

## BOTANICAL GAZETTE

MARCH, 1905

GAMETOPHYTES AND EMBRYO OF *TORREYA TAXIFOLIA*.CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.  
LXIX.

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(WITH PLATES A, I, II, III)

*Torreya taxifolia* Arnott<sup>1</sup> occurs in a narrow belt along the eastern side of the Apalachicola River, extending from the southern boundary of Georgia for about thirty miles southward (1). In April 1904 this region was visited by H. C. COWLES of this laboratory, among whose notes the following are of interest in this connection:

My visit was to the northernmost colony, west of Chattahoochee village, and close to the Georgia line. The distribution lines on CHAPMAN'S map (1) would lead one to suppose that the tree is xerophytic and frequents the steep and dry eastern bluffs. I was much surprised to find that it was confined (in the Chattahoochee station at least) to the extremely mesophytic slopes of ravines, growing exclusively in the shade of trees, and in places that are continually moist, preferably on slopes facing north. The northern and southern known limits of the tree are only about thirty miles apart, and the east-west range is much less. Furthermore, on account of the great economic value of the wood, and the familiarity of the tree to all the inhabitants of the region, the likelihood of finding other areas is very slight.

It is associated with a remarkable and somewhat extensive group of northern mesophytic plants, and the conclusion is irresistible that *Torreya* is a northern plant of the most pronounced mesophytic tendencies, and to be associated with

<sup>1</sup> Unfortunately, *Torreya* was used at least three times as a generic name, in as many families, before 1838, the date of the publication of *Torreya* Arnott. Hence ARNOTT'S genus has been replaced by *Tumion* Raf., and our species becomes *Tumion taxifolia* Greene. In the present paper, however, the more familiar name is used for convenience.

such forms as the beech-maple-hemlock forms of our northern woods, our most mesophytic type of association. Probably it never becomes a large tree, although farmers always cut the trees as soon as they become at all usable. Rarely were any found over 30<sup>cm</sup> in diameter or more than 9 to 12<sup>m</sup> high. It has remarkable capacity for vegetative reproduction, almost equaling the redwood in this respect. Many suckers issue from cut stumps, and even from fallen trunks; even rotten stumps show vigorous suckers, and it seems to be as tenacious of life as the poplar. Staminate trees appear to be the more numerous; however, when in blossom they are far more conspicuous than are the pistillate trees, and the conclusion as to proportion may not hold.

COWLES secured collections of staminate and ovulate material April 4 and 5, and arranged for subsequent collections to be sent to the laboratory. These collections were made at intervals of approximately two weeks up to October 21. The strobilus-bearing twigs were packed in damp cotton in tightly closed tin buckets and reached the laboratory in good condition in about five days. The length of the intervals between collections and between collecting and killing has prevented so close a series as is desirable at certain stages, but the gymnosperm periods are so long that a fair outline of morphological events was secured.

#### SPERMATOGENESIS.

STROBILI.—The staminate strobili occur in the axils of leaves of young shoots. Their appearance in October, on a shoot of the same season, is shown in *fig. 1*. At this time they are small and ovoid, solitary in the axil of each leaf, and by a curving of the short peduncles are displayed along the under surface of the shoot. The strobilus consists of a series of closely overlapping sterile bracts, in four vertical rows, completely enveloping the tip of the axis bearing numerous stamens. It is thus distinctly sterile below and fertile above, the sporophylls continuing the spiral succession developed by the bracts (*figs. 6 and 7*).

The young strobili were first observed in July, and at that time no primordia of sporophylls were evident; but in August these were beginning to appear. *Fig. 7* is from a longitudinal section through a strobilus at this period (August 12), showing the overlapping sterile bracts and the beginning of staminal primordia. A remarkable development of the pith region of the axis below the staminate por-

tion of the strobilus, with its investing vascular cylinder, may be observed, apparently an important storage region for the strobilus.

STAMENS.—At the stage of the staminal primordia observed August 12, there is no evident differentiation of tissues (*fig. 8*), but early in September the young sporangia become distinct, the sporogenous tissue being represented in each case by a single primary sporogenous cell. At this stage the further development of the young sporangia differs. Seven sporangia are seen in the primary sporogenous cell stage, three adaxial and four abaxial (*fig. 10*), radially arranged about the central axis as are the five or more sporangia in the allied *Taxus*. The four abaxial sporangia develop in the usual way, and become the pendent abaxial sporangia characteristic of *Torreya*. The three adaxial sporangia, however, do not develop further, the primary sporogenous cell not dividing, and its nucleus showing signs of disorganization (*fig. 9*). The disorganization continues until it involves all the cells separating the three adaxial sporangia, which are thus replaced by a single large flattened cavity, which becomes a resin cavity. Intermediate stages were found in which the three sporogenous regions were still distinct (*fig. 10*). In *fig. 11* (October 21) is shown a median longitudinal section through a stamen, in which the large resin cavity is seen above and the normally developing sporogenous tissue below. As a result, the mature stamen of *Torreya* is characterized by four abaxial sporangia and a large adaxial resin cavity.

In one instance the two lateral sporangia were smaller than the middle ones (*fig. 10*), suggesting a tendency to still greater reduction in the number of sporangia. Accordingly *Pinus Laricio* was examined, and two resin cavities were found related to the two sporangia exactly as the two lateral sporangia of *Torreya* are to the middle ones; and in early stages the tissue on the sites of these two resin cavities of *Pinus* resembles that of sporangia. It is interesting to note that in *T. californica* Miss ROBERTSON (8) found occasional stamens bearing six or seven mature sporangia, indicating that in these cases some or all of the usually abortive sporangia reached maturity.

There is evident a tendency to reduce the number of functioning sporangia by abortion, a reduction that has proceeded farther in *Pinus* than in *Torreya*; and in the latter farther than in *Taxus*. That

the cavities formed by the breaking down of young sporogenous and adjacent tissue should become resin cavities in conifers is to be expected.

It was only in the abortive sporangia of *Torreya* that stages were found indicating the history of the sporangium up to the primary sporogenous cell stage. For example, from *fig. 9* it seems evident that there was a single hypodermal archesporial cell, which at this stage had given rise to two layers of wall cells and a single primary sporogenous cell. In the mature sporangium there are three or four wall layers. The epidermis contains numerous stomata, and its cells become prominent and have thickened walls. The sporangia are bluntly four-angled from pressure, and the epidermal cells at the angles are much larger than elsewhere.

MALE GAMETOPHYTE.—We secured no stages between the very young sporangium and the shedding of the pollen, which occurs late in March and early in April. Miss ROBERTSON (8) has observed the mother-cell stage and the formation of tetrads in *T. californica*.

In germination the microspore of *T. taxifolia* cuts off no prothallial cell, a feature common to all the Coniferales except Podocarpeae and Abietaeae. After the first division the generative and tube cells are distinct and separated by a very delicate wall or membrane. The tube nucleus is sