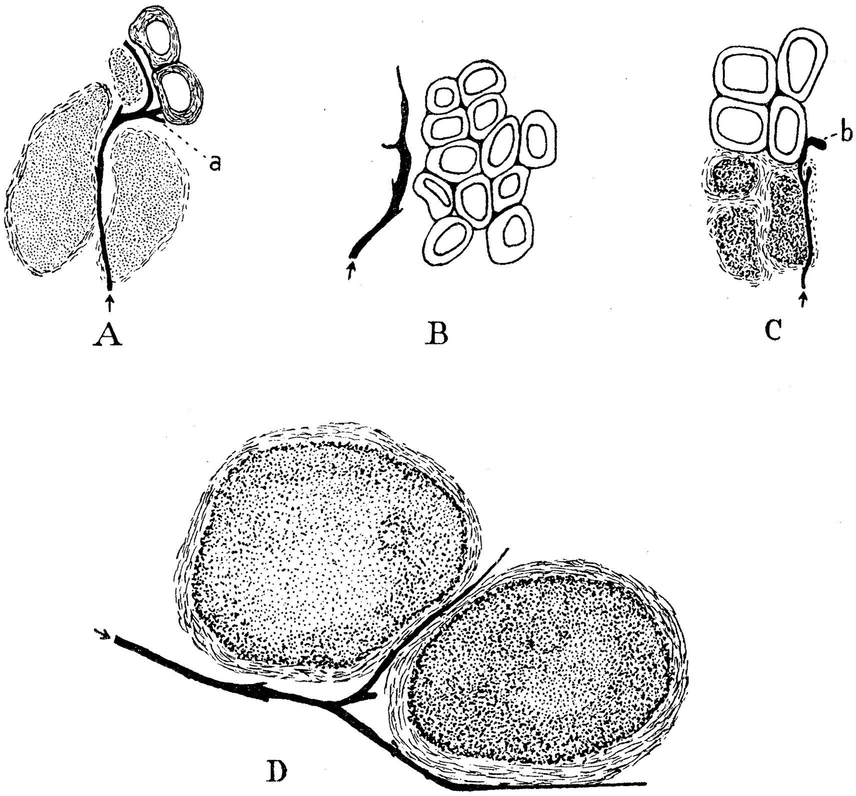
The Stylet and its Sheath.

BÜSGEN was the first writer to demonstrate in the case of the Aphids, that simultaneously with the entry of the stylets into the plant tissue, salivary juice was ejected, and a stylet-sheath was formed which he regarded as being albuminous in nature, staining a deep red with Millon's reagent, and also with Safranin. This stylet sheath remained clearly visible in the tissue after the withdrawal of the stylets themselves, and indicated their former presence and direction in the tissue. The occurrence of a stylet-sheath has been demonstrated by later writers, *e.g.*, in the case of *Phylloxera* by PETRI, in Aphids of several genera by ZWEIGELT, and recently by DAVIDSON (7) in *Aphis rumicis*. MORSTATT (15), however, was unable to demonstrate its presence in the Oyster Shell Scale, *Lepidosaphes*, and PETRI likewise failed to find it in the case of the Olive Scale *Mytilaspis fulva* Targ.



TEXT-FIG. 6.—Outline drawings of stylet-sheaths in silver-fir tissue to show the branching nature of the sheath.

The stylet-sheath in *Dreyfusia* is a colourless tube which closely surrounds the stylet, and which when the stylet itself has been withdrawn, remains in the tissue. Such empty sheaths are to be found ramifying in all directions throughout the tissue, and they afford a means of tracing the path of the stylet (text-fig. 6) (Plate 6, fig. 4) (Plate 5, fig. 4). This ramification of the sheaths represents the varying tracks followed by the stylet in its search for nutriment. The multiple branching of the sheath from a single track which is often observed gives evidence of repeated partial withdrawal of the stylet followed by reinsertion in another direction. Each forking of the sheath in such cases probably indicates the point to which retraction of the stylet has proceeded.



TEXT-FIG. 7.—Stylet sheaths in relation to surrounding tissue. A, showing sheath stopping short at lignified xylem cells at "*a*" and then branching off in another direction; B, stylet sheath lying alongside a group of xylem cells; C, stylet sheath turning abruptly away from xylem cell at "*b*"; D, intercellular passage of the sheath.

NOTE—The arrows indicate direction of the stylets passage.

The stylet-sheath itself is probably derived at least in part from the saliva secreted by the salivary glands. Its composition, however, may be complex, one or more plant substances contributing to its formation in addition to the original salivary constituent. PETRI* considers that in *Phylloxera* the sheath is composed of callose and insoluble calcium pectate, derived from the middle lamellæ of the cell walls, and of tannin deposited externally after the wall of the sheath has been formed.

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* In a scale insect of the genus *Dactylopius* PETRI (18) found that the sheath coloured red with Millon's reagent, and further investigation indicated that here probably both Proteid substances and Tannin entered into its composition.

In DAVIDSON's opinion, in the case of the Aphids, watery solutions of a non-colloidal nature pass through the walls of the plant cells, and, mixing with the saliva, cause a precipitation or coagulation of substances giving rise to the well-defined stylet track (DAVIDSON, 'Ann. App. Biol.,' vol. 10, p. 42).

In the examination of the sheath in *D. nüsslini* I have employed a considerable number of micro-chemical tests, and the following table shows the principal results obtained :—

TABLE I.

Nature of Material.	Reagent.	Reaction.
1. Alcoholic	Aniline blue	Sheath coloured pale blue.
2. Fresh	Millon's reagent	Pinkish red. Good reaction. Sheaths can be clearly picked out by this means in thick dense sections.
3. Fresh	Iodine in K.I., followed by concentrated sulphuric acid	Cellulose walls deep blue. Sheath shows dark brown colour, and can be traced sometimes for long distances, running in all directions between the cells.
4. Fresh	Azo blue	No result.
5. Fresh	Schweizer's reagent followed by carbol fuchsin	Sheaths stain purplish red. Sections show intercellular course very clearly. The stain goes brighter if left longer—say up to 30 minutes in Schweizer.
6. Fresh	Ammonia (conc.), followed by carbol fuchsin (Plate 5, fig. 3)	Sheaths swell. Stain purplish red, the colour deepening as section is left longer.
7. Fresh	Ammonia, followed by Safranin	Slight staining—red.
8. Fresh	Iodine	Sheath stained light yellow.
9. Fresh	Nitric acid (Xanthoproteic reaction)	Sheath and cell contents of abnormal tissue stained yellowish brown.
10. Fresh	Coralline in 4 per cent. Na_2CO_3. (test for callose).	No result.
11. Fresh	Eosin (followed by glycerine)	Pinkish red.
12. Fresh	Picric acid (conc.)	Very marked yellow colour (Plate 5, fig. 4). Sheaths stained both in connection with the stylet and also apart.
13. Imbedded and microtomed	Iodine green and acid fuchsin	Sheaths coloured a bluish green, very distinct.
14. Imbedded and microtomed	Eosin and Delafield's hæmatoxylin	Eosin picks out the areas of abnormal tissue very clearly. Sheaths stain pink (Plate 5, fig. 2).
15. Fresh	Eau de javelle	Dissolves the stylet sheath.

The results obtained in the majority of the above tests indicate two things :—

(1) That there is a great concentration of substances of a proteid nature in the abnormal cells which to some extent resemble secretory cells in appearance, and

(2) That similar substances also form a part of the stylet-sheath itself.

The results of the above tests showing the presence of abundant proteid substances in the cells of the abnormal tissue and in the stylet-sheath itself received further support from the following experiments recently carried out at Kew, on the action of the proteolytic enzymes of gastric juice (or pepsin) and of pancreatic juice (pancreatin or trypsin) upon the contents of the abnormal cells and the stylet sheath. For the pepsin experiment, the following formula by BRÜCKE was employed, viz. :—

Liquor Pepticus Benger	1 vol.
HCl, 0 ·2 per cent.	3 vol.
Thymol, a few crystals.	

Tissues were cut from fresh material and allowed to remain in the solution for varying periods, from 3-4 up to 24 hours, at a temperature of 37°–40° C., a control solution consisting of the last two components, together with the glycerine solvent contained in the pepsin preparation, being used at the same time.

The results of several tests indicated that the contents of the abnormal cells were certainly gradually dissolved, but at no time was the action a rapid one. The sheaths, on the other hand, seemed to be so slightly affected that positive evidence of action was difficult to obtain.

For the Pancreatin experiment, the following formula was employed, viz. :—

Pancreatin, 0 ·2–0 ·4 per cent. in distilled water.
Carbonate of soda, 0 ·3 per cent.
Thymol, a few crystals.

In this experiment the tissues were treated in a similar manner to that already described in the pepsin tests. The results with the pancreatin were much more positive than in the pepsin experiment. Tissues after only 4 hours in the solution at a temperature of 38°–40° C. showed marked dissolution of the cell-contents in the abnormal tissue, and also signs of splitting up in the stylet-sheaths. Prolonged action, up to 24 hours intensified this result, the cells becoming practically empty, while the sheaths, though not completely dissolved, had largely disappeared, leaving only scattered remnants behind.

The conclusion drawn from these experiments was that the action of the proteolytic agents had confirmed the proteid nature of the dense contents of the abnormal cells, and showed that the stylet-sheath consists largely of albuminous substances and that Pancreatin was the more active digestive agent of the two employed.

As regards the question of the presence of pectic substances in the stylet-sheath, DAVIDSON, in his work on *Aphis rumicis*, treated sections for 24 hours in 22 per cent. HCl in 95 per cent. alcohol, and afterwards stained with aqueous methylene blue, which he found brought out the stylet track very distinctly in relation to the cells. As a result of this reaction he suggests the probability that pectic substances form a part of the sheath substance in *A. rumicis*, as PETRI says they do in the case of *Phylloxera* quoted above (p. 44).

With *Dreyfusia nüsslini* I also employed the methylene blue stain as used by DAVIDSON, and obtained good staining of the sheath, but I am unable to deduce from this fact the presence of pectic substances, for the following reasons :—

(*a*) As a confirmatory test for Pectin and its compounds, I used Ruthenium red, which is recommended by MOLISCH* as one of the best tests for this purpose. My results showed that although I got good staining of the middle lamellæ of the cell walls, in no case did I detect any sign of staining in the numerous stylet-sheaths present in the abnormal tissue.

(*b*) Methylene blue cannot be regarded as a distinctive stain for pectic compounds, and its action on the sheath may be due either to the presence of some animal product, perhaps of the nature of mucin, derived from the saliva, or, on the other hand, to certain plant substances extracted from the cells.

(*c*) The dissolution of the stylet-sheaths as a result of the action of pancreatin, indicates that pectic substances are either absent or only present in small proportion.

The penetration of the cell walls along the middle lamella by the stylet is probably due to or aided by the action of a ferment contained in the saliva, while the presence of a second ferment is assumed, its function being to attack the proteid cell contents and by bringing them into solution to render them available as food for the insect.

The consideration of the problem as to the nature of the food material derived from the plant by the insect, brings us to the important question as to the diastatic action of the salivary secretion in the *Hemiptera*, upon the starch in the plant cells attacked. Here, the work of PETRI, ZWEIGELT, DAVIDSON and other writers has shown that the conversion of starch into sugar by this agency is a common property of the saliva among these insects. In the case of *D. nüsslini*, while I have not been able to secure definite evidence of an increase in sugars near the stylet-sheath, and thus can give no proof of the presence of a diastatic ferment, I have found that in affected tissues, treatment with iodine shows that starch is definitely rare or absent in the abnormal cells, and that in cells lying near by the affected areas the starch grains showed decided diminution in size. From analogy with other hemipterous forms we may assume that the insect takes up sugars as well as proteid substances in its food, but we have to consider also that in the case of the abnormal parenchymatous tissue, the disappearance of the starch in these cells may have been effected by ferments present in the cells themselves, and may be connected with the thickening of the cell walls, this thickening being perhaps a response to the irritation caused by the insect.

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* H. MOLISCH, 'Mikrochemie der Pflanze,' Zweite Auflage, p. 351 (1921).

Time of Attack.

The production of abnormal tissues begins at a very early stage in the development of the stem. As has already been noticed, the young larvæ are found at the base of the buds, and sometimes on the buds themselves. Serial sections of infested buds were made, and it was found that very early in the development of stem tissue from the growing point, before any definite differentiation into xylem and phloem had begun, large masses of abnormal tissue were already formed. The result at a later stage is seen in Plate 5, fig. 1, representing a transverse section taken at the base of the bud, where the abnormal tissue already occurs making a gap in the xylem ring, and extending into the pith.

This early commencement of the attack upon the stem explains the extensive interference with the xylem formation, which is so characteristic of injured stems. So far as my experience has gone, the insect is unable to produce any marked effect upon tissue elements already lignified. In one or two cases indeed, marked sign of arrested progress and actual deflection of the stylet, has been observed in the vicinity of lignified tissue. This point has already been touched upon on page **43**.

The extent to which the insect can affect the xylem ring is limited when attack takes place at some distance below the terminal bud, in a stem which has not been previously attacked. A complete xylem ring having already arisen, the insect can only penetrate as far as the cambial layer and interfere with the later increments of wood.

Is Dreyfusia nüsslini the Sole Cause of the Appearance of Abnormal Tissue in the Silver Fir?

Experiments at Kew.—Two series of experiments were carried out in this connection during the past year (1924) at Kew, the one dealing with young seedling plants of *Abies pectinata*, and *A. nordmanniana*, and the other with older plants (three years old), of the same species. The details of these experiments follow :—

Experiment B.—A. pectinata and *A. nordmanniana. Seedlings* (Plate 3, fig. 3), infected in April, 1924, to ascertain :—

(1) If *D. nüsslini* is the sole cause of the appearance of abnormal tissue, and if so how soon such tissue shows signs of appearing ?

(2) If a young seedling of Abies is very heavily infested by *D. nüsslini*, can the insects cause sufficiently severe damage in one season to kill the leading shoot ?

The young larvæ of *Dreyfusia* settled in good numbers on the shoots, especially concentrating at the bases of the buds. The first examination of the infected stem was made on May 21 about five to six weeks after the experiment began, at which time the stylets were found in the cortical tissue surrounded by their sheaths, and the cells of the cortex showed signs that the development of abnormal tissue was beginning. The attraction of the cell nuclei towards the part of the cell adjoining the stylet tract was specially noticeable, and the sheaths were already well developed and much branched, showing considerable activity on the part of the insect. The intercellular course of the stylets was again very evident.

The second examination of infected seedlings was made on August 9, with the following results :—

(A) *Sections.*—The development of abnormal tissue during the past $2\frac{1}{2}$ months had been very considerable (Plate 7, fig. 2). Sections cut at the base of the bud, and stained with methyl green and carmine, showed almost total suppression of the xylem elements. Sections lower down the stem showed the peripheral outline of the xylem to be irregular owing to a varying number of indentations due to abnormal parenchymatous tissue replacing the tracheids. The xylem of the leaf traces in the cortex was also partially suppressed. The stylet-sheaths ramified all through the abnormal tissue, and the indications were that lignified tissue could not be affected by the insects' attack.

(B) To ascertain the effect of xylem reduction on the transpiration current, seedling stems, cut under water, were kept with the cut end in eosin solution for varying periods, after which the distribution of stained tissue was observed in transverse and longitudinal sections.

This experiment showed that where a patch of abnormal tissue interrupts the xylem, the upward conduction of water practically ceases at that point, and where there is extensive interruption of the xylem, the upward conduction of water becomes restricted to the small amount of xylem remaining, so that the water supply is quite insufficient for the requirements of that portion of the stem above this point. The condition last described was found near the terminal points of the young seedlings in question, where the reduction in xylem was clearly brought out by staining sections in methyl green and carmine.

Since the abnormal tissue previously described is never found in unattacked stems of silver fir, and only makes its appearance shortly after the commencement of *Chermes* attack, the insect must therefore be regarded as the sole cause for the appearance of this tissue.

It is also very probable from the effects observed above, that a seedling of *Abies* heavily attacked by *Dreyfusia*, would have its terminal shoot tissue so affected owing to the lack of xylem as to preclude further development next year.

A. pectinata. A. nordmanniana. Older plants.—Infected in March and April, 1924. This experiment was a confirmatory test of that applied to the young seedlings. Sections of infected stems cut from these plants showed extensive occurrence of abnormal tissue, as in the case of the seedlings, and it was also seen that in some plants a good deal of interference with the growth of the xylem occurred within a period of three to four months. The extent of this, however, very largely depended on the original state of health of the young plant, as there was no doubt that the weakest growing plants in this experiment were far more heavily attacked than the stronger ones. Some of the stronger plants, although their young twigs were heavily infested by *Chermes*, still looked green and healthy, while the weaker plants, whose shoots were already of poor growth, had succumbed to the attack. Whether the plants which, at present, show no sign of wilting under the *Chermes* attack will develop new shoots next year is still uncertain.

The experiment of protecting certain branches from attack by means of tanglefoot rings was tried, and was successful in several cases in keeping these clean, such branches showing quite normal growth.

The Effects of Injury to the Stem upon the Transfer of Water and Food Substances in the Silver Fir.

It is now generally agreed among plant physiologists that the conduction of water, the so-called transpiration stream, is effected in the stems of plants through the tracheal tubes of the xylem, although the precise mechanism involved in the raising of water is still a subject of controversy. In addition to their function as conductors of water, however, the tracheids and vessels of the xylem were recognised by HARTIG in 1858 to be important channels of transport for the soluble products of the wood parenchyma and medullary rays, from the lower to the higher regions of the plant.

HARTIG'S work was followed by that of FISCHER (10), who in 1890 showed that in *Pinus sylvestris*, *Larix europæa*, and *Thuja occidentalis* the tracheids contained much glucose, which was higher in amount during the summer season of growth, but which even in winter was relatively large. More recently, DIXON and ATKINS (8, 9) have found sugars (mono- and di-saccharides) present in the tracheæ of a large number of evergreen and deciduous trees, and they have drawn the following important conclusions as a result of their work :—

(*a*) " The transfer of carbohydrates upwards is a continual and primary function of the tracheids. This great function of the tracheids is never put out of action even in times of greatest drought."

(*b*) " The column of tracheids forms a permanent channel for the rapid carriage of water and salts, and to a less degree for the transfer of organic substances."

GROOM (11), working on silver fir, scots pine, larch and other coniferous trees, has carried out some experiments, using the ordinary eosin method, upon the rate of ascent of water in the tracheids, and has arrived at the following conclusions :—

(*a*) Evergreen conifers can and do conduct water at a high speed through the tracheidal elements.

(*b*) Under identical atmospheric conditions, a species such as larch may conduct water at the same, or even a slightly higher rate, than obtains in a very rapidly transpiring dicotylous tree such as beech.

(*c*) Next to larch, scots pine and silver fir showed the highest rate of flow, although the experiments were not sufficiently extensive to lay down a relative rate.

(*d*) In normal trees the tracheidal structure of the wood is at one and the same time an efficient medium for the relatively rapid transfer of water, and also a safety mechanism providing against over-rapid loss of water in seasons of drought.

(*e*) The coniferous mechanism is more easily deranged and killed by sudden injuries than the dicotylous, *e.g.*, fungi, leaf-feeding and bark-boring insects may kill coniferous trees more rapidly than dicotylous trees.

Consideration of the results contained in the above quotations makes it abundantly clear that the importance of the xylem tracheids is paramount, and that extensive reduction in amount of these elements will seriously affect not only the upward conduction of water and salts to the growing region, but also the transference of soluble carbohydrates, etc., in both directions to those points in the stem where they are needed.

The following further experiments dealing with this point were conducted at Kew by the eosin method, already described on page 49, in the case of young seedlings, branches of silver fir both normal and infected being used in this case.

I. *Normal Stem.*—Four separate experiments were made with normal stems from 6 to 10 inches long, cut under water, and kept with the cut ends in eosin for varying periods of 12 to 36 hours.

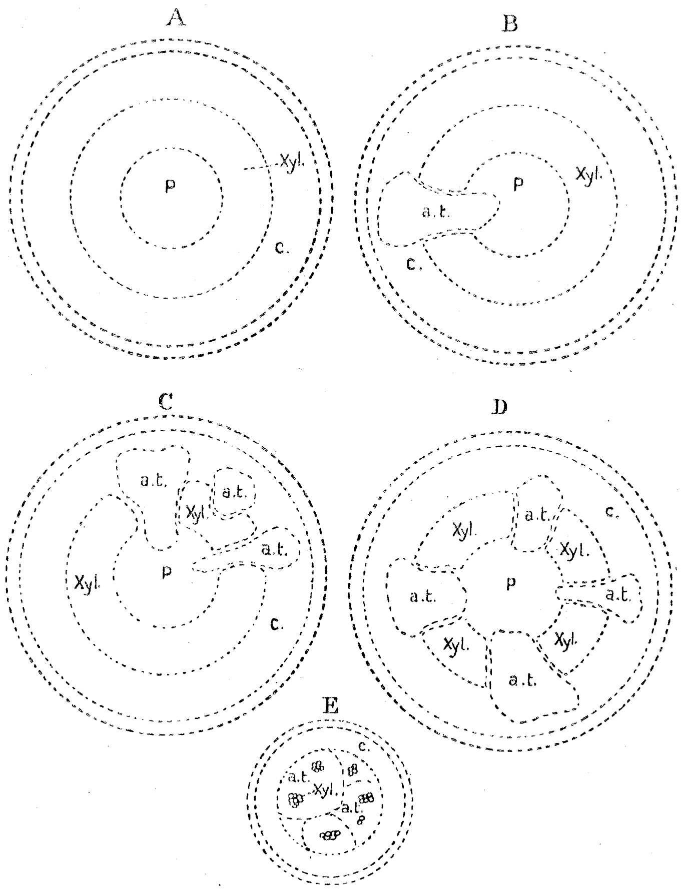
In all these experiments transverse and radial sections showed the path of the eosin through the xylem quite well defined. Text-fig. 6 shows a diagrammatic transverse section of one of these, and text-fig. 9 the longitudinal aspect.

II. *Infested Stem.*—From six experiments similarly conducted upon infested stems the following general result was obtained :—

That abnormal tissue cells are a bar to the passage of water, this being indicated by the following conditions found in the sections of infected stems.

Transverse.—In slightly attacked stems, while the xylem area showed regular passage of the eosin, the areas occupied by abnormal tissue showed no colour at all.

In severely attacked stems, especially near the tips of the branches, the amount of abnormal tissue was so great that the only xylem present consisted of scattered patches of tracheids, which were the only elements showing evidence of eosin conduction (text-fig. 8).

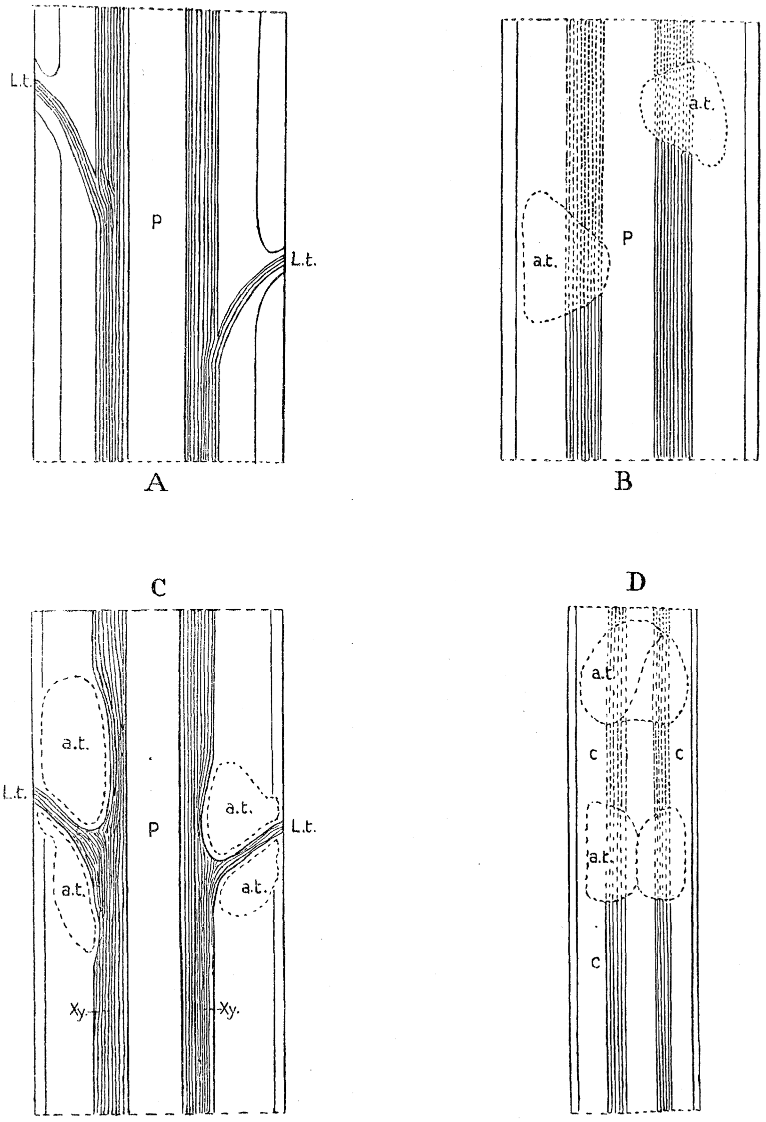


TEXT-FIG. 8.—Diagrammatic transverse sections of normal and infected silver fir stems, showing the relation of the abnormal tissue to the other tissues of the stem. A, normal stem; B, C, D, E, infected stems showing a progressive increase in the amount of xylem replaced by abnormal tissue; C, cortex-phloem region; Xyl., xylem; P, pith; a.t., abnormal tissue.

Longitudinal Sections.—In longitudinal sections, where a few tracheids were left surrounded by abnormal tissue, the path of the eosin ran through the former, while the surrounding abnormal tissue showed no sign of passage (text-fig. 9).

Where abnormal tissue occurred around leaf traces, the staining with eosin brought out clearly the reduction of the conducting tissue in the leaf trace, and showed that the amount of water reaching the leaf in such a case would be considerably reduced. This was confirmed by the degree of staining of the leaves themselves.

In stems attacked at some distance below the bud, the treatment with eosin emphasized the effect produced by the insect upon the plant tissues as already described on page 48. The stain being restricted to the conducting tracheids, it is readily seen in such cases that the tissues have often been affected as far inwards as the cambium layer, which has become replaced by abnormal parenchyma. The resulting cessation of growth of the xylem ring at this point, while its growth has continued elsewhere, has caused the appearance of a local constriction of the ring. (Text-fig. 9.)



Text-fig. 9.—Diagrammatic longitudinal sections of normal and infected silver fir stems, showing the relation of the abnormal tissue to the other tissues of the stem. A, normal stem; B–D, infected stems; L.t., leaf trace; other letters as in text-figure 8.

Dreyfusia nüsslini on Abies grandis.

Experiments at Kew.—The following experiment was devised to ascertain the behaviour of *D. nüsslini* towards the species *A. grandis*, which hitherto was supposed to be at least partially immune from its attacks.

Experiment A.—Abies grandis.

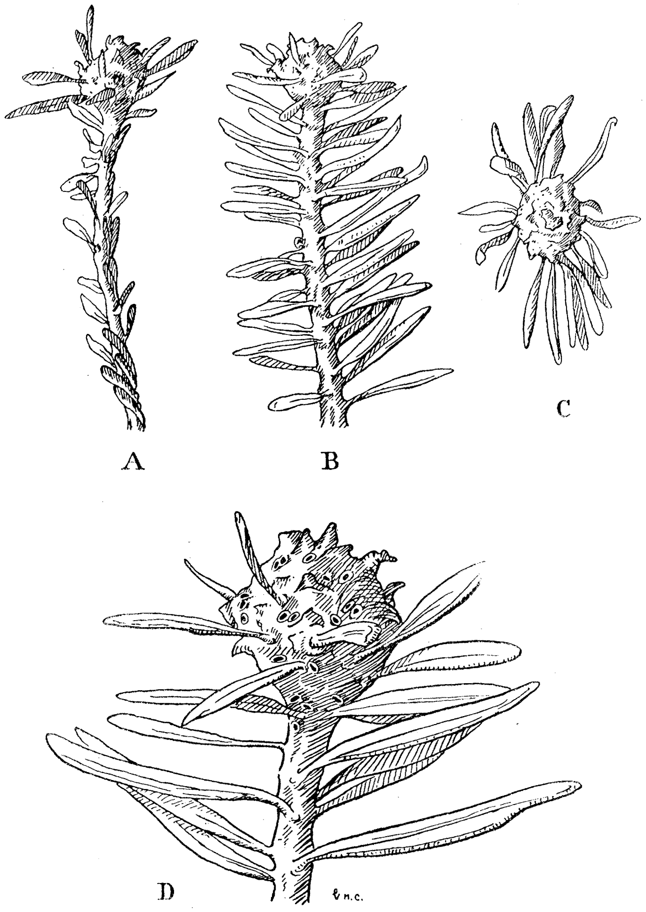
Six young plants of *Abies grandis* were exposed to infection by *D. nüsslini* in March and April, 1924, to establish the following points:—

(1) Does *D. nüsslini* attack *Abies grandis*?

(2) If so, does it cause similar injury to the stem as is found in *Abies pectinata*?

(3) If the *Chermes* does not attack this tree, are there any signs of natural protection, such as hairs on the stem, &c., or internal characteristics which prevent such attack ?

All the plants of *A. grandis* used in this experiment were readily attacked by *D. nüsslini*. The young larvæ settled quite freely and were certainly not impeded in any way by the presence of short hairs on the young stem. It was noticeable, however, that they concentrated in greater numbers round the buds and stem nodes, than along the whole length of the shoot, and again that in many cases the stem had swollen considerably in size locally following the attack, and showed a similar character to that assumed by *Abies nobilis* when attacked by *D. piceæ*. This has been observed on all the plants affected, the terminal buds of which become quite globular in shape (text-fig. 10), and show large numbers of larvæ settled upon them. In fact, wherever one finds a bud upon which the *Chermes* have settled this swelling begins to appear within a few weeks.*



TEXT-FIG. 10.—*Dreyfusia nüsslini* on *Abies grandis*. Experiment A, Kew, 1924. Showing the swollen buds produced as a result of the insect's attack.

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* This applies even to small buds occurring at different points on the stem.

The swelling of the stem is not only noticeable in the bud region, but may occur also at the branch nodes, and also at other points on the main stem, which may therefore show several nodular thickenings. Sections of such stems showed the following characters :—

(*a*) Some of the cortical cells are markedly affected and become considerably enlarged and swollen, although they do not always exhibit the dense contents and enlarged nuclei observed in *A. pectinata* (Plate 7, fig. 3).

(*b*) The phloem region is very much stimulated and increased as a result of attack. The normal function of the cambium is interfered with, and the formation of xylem may cease at the attacked point (Plate 7, fig. 3).

(*c*) In very young stems where the xylem has been isolated into patches the cambial layer may be found turning in towards the pith where it is continuous with a strip of abnormal medullary cambium peripheral to the pith. A ring of cambium may thus completely surround each isolated xylem patch (Plate 7, fig. 4).

(*d*) As in *A. pectinata*, the leaf-trace bundles are markedly affected.

It is evident then, that the swelling of the stem is due partly to the enlargement of the cortical cells, and partly to the increase in the phloem region.

It is also shown by this experiment that *D. nüsslini* on *A. grandis* produces swellings of the buds and stem similar to those found upon *A. nobilis* attacked by the so-called *D. piceæ* var. *bouveri*, and that on *A. grandis* the signs and effects of attack are somewhat different from those already seen on *A. pectinata*. The reason for this is unknown. It is also remarkable that all this deformation of tissue is the work of the sistens larvæ in their first stage, no further development having taken place throughout the summer in the stem forms.

The foregoing results on *A. grandis* received confirmation in the following experiment, conducted with this and two other species of silver fir :—

Experiment C.—A. grandis. A. pectinata. A. nordmanniana.

Specimens of these three species were placed together in close contact and infected with *D. nüsslini*, with the object of establishing whether or not the *Chermes* would seek one species in preference to the others.

A. grandis.—The young larvæ settled on this species to some extent, but not in such numbers as upon the other two. They showed a marked preference for the bud regions, and caused these to swell markedly as already noticed in Experiment A. Those larvæ which were not on the buds or near them did not cover the stem as in *A. pectinata* and *A. nordmanniana*, but lay close to the needle bases and considerably affected the adjoining tissues.

A. pectinata.—The plant selected turned out rather a poor specimen, the young larvæ attacked it in greater numbers than in the case of *A. grandis*, and covered the whole length of the young shoots.

A. nordmanniana.—This plant was an excellent specimen, and the young larvæ settled freely on the new shoots and also lower down on the stem. There was no marked swelling of the buds as in *A. grandis*, and the main stem was attacked more heavily.

The inferences to be drawn from this experiment may be divided into three :—

(*a*) That in *A. grandis* the attack affects the buds and immediately adjacent portion of the stem causing swelling.

(*b*) That, as far as location on the main stem is concerned, *A. pectinata* and *A. nordmanniana* were more heavily infested than *A. grandis*.

(*c*) Examination of the young larvæ causing swelling of the buds in *A. grandis* showed them to possess the characters of *D. nüsslini* as laid down by BÖRNER, and confirmed the result of Experiment A that this type of deformation is not confined to the *D. piceæ* form.

GENERAL DISCUSSION OF RESULTS.

The above observations and experiments show that the silver fir *Chermes* causes serious injury to the young tree, and it is especially during the first 15–20 years of its life that the evil effects of the insects' attack are most pronounced. This injury is directly attributable to the interference with the normal conduction of water and dissolved substances in the stem, in consequence of the partial replacement of the xylem elements by abnormal parenchymatous cells, incapable of carrying out this function satisfactorily. As a direct result of the reduced water supply to the terminal portions of the shoots (*i.e.*, in those regions below which abnormal tissue has been developed), gradual withering of these, together with defoliation, is to be anticipated, and this is precisely what is found in the case of heavily infested trees under natural conditions. A second source of injury to the plant no doubt arises from the fact that food substances are continually being removed from it by the insect, but this, in my opinion, is probably the less important factor of the two, the inadequate supply of water being the primary cause of injury.

The commencement of the attack at an early stage in the differentiation of the tissues of the stem, before the first xylem and phloem have been laid down, is an important point, as the normal development of the xylem in the young shoots is interfered with in its inception, with a consequent initial handicap to all the young growth. This, as time goes on, is gradually increased by the attacks of succeeding generations of stem-feeding forms.

How numerous these *Chermes* can become in the case of *D. nüsslini*, may be seen in the following table, showing the different generations produced in a single year's cycle and their respective feeding places on the fir :—

TABLE II.

Generation.	Place of feeding.	Time of feeding.
Winter larvæ, 1922	Stem.	Spring, 1923.
Progeny of Stem Mothers. Spring, 1923	Needles (Progrediens L.) . . . . . Stem (Sistens L.).	Summer, 1923.
Stem Mothers of Sistens larvæ and their progeny	Stem	Late Summer, 1923.

From the above, we see that out of four generations of young larvæ, the stem is the chosen settling place of three, only a small and relatively insignificant proportion of the total number of young larvæ produced seeking out the needles. This results, in the case of badly-infested trees, in an overwhelming concentration of the larvæ on the young stems and buds, with the result that serious damage is done at the growing points.

The rapidity with which the stem can become affected has been shown in Experiment B, where in the short space of 2½ months a considerable amount of deformation in the tissue had taken place. This can easily be accounted for by the extreme mobility of the stylet, which, as we have seen, moves by repeated partial retraction and reinsertion in all directions through the tissues, leaving its tracks behind. When, therefore, in a localised area of stem, to the extensive operations of a single stylet one adds those of ten or even a hundred other stylets, it is not difficult to imagine the effect of such an invasion on the tree.

Of all the species of silver fir examined *A. pectinata* and *A. nordmanniana* are the two which show the effects of *D. nüsslini* attack in its worst form. In species like *A. grandis*, *A. nobilis* and others, it is found that the injury is confined to the buds, and this type of damage has, in the past, been referred to the form *D. piceæ* var. *bouveri*. Whether this subdivision of the genus into several species is sound from the biological point of view, is in the author's opinion open to question, but at any rate one thing is quite clear, namely, that the factors at work on the deformation of the stem in *A. pectinata* and *A. nordmanniana* are virtually similar to those operating in *A. grandis* and that type of tree. It is probable also, that, on trees of the *A. grandis* type, the localisation of the attack to the buds and older portions of the stem, and the evident avoidance of the young stem, has to do with some factor possibly inherent in the host plant, the nature of which is obscure. It is remarkable to find that *D. nüsslini* when placed on *A. grandis* will produce the *D. piceæ* type of deformation, and this suggests the possibility, that the two forms (*D. nüsslini* and *D. piceæ*) may be, as NÜSSLIN believed, only biological races.

SUMMARY OF RESULTS.

(1) The life-history of *Dreyfusia nüsslini*, BÖRNER, one of the silver fir *Chermes*, has been studied at Kew and elsewhere, during the last two years, and a complete series of generations on the silver fir has been observed. Winged forms were produced in small numbers on the fir, and these were proved both morphologically and biologically to be sexuparæ. Experiments showed that they would settle upon the Oriental spruce (*P. orientalis*), and lay eggs there, which produced the young larvæ of the sexual forms, but they did not breed through to maturity.

(2) In the open, no sexuparæ of this species were found, and it is considered that they are rare in Britain, at least in certain years.

(3) No winged "alata non migrans" form has been found in the life-history of *D. nüsslini* in Britain.

(4) The life-history of the silver fir *Chermes*, *Dreyfusia piceæ* (Ratz) BÖRNER, has also been studied at Kew. This form has been studied upon the older stems and branches of silver fir, and also upon the buds on which it causes deformation.

(5) No winged form (alata non migrans) has been found in this species. The life-cycle has consisted of wingless, parthenogenetic forms only.

(6) The majority of the larvæ of *D. nüsslini* are stem feeders, and as a result of this they cover the greater portion of the main stem and smaller branches of young trees when present in large numbers. The effects of their feeding upon the tissues have been observed, and it has been shown that considerable malformation results, which, commencing in the bud, causes the xylem or conducting tissue to be suppressed, often to a considerable degree, both in the main stem and also in the leaf-trace bundles.

(7) Entry into the tissue is made at the junction between two epidermal cells, the stylet passing down the middle lamella of the cell walls. The course of the insect's stylet in the tissues is intercellular throughout, and round the stylet there is formed a sheath which remains in the tissue after the stylet tube has been withdrawn.

(8) The stylet-sheath responds to a wide variety of micro-chemical tests, some of which indicate that it is composed at least, in part, of substances of a proteid nature.

(9) The cells of the affected tissue are abnormally large, show much thickened walls, dense proteid contents, and an enlarged nucleus. The nuclei of those cells which lie near to the path of the stylet are seen in many cases lying on the side of the cell nearest to the stylet.

(10) The area of the abnormal tissue in the stem spreads gradually both transversely and longitudinally, and experiment has shown that this tissue restricts the passage of water through the stem. In the cambium region the cambial cells are prevented from functioning normally in most cases.

(11) It has been shown by experiment that *D. nüsslini* is the sole cause of the appearance of abnormal tissue. The formation of this tissue begins very soon after the insects have settled on the young shoots in spring, and its area increases as the summer advances.

(12) Weakly young plants heavily attacked by *D. nüsslini* will undoubtedly perish

in a single season, but healthy plants can probably hold out for a longer period without sustaining fatal injury. It is, however, only a matter of time until the plants succumb, as the deficiency of water due to imperfect conduction must already have its effect upon the production of new growth next year, when the attack unless checked will be renewed upon the weakened stem. The removal of food substances by the insect no doubt also contributes in some measure to the injury caused.

(13) *D. nüsslini* has been shown by experiment to attack *A. grandis*. The insect does not, however, spread over the stem to the same extent as on *A. pectinata* and *A. nordmanniana*, but settles principally at the bases of needles and on the buds. As a result of this the latter swell up and become distorted, as do also other portions of the stem upon which the insect settles. This swelling has been shown to be due to the enlargement of the cortical cells, combined with considerably increased growth of the phloem in the affected parts.

In conclusion, I desire to express my thanks to the Director of the Royal Botanic Gardens, Kew, for the facilities granted to me during the course of this work. I am also much indebted for valuable suggestions and advice to Dr. J. W. MUNRO, Entomologist to the Forestry Commission, under whose supervision the work was carried out; to Mr. L. A. BOODLE, Assistant Keeper of the Jodrell Laboratory, Kew, for his assistance and criticism of the work; and lastly to Mr. G. ATKINSON, Botanical Artist at Kew, for his share in the preparation of the photographs.

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DESCRIPTION OF PLATES.

Plate 3.

Fig. 1.—*A. pectinata*, showing terminal shoots attacked by *D. nüsslini.*

Fig. 2.—*A. nobilis*, showing galls produced by *D. piceæ.*

Fig. 3.—*D. nüsslini* on seedlings of *A. nordmanniana.* (Experiment B, Kew, 1924.)

Fig. 4.—*D. nüsslini*—stem mothers and young larvæ on *A. pectinata*, Kew, 1924.

Plate 4.

Fig. 1.—Transverse Section, 1 year stem, *A. nordmanniana* showing normal structure. *A*, Epidermis. *B*, Periderm. *C*, Collenchyma. *D*, Cortex. *E*, Primary Phloem. *F*, Cambium. *Xy*, Xylem. *P*, Pith. *R*, Resin Canal. × 41.

Fig. 2.—Transverse Section. 1 year stem, *A. nordmanniana* showing groups of abnormal cell tissue at *A* and *B*, where the Chermes have been feeding. *St, Stylet.* × 43.

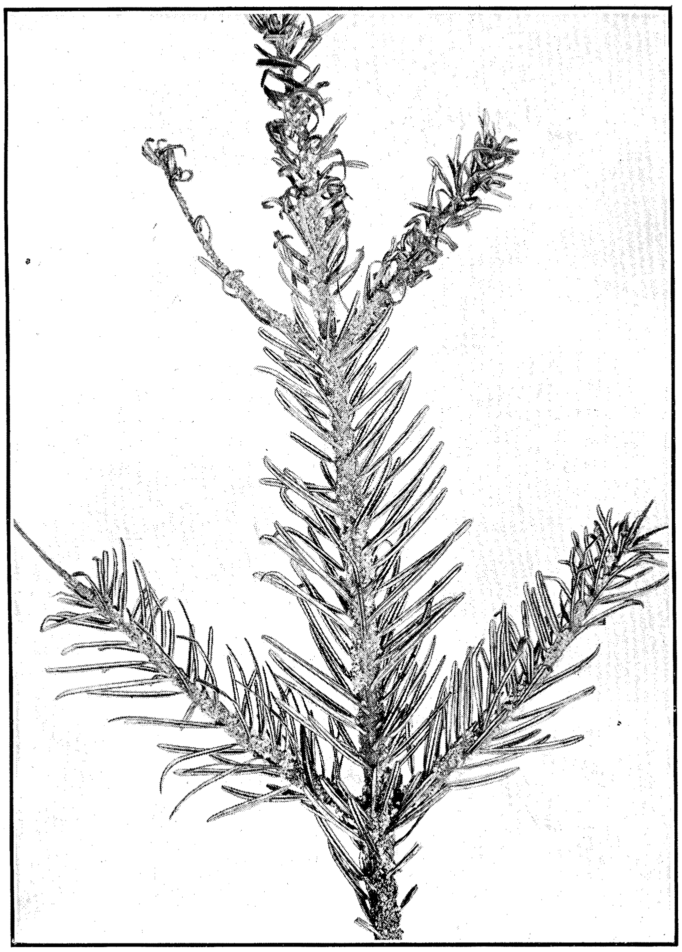
Fig. 3.—Transverse Section, 1 year stem, *A. nordmanniana* showing the breaking up of the xylem ring as a result of severe attack. × 41.

Fig. 4.—Transverse Section, 1 year stem, *A. nordmanniana,* showing the stylet of the *Chermes* in the tissue. × 175.

Plate 5.

Fig. 1.—Transverse section at the base of the bud in *A. nordmanniana,* to show the abnormal tissue separating the xylem at an early stage.

Fig. 2.—Longitudinal section through the apex of the stem in *A. pectinata,* showing three patches of abnormal tissue (A.B.C.) lying at the base of the bud. These areas have been deeply stained with eosin. × 33.



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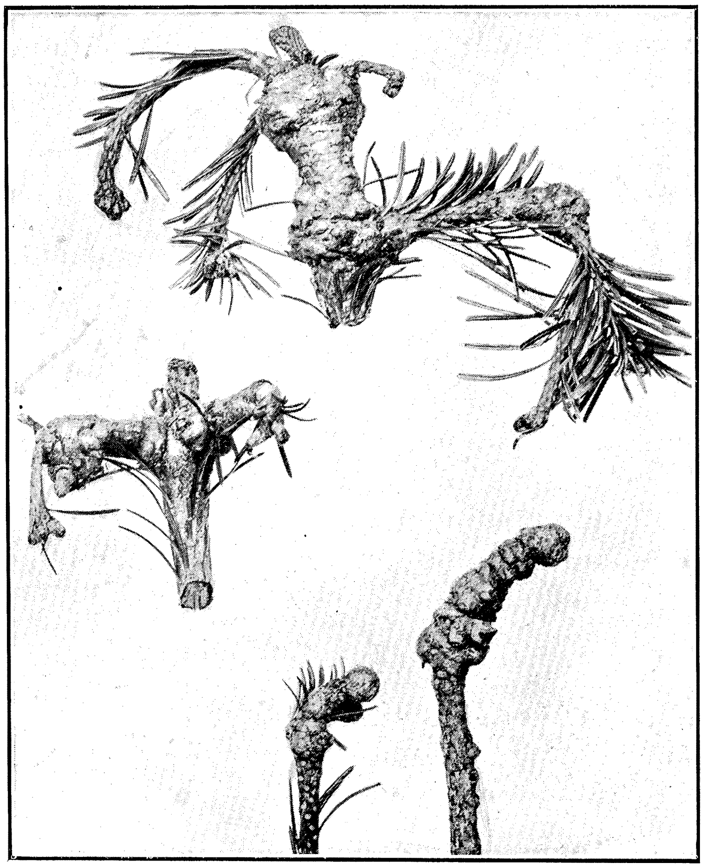

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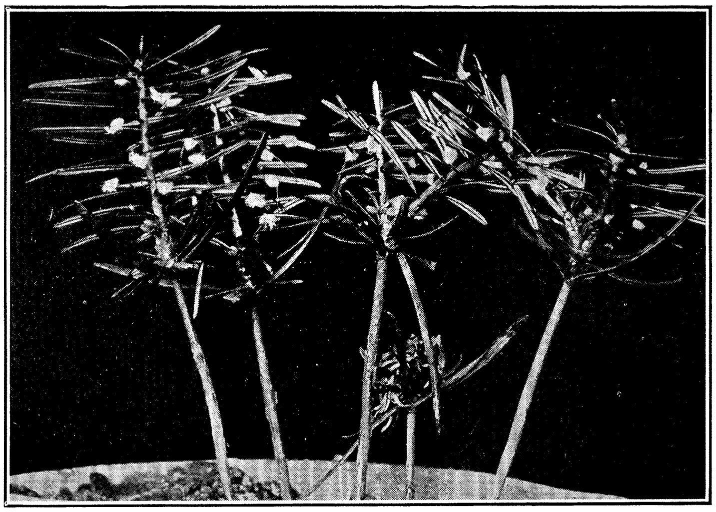

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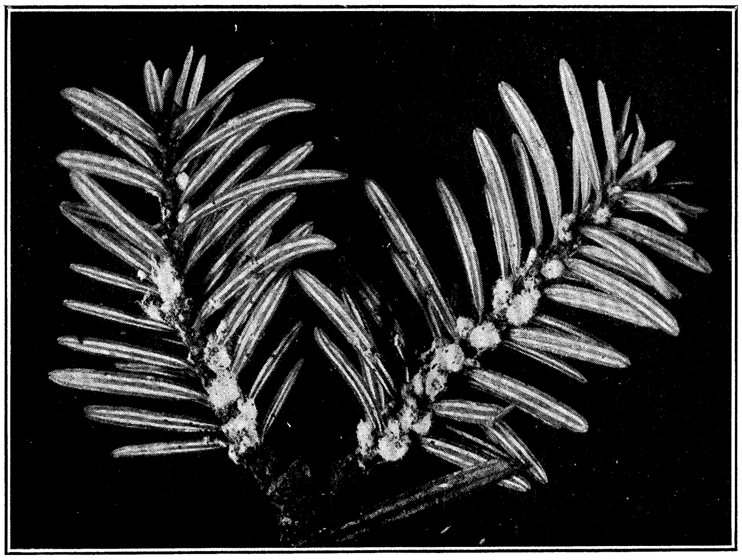

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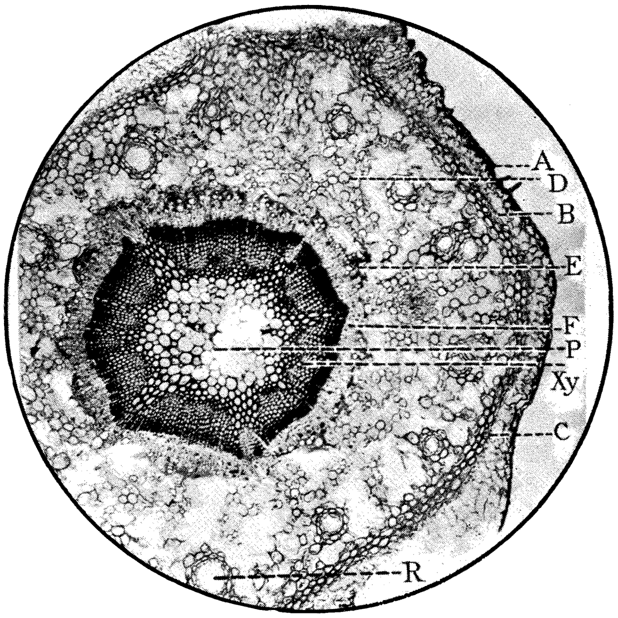

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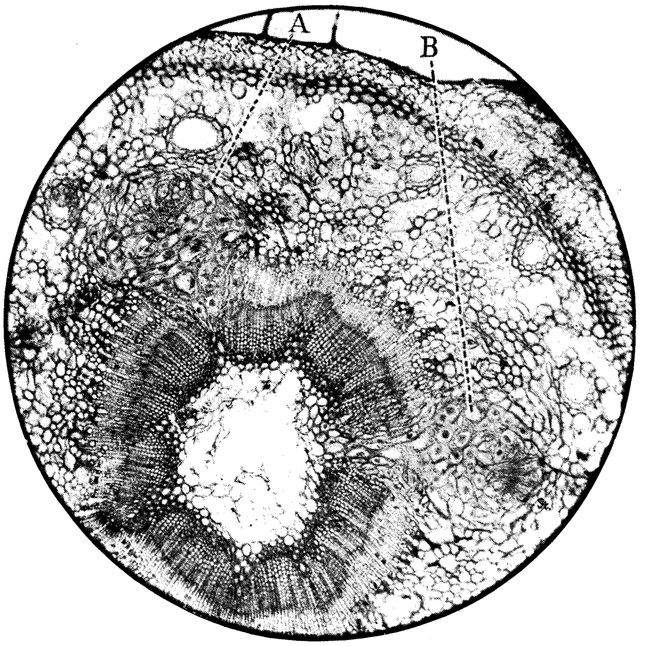

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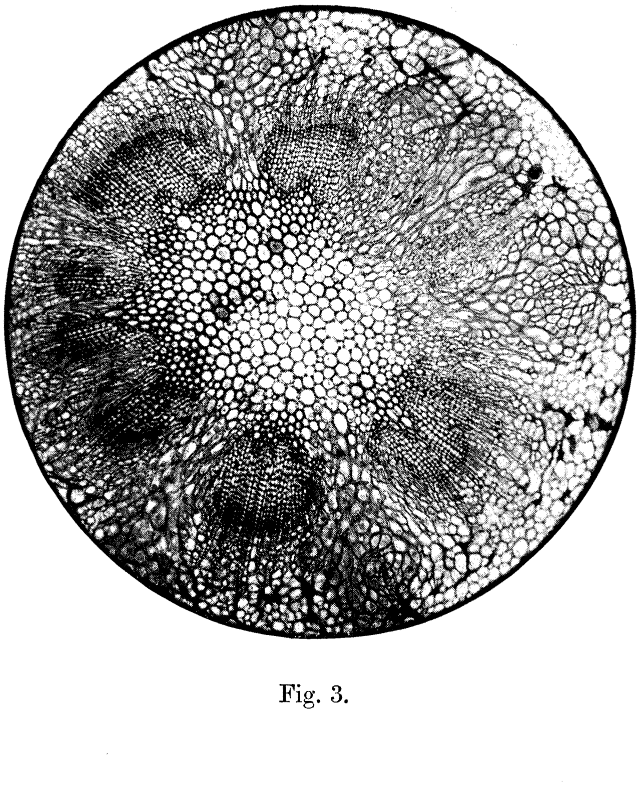

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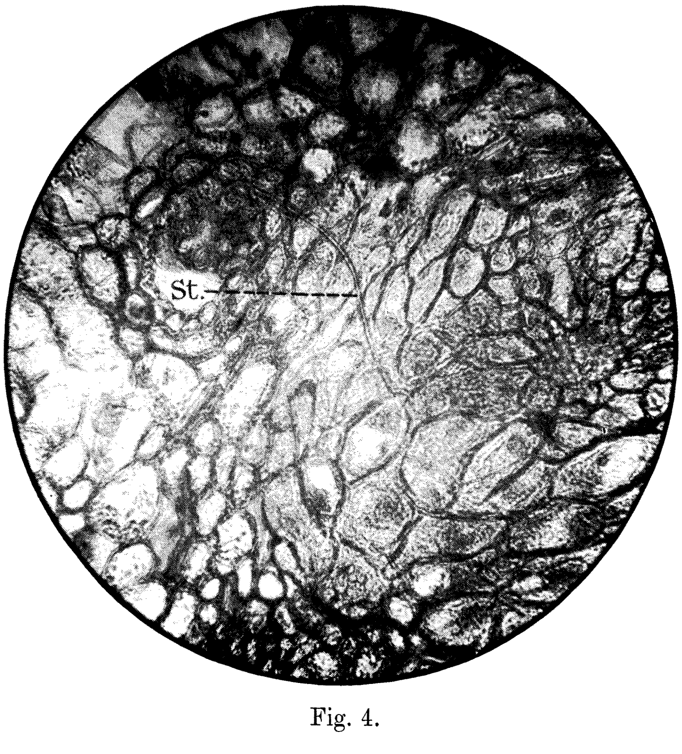

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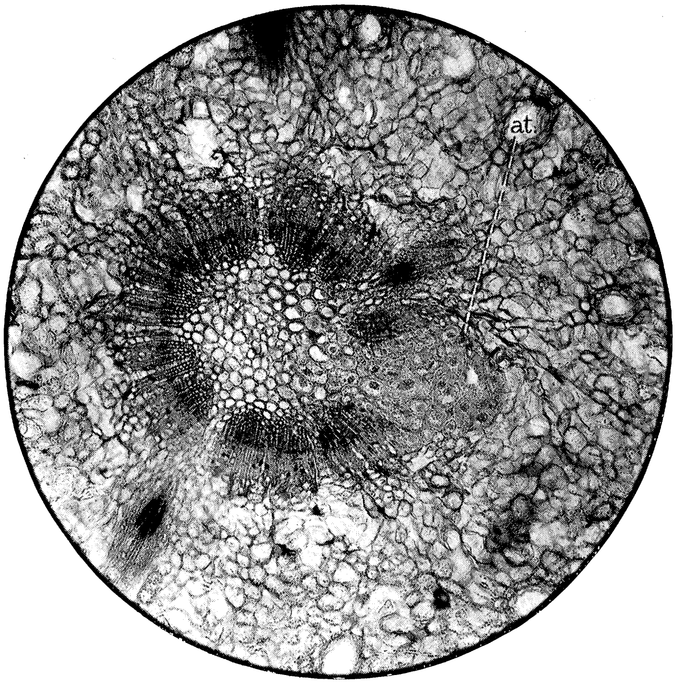

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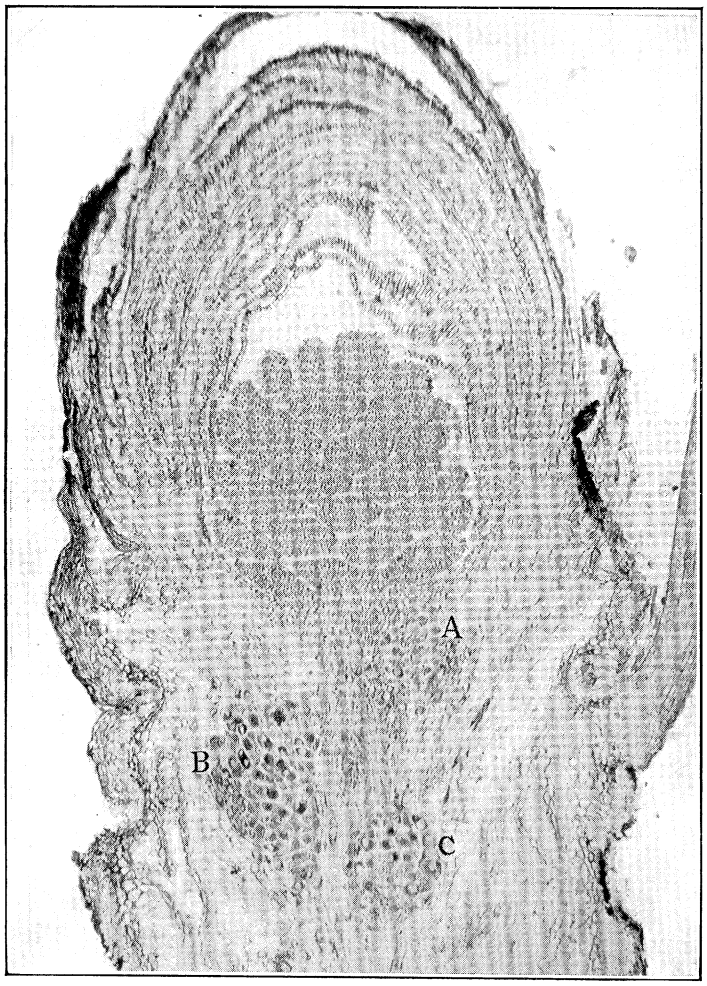

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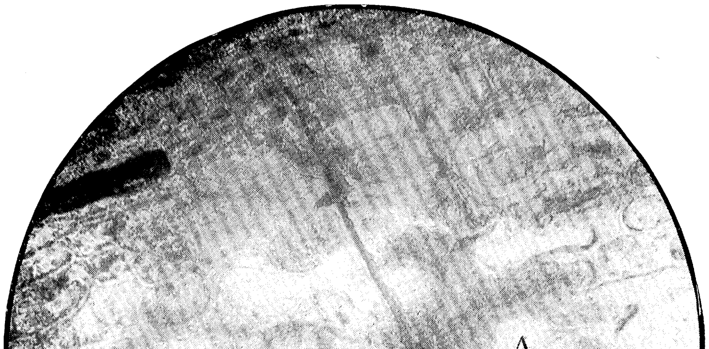

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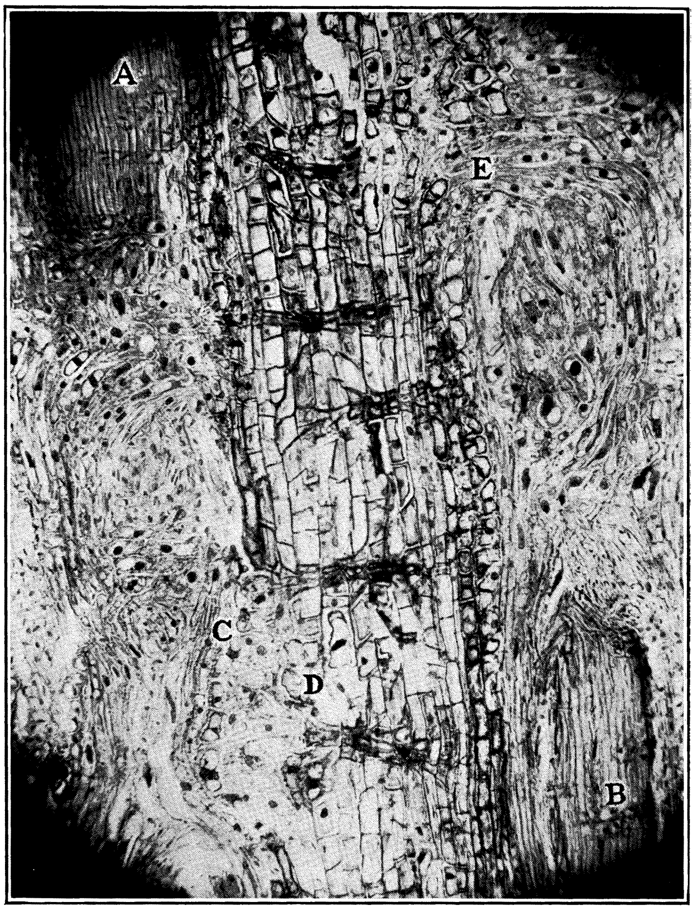

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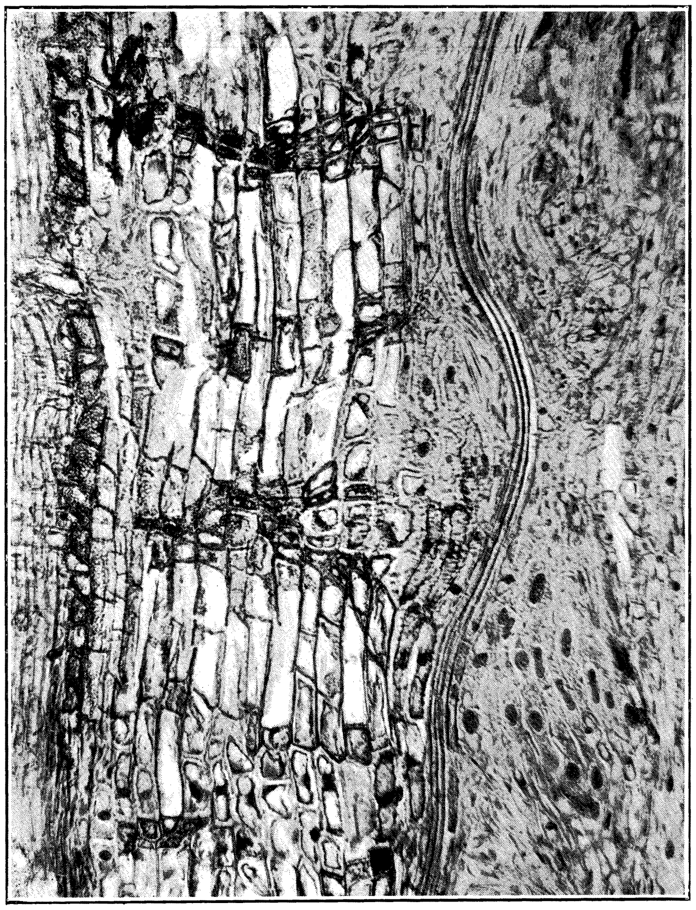

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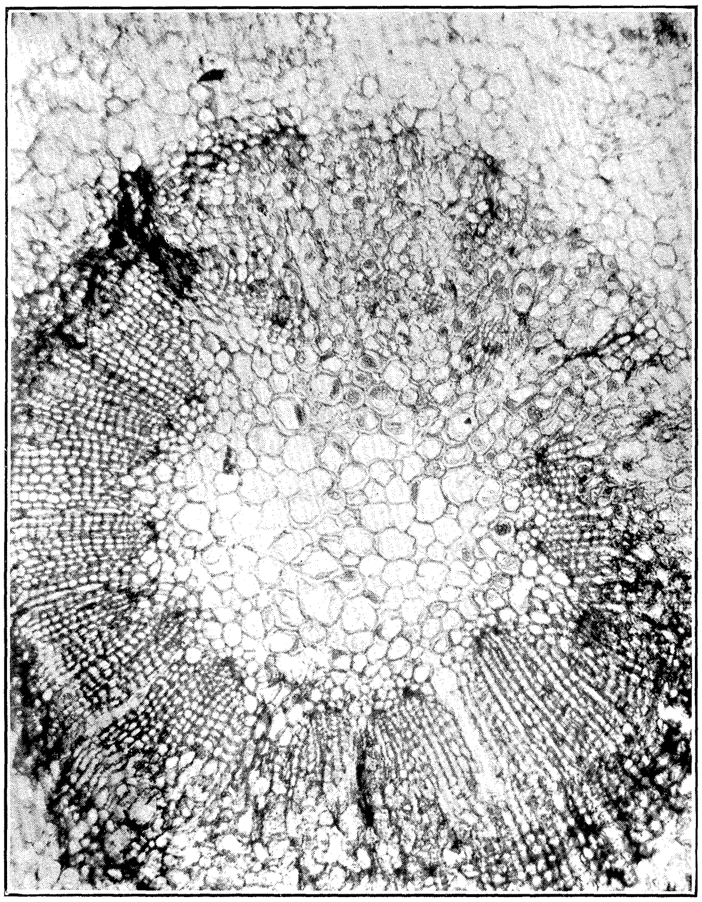

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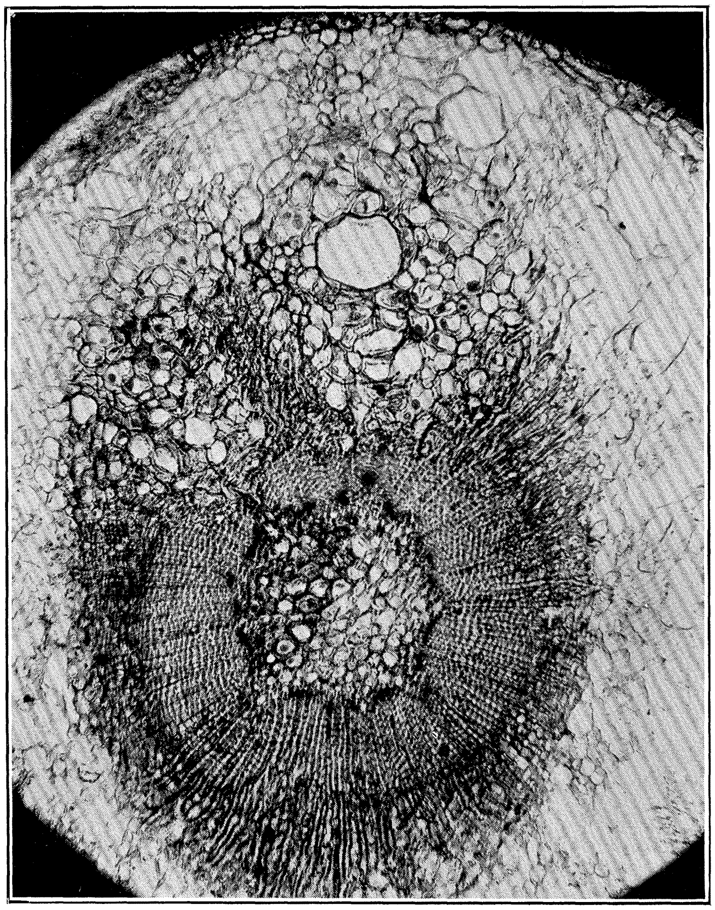

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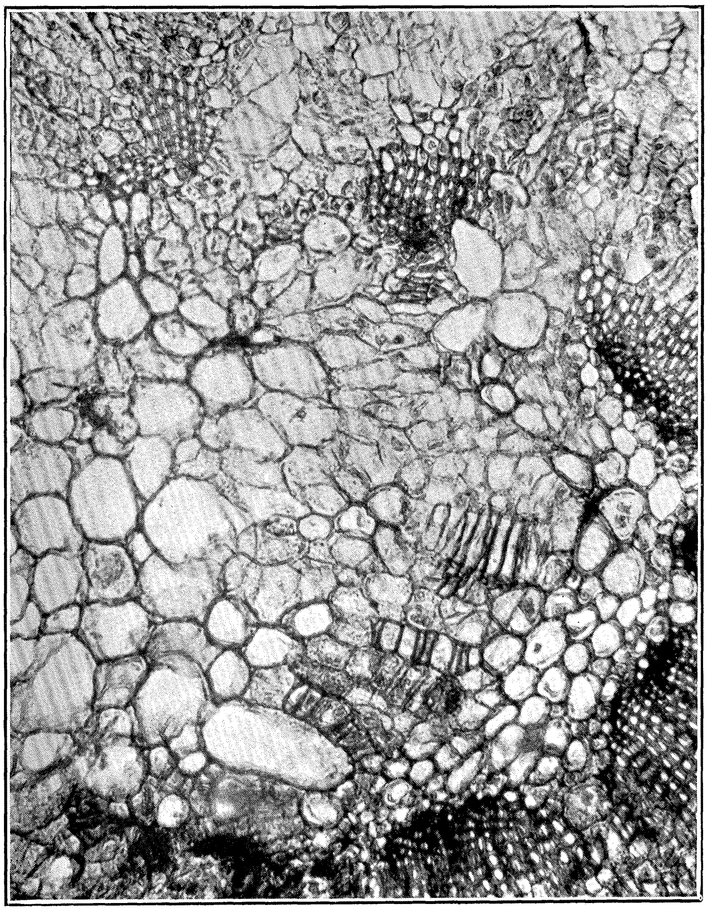


Fig. 4.

Fig. 3.—Longitudinal section of the stem in *A. pectinata* treated with ammonia and followed by Carbol fuchsin, showing stylet sheaths (A, B, C, etc.), among the cells of the abnormal tissue. × 358.

Fig. 4.—Longitudinal section of the stem in *A. pectinata* stained with concentrated picric acid, showing stylet surrounded by its sheath and empty sheaths at the point A. × 183.

PLATE 6.

Fig. 1.—Longitudinal section, *A. pectinata,* through central axis of the stem. × 60. *A, B,* Xylem tracheids cut off by abnormal tissue. *C,* Xylem tracheids bent by the pressure of the surrounding abnormal cells. *D,* Abnormal cells in the pith region. *E,* Abnormal cells in a medullary ray.

Fig. 2.—Longitudinal section, *A. pectinata,* showing large area of abnormal tissue (*a.t.*) extending across the pith region, and the remains of the tracheids (*T*), whose course has been interrupted. × 90.

Fig. 3.—Longitudinal section, *A. pectinata,* showing tracheids bent by the pressure of the abnormal cells. × 90.

Fig. 4.—The stylet-sheaths of *D. nüsslini* in the tissue of *A. pectinata.* × 600.

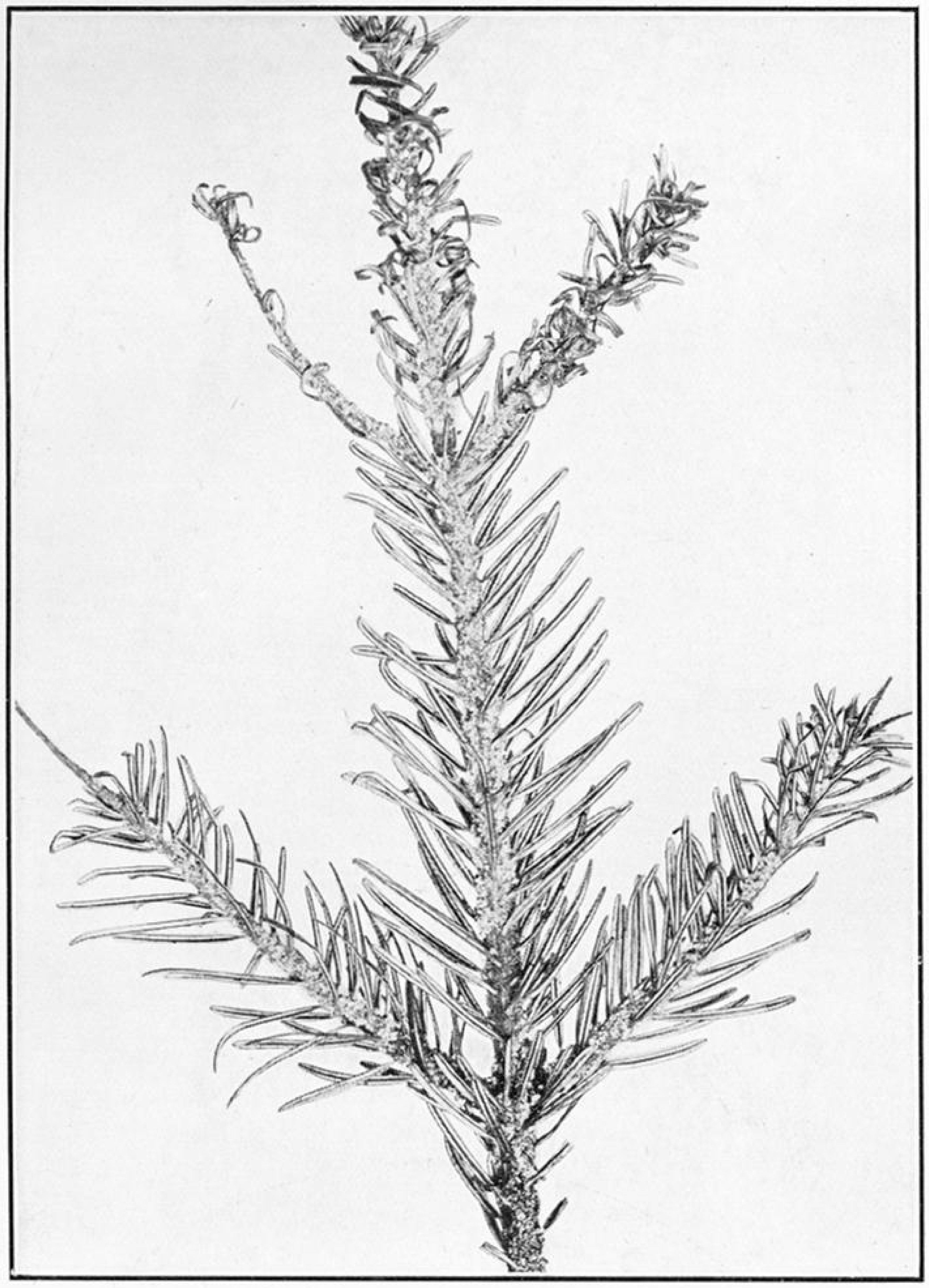
PLATE 7.

Fig. 1.—Longitudinal (Tangential) section, *A. pectinata* showing abnormal tissue (*a.t.*) penetrating the xylem region. × 110.

Fig. 2.—Transverse section of seedling, *A. nordmanniana* infected in May, 1924. The section was cut in August, about 2½ months later. The whole of this injury to the stem has been done between these dates. × 110. Experiment B, Kew, 1924.

Fig. 3.—Transverse section, stem, *A. grandis,* showing abnormal increase in the cortex and phloem, combined with replacement of the xylem, as a result of attack by *D. nüsslini.* × 60. Experiment A, Kew, 1924.

Fig. 4.—Transverse section, stem, *A. grandis* showing formation of abnormal cambial tissue in the pith as a result of attack by *D. nüsslini.* × 160. Experiment A, Kew, 1924.



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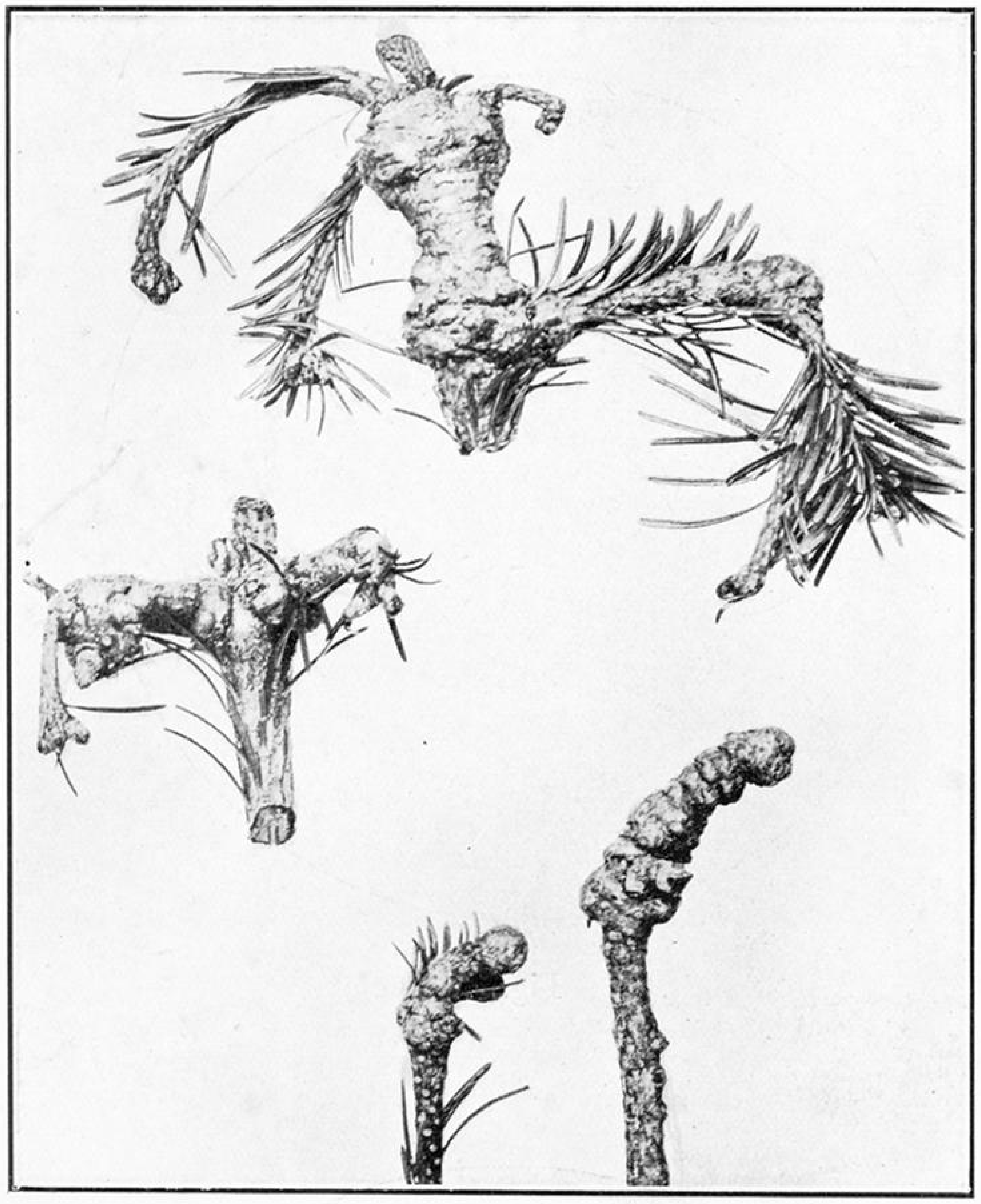

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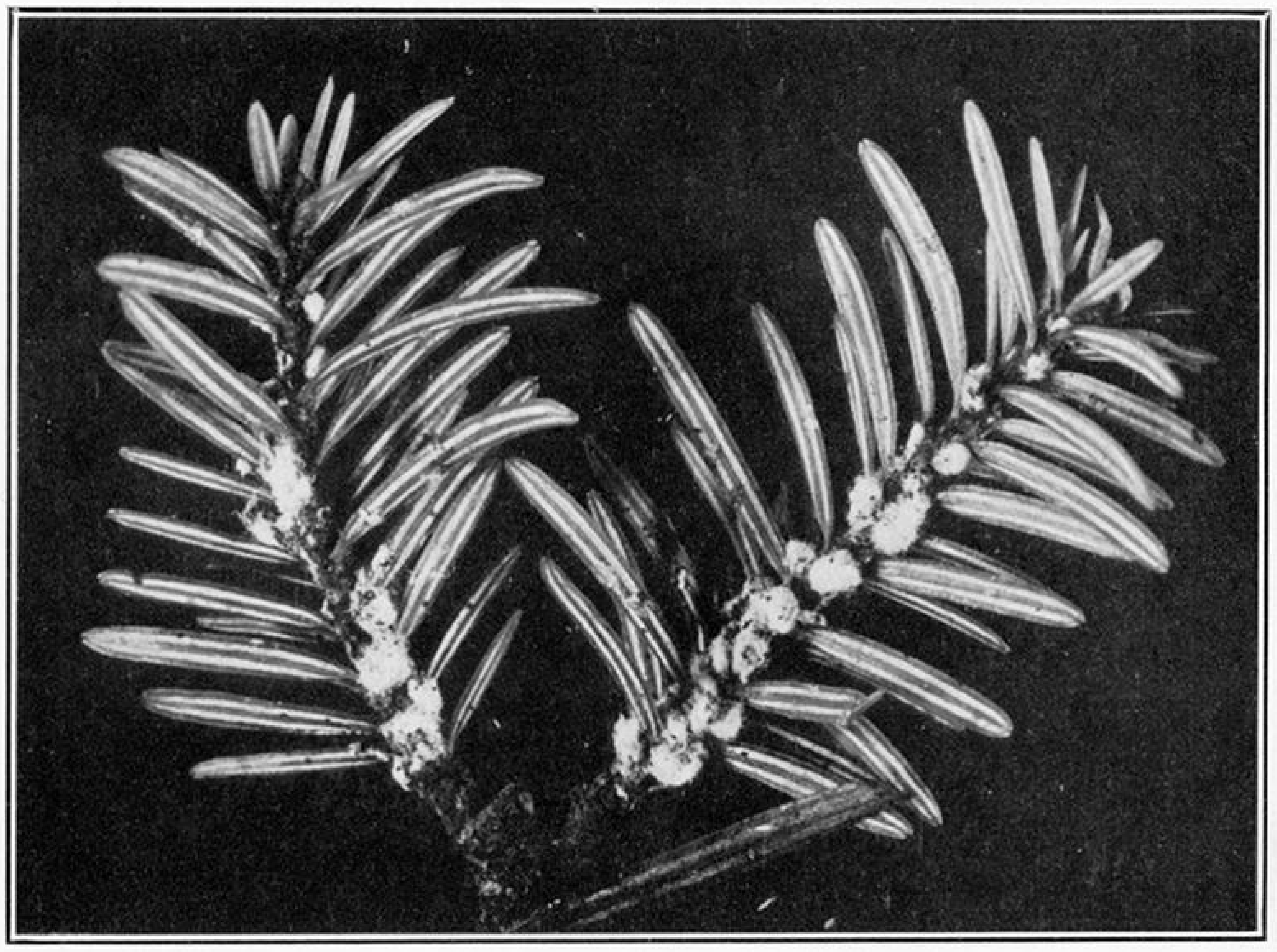

Fig. 4.

PLATE 3.

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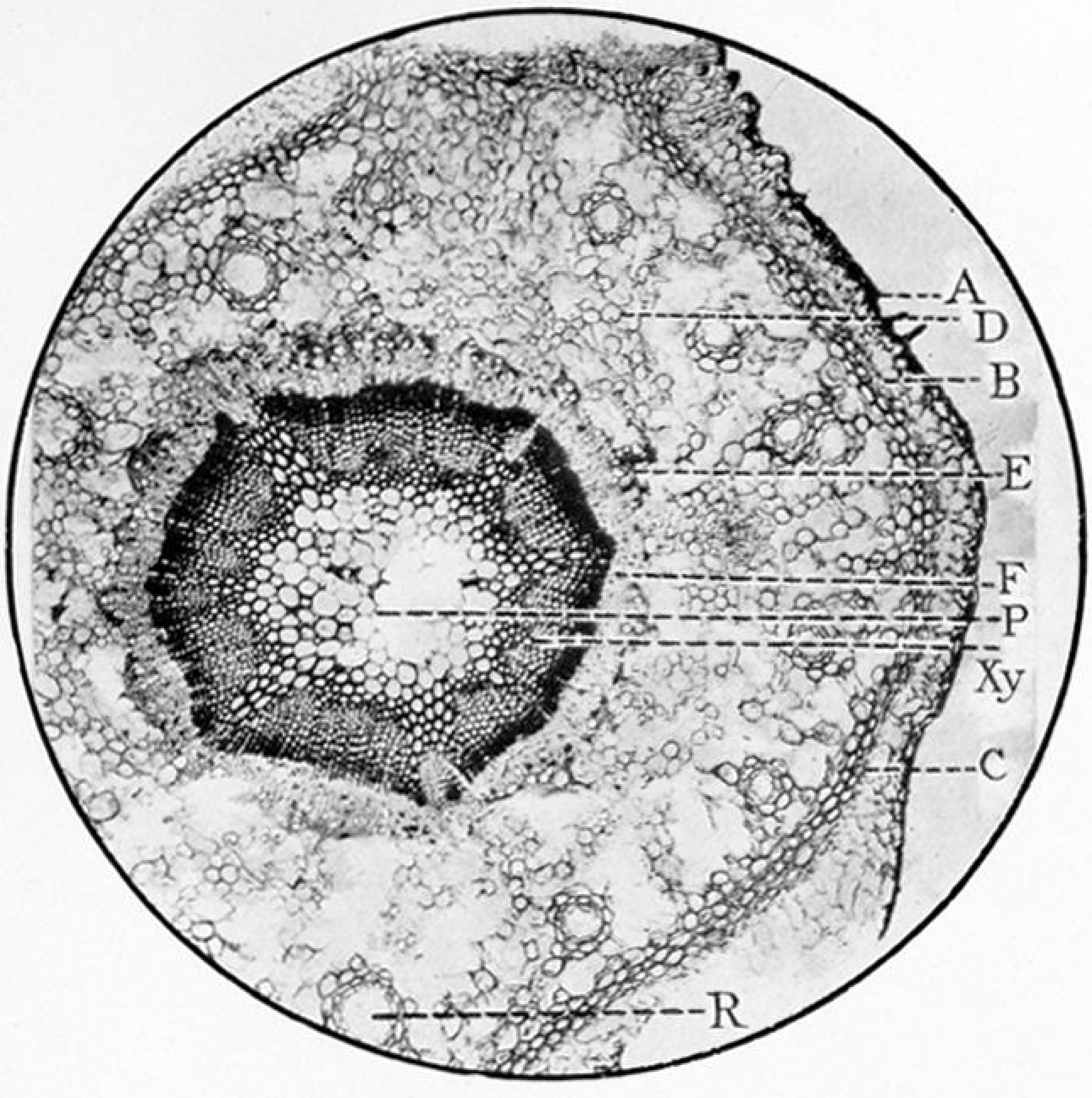

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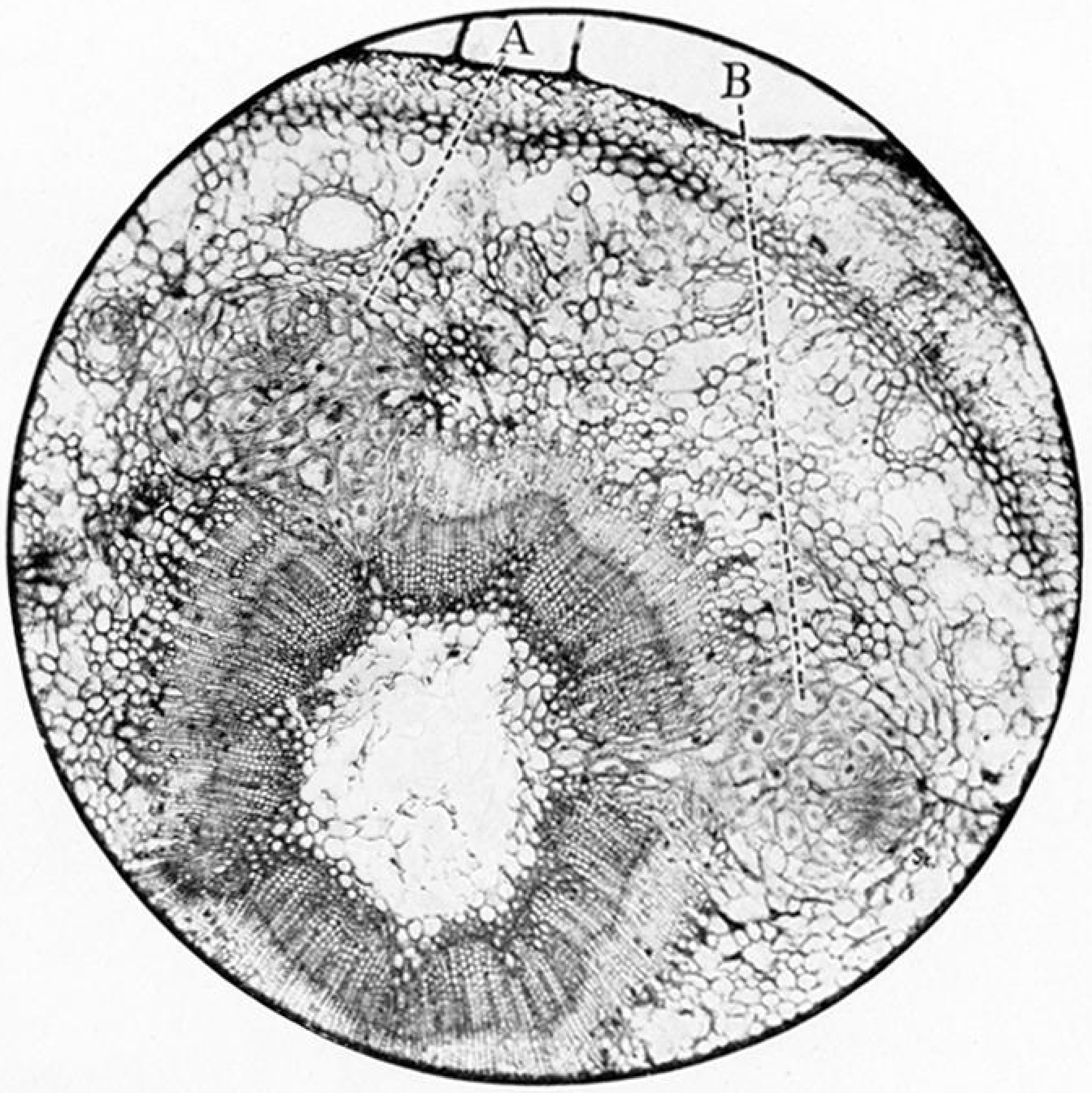

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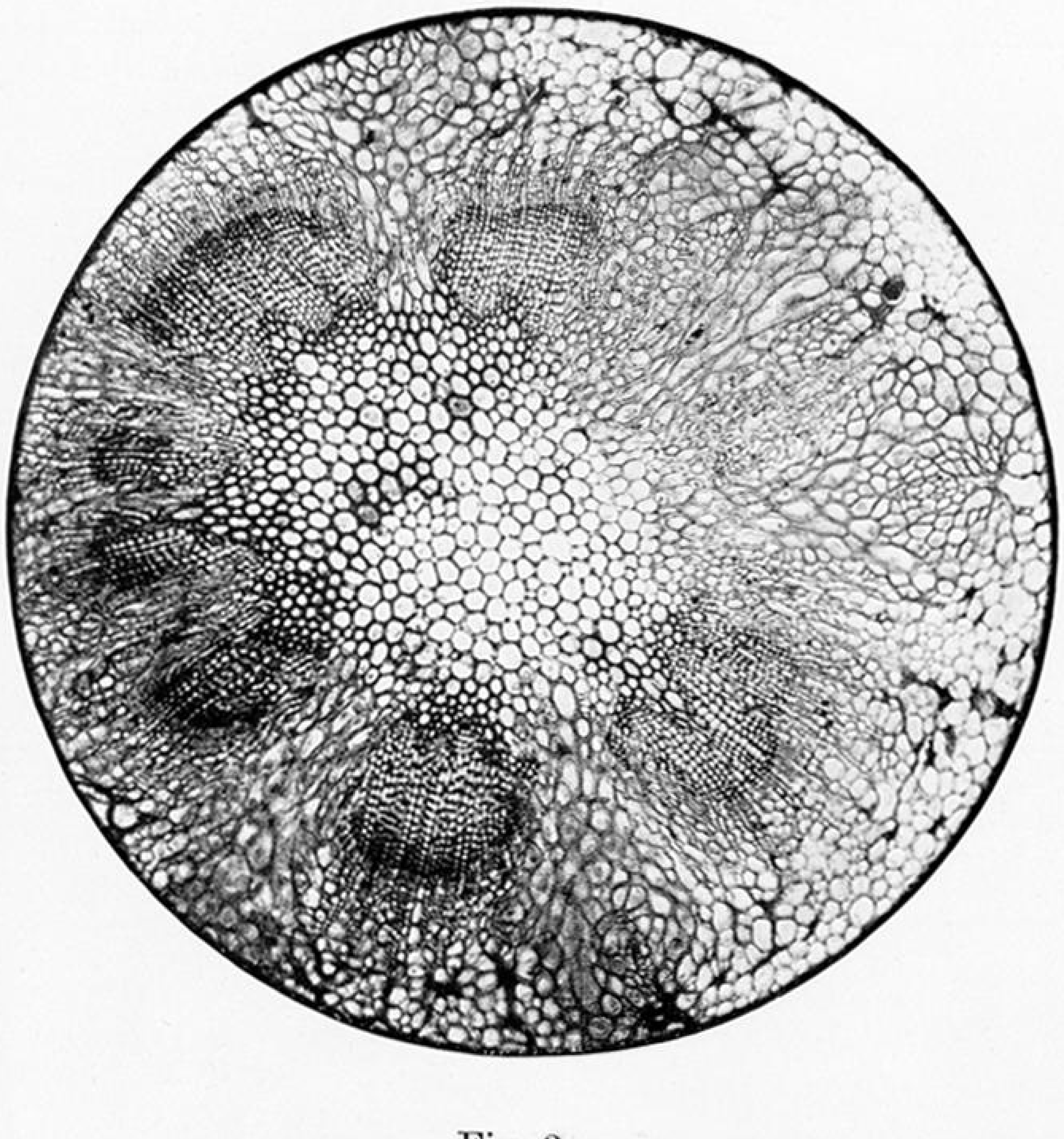

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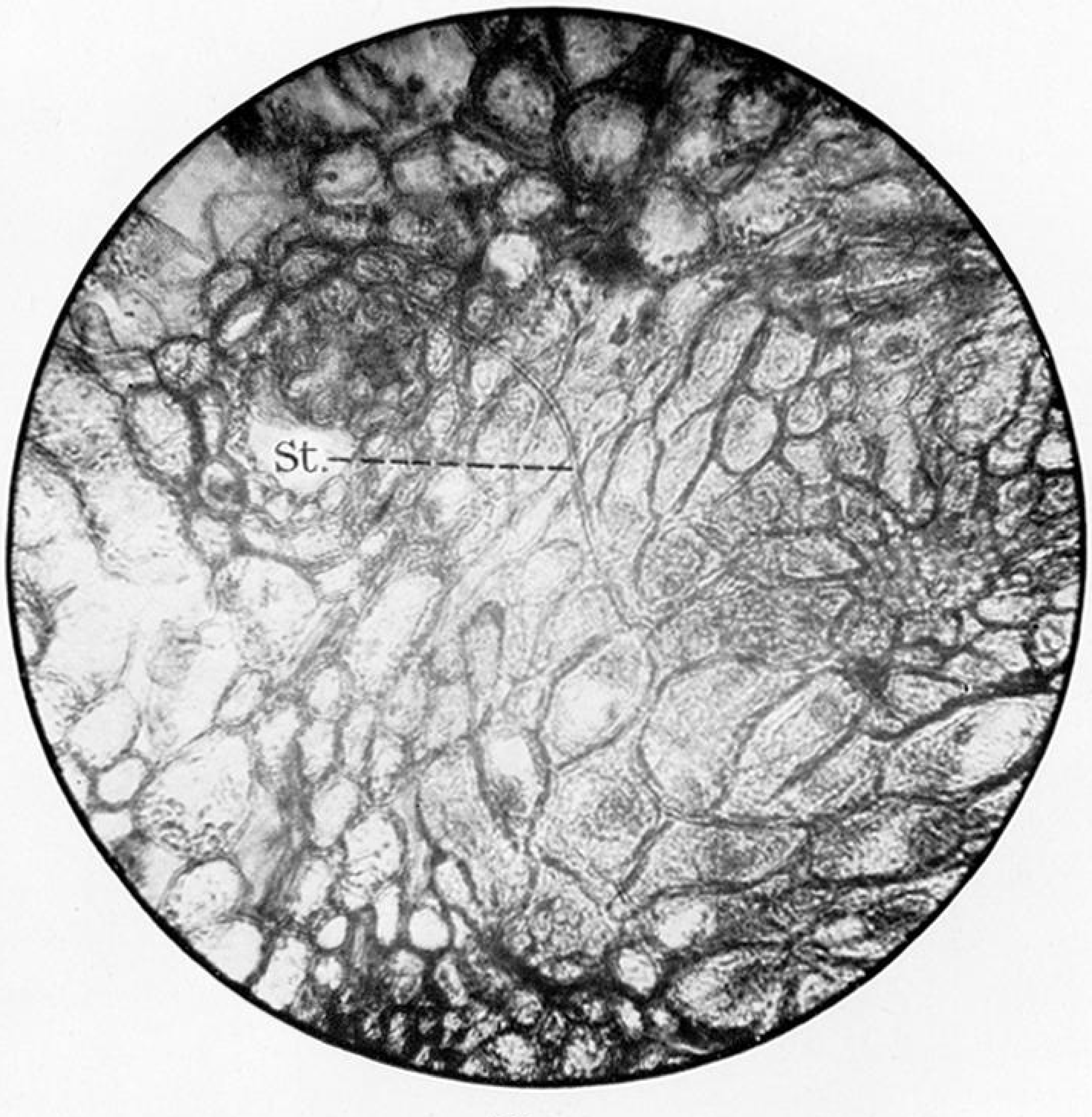

Fig. 4.

PLATE 4.

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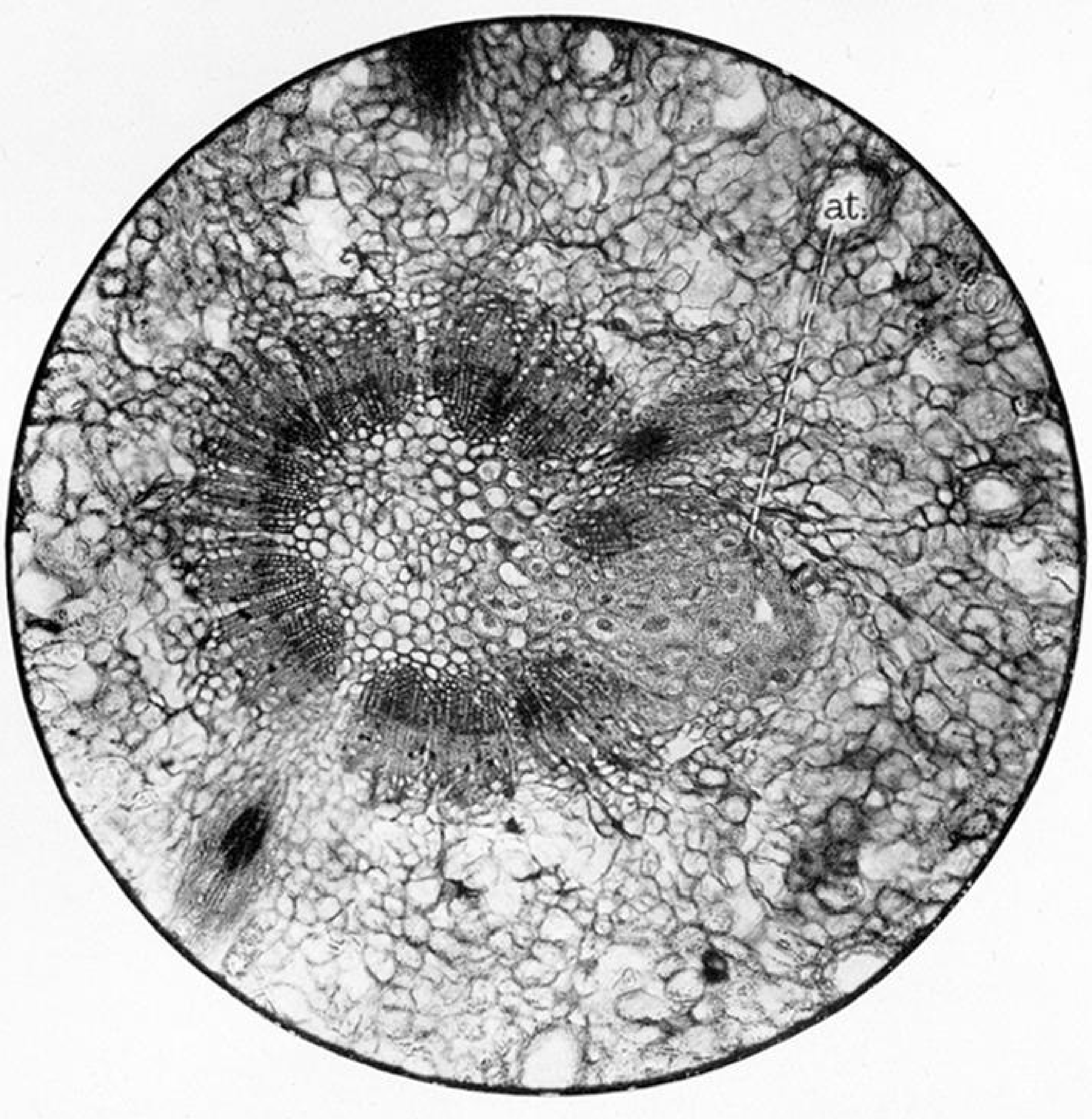

Fig. 1.

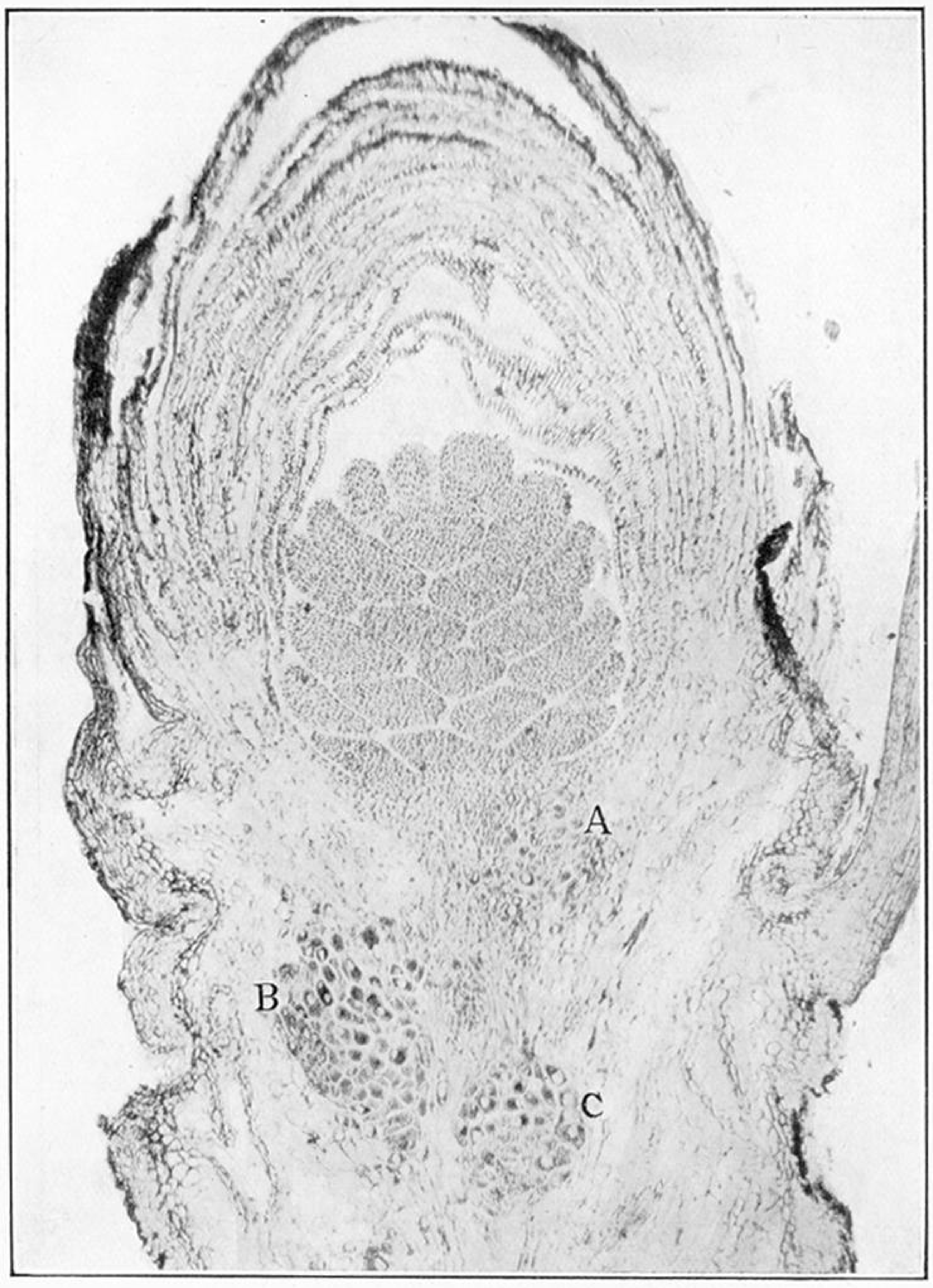

Fig. 2.

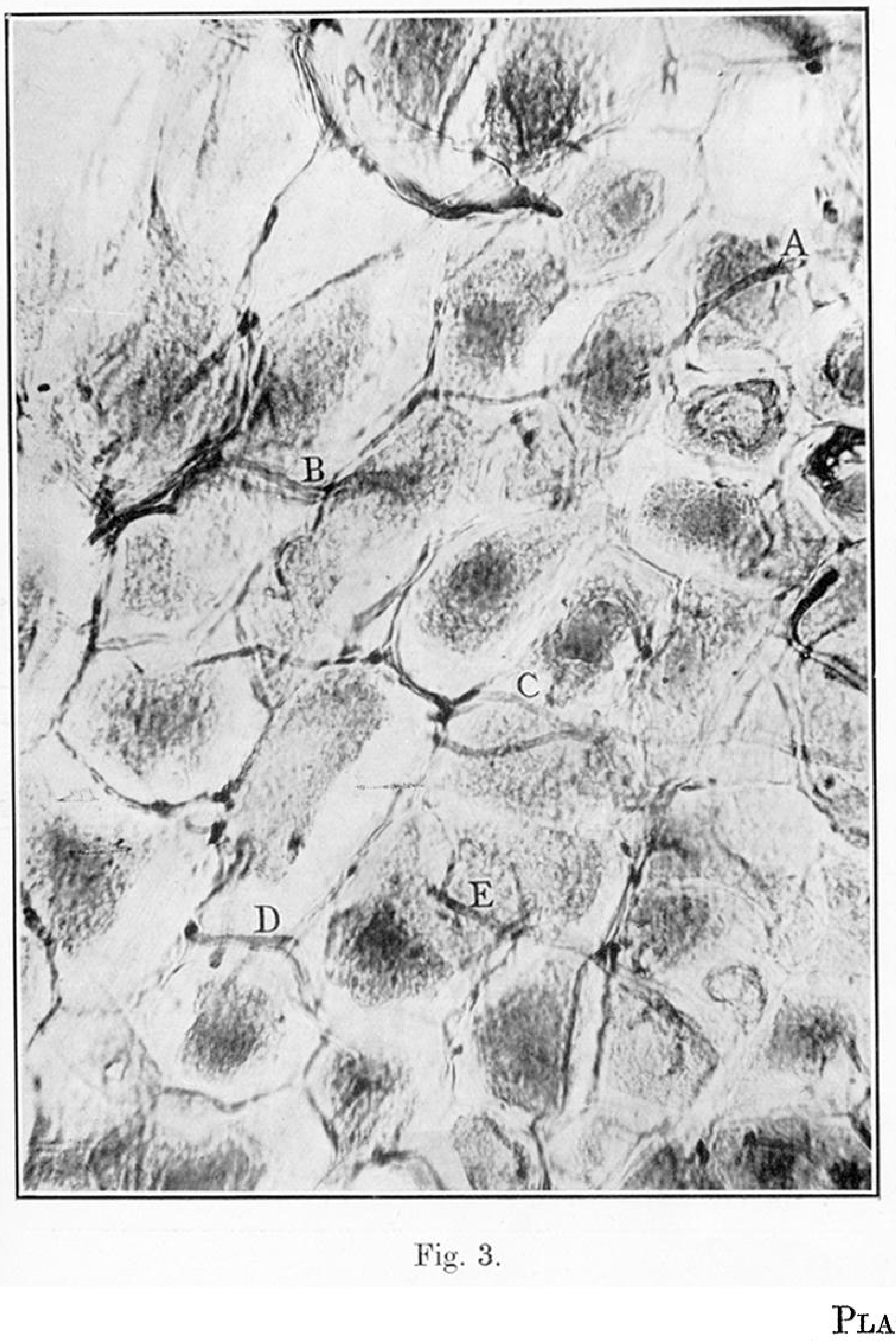

Fig. 3.

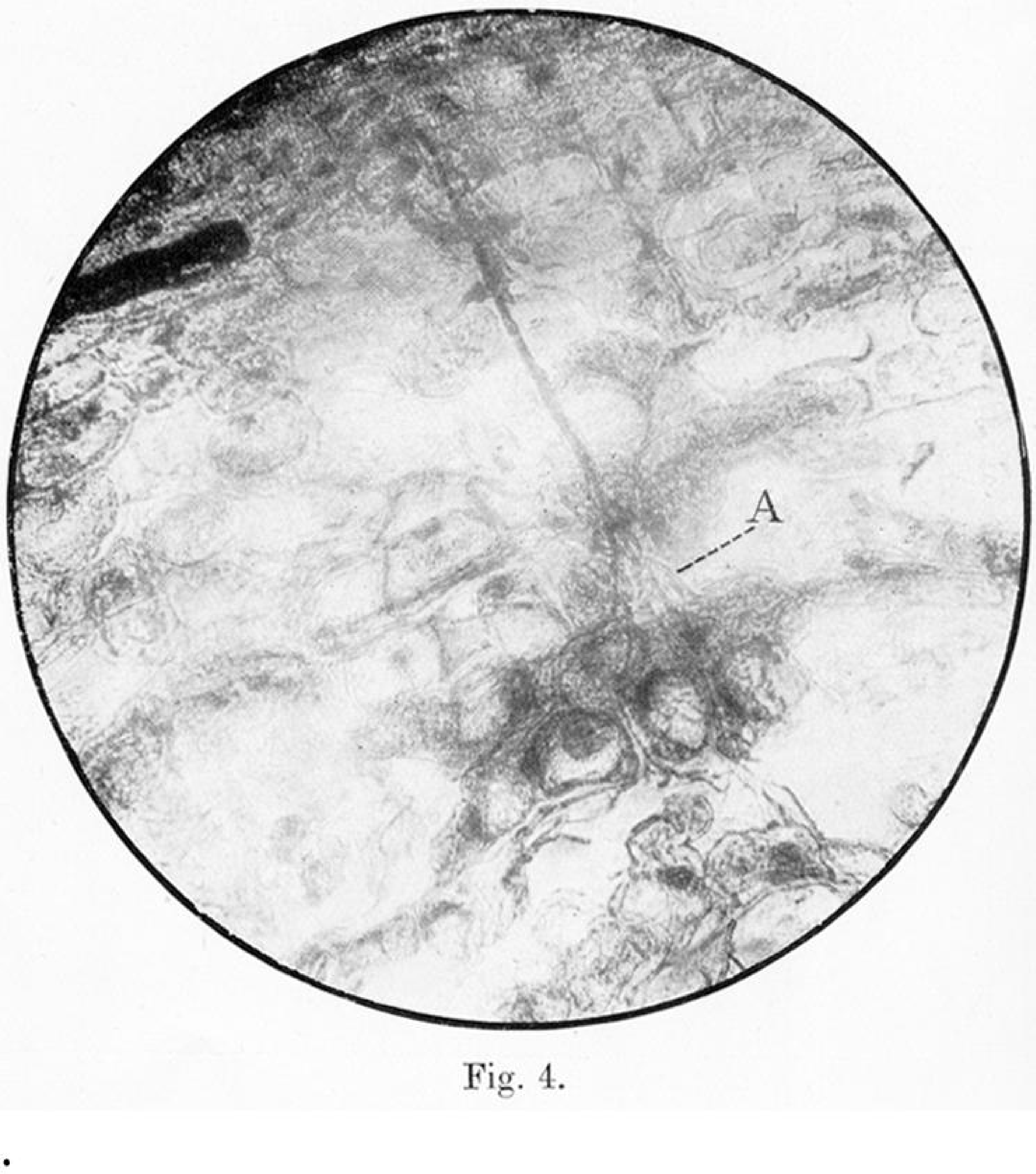

Fig. 4.

PLATE 5.

Fig. 1.—Transverse section at the base of the bud in *A. nordmanniana,* to show the abnormal tissue separating the xylem at an early stage.

Fig. 2.—Longitudinal section through the apex of the stem in *A. pectinata*, showing three patches of abnormal tissue (A.B.C.) lying at the base of the bud. These areas have been deeply stained with eosin. × **33.**

Fig. 3.—Longitudinal section of the stem in *A. pectinata* treated with ammonia and followed by Carbol fuchsin, showing stylet sheaths (A, B, C, etc.), among the cells of the abnormal tissue. × **358.**

Fig. 4.—Longitudinal section of the stem in *A. pectinata* stained with concentrated picric acid, showing stylet surrounded by its sheath and empty sheaths at the point A. × **183.**

Fig. 1.

Fig. 2.

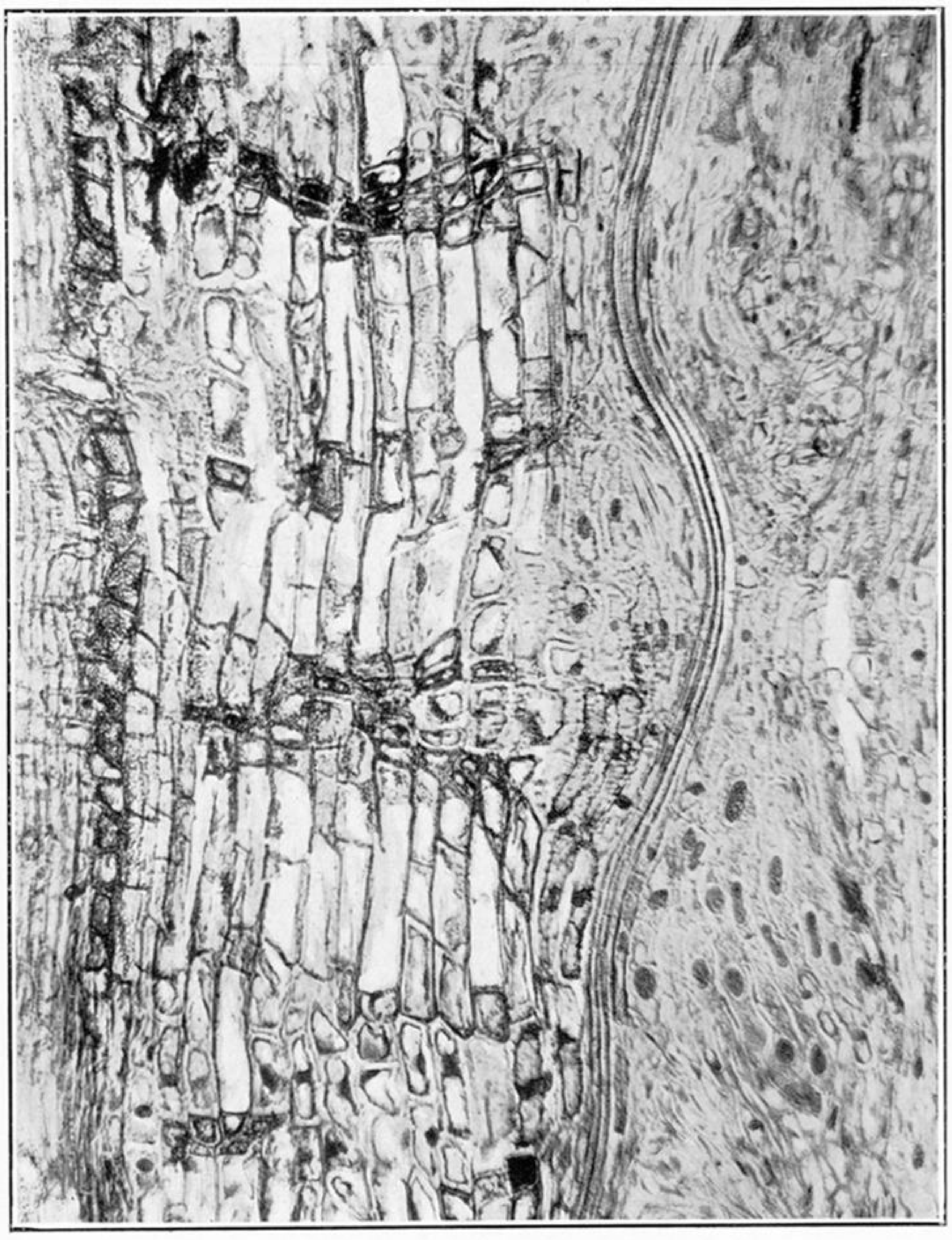

Fig. 3.

Fig. 4.

Plate 6.

Fig. 1.—Longitudinal section, *A. pectinata,* through central axis of the stem. × 60. *A*, *B*, Xylem tracheids cut off by abnormal tissue. *C*, Xylem tracheids bent by the pressure of the surrounding abnormal cells. *D*, Abnormal cells in the pith region. *E*, Abnormal cells in a medullary ray.

Fig. 2.—Longitudinal section, *A. pectinata,* showing large area of abnormal tissue (*a.t.*) extending across the pith region, and the remains of the tracheids (*T*), whose course has been interrupted. × 90.

Fig. 3.—Longitudinal section, *A. pectinata,* showing tracheids bent by the pressure of the abnormal cells. × 90.

Fig. 4.—The stylet-sheaths of *D. nüsslini* in the tissue of *A. pectinata.* × 600.

Fig. 1.

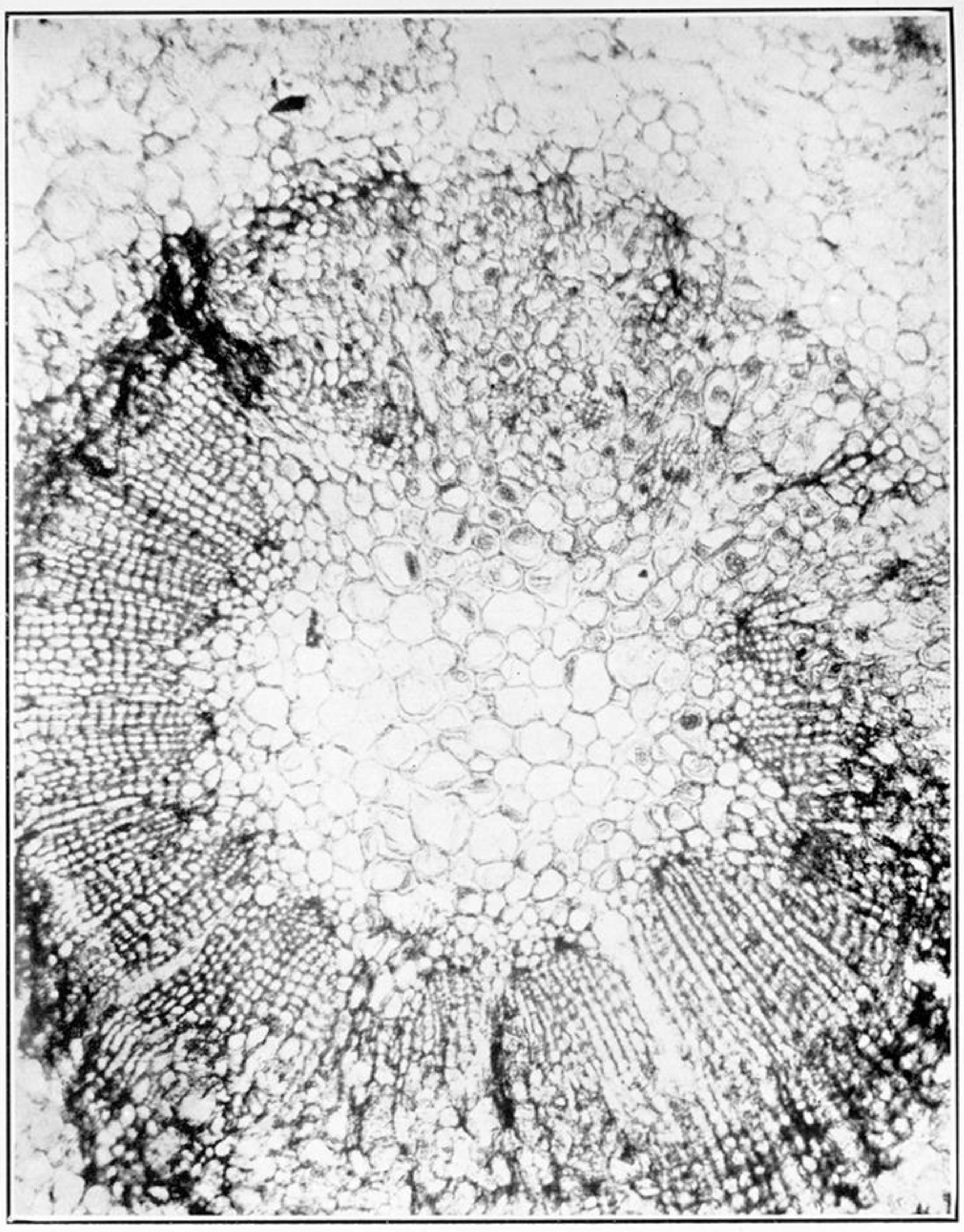

Fig. 2.

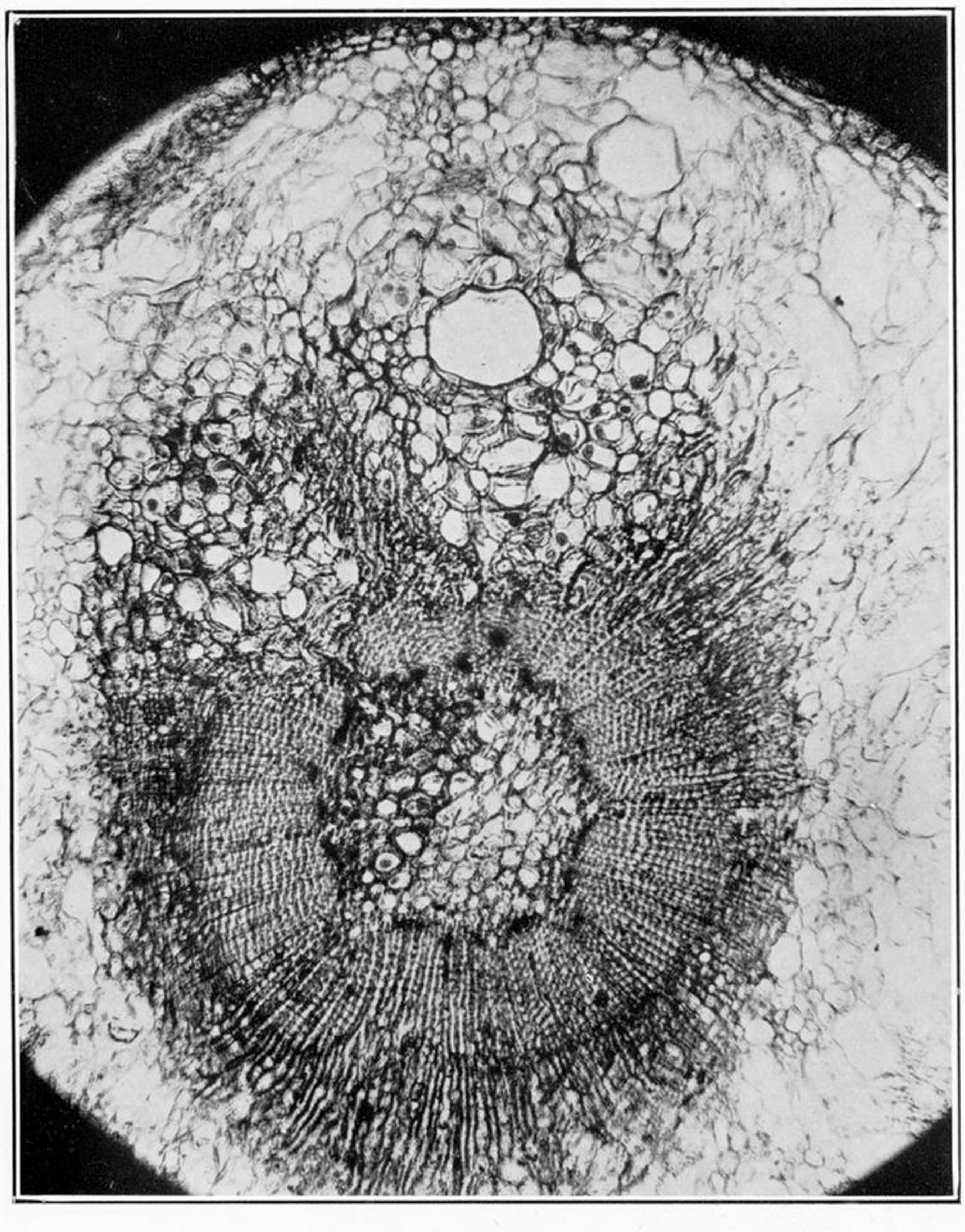

Fig. 3.

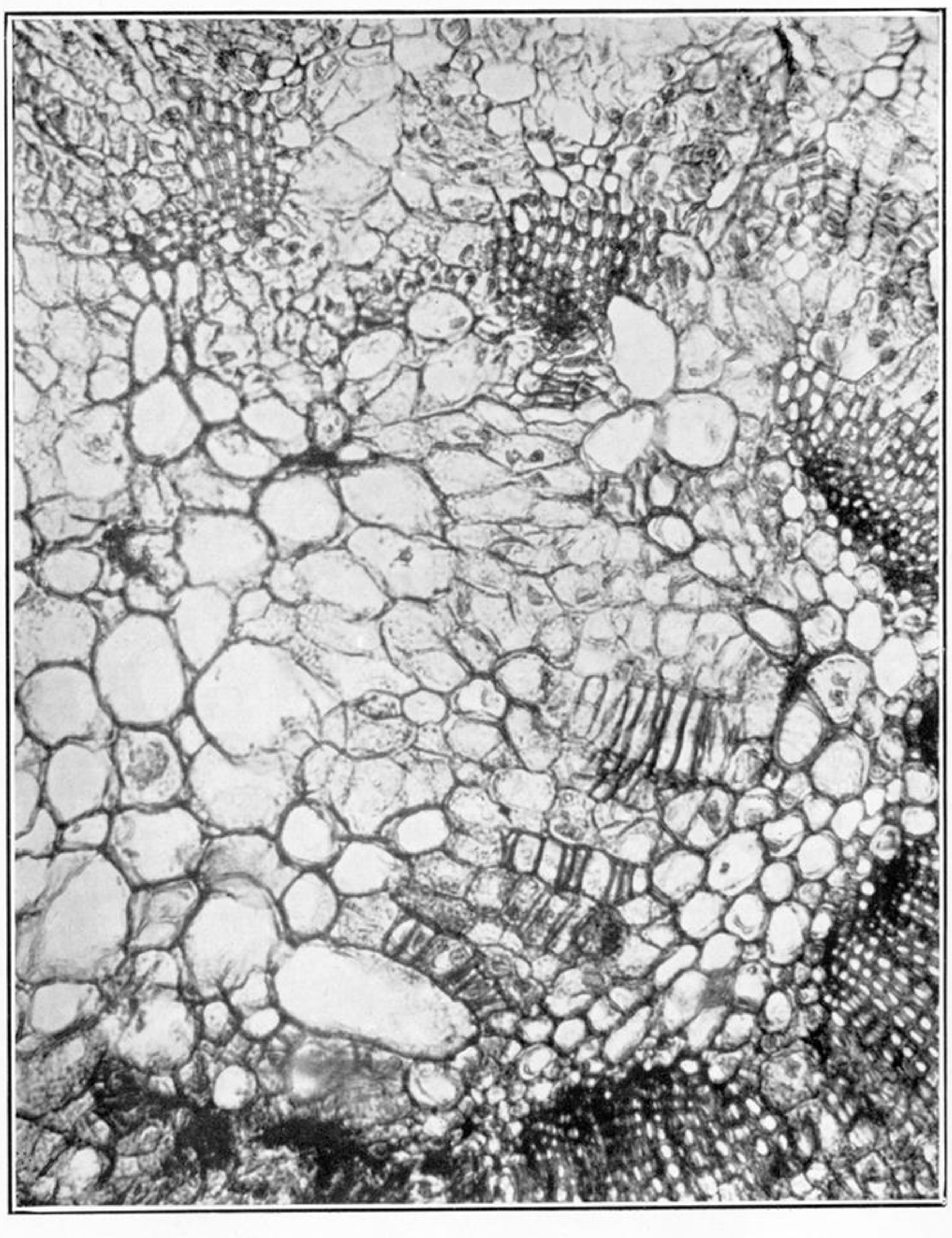

Fig. 4.

Plate 7.

Fig. 1.—Longitudinal (Tangential) section, *A. pectinata* showing abnormal tissue (*a.t.*) penetrating the xylem region. × 110.

Fig. 2.—Transverse section of seedling, *A. nordmanniana* infected in May, 1924. The section was cut in August, about $2\frac{1}{2}$ months later. The whole of this injury to the stem has been done between these dates. × 110. Experiment B, Kew, 1924.

Fig. 3.—Transverse section, stem, *A. grandis*, showing abnormal increase in the cortex and phloem, combined with replacement of the xylem, as a result of attack by *D. nüsslini*. × 60. Experiment A, Kew, 1924.

Fig. 4.—Transverse section, stem, *A. grandis* showing formation of abnormal cambial tissue in the pith as a result of attack by *D. nüsslini*. × 160. Experiment A, Kew, 1924.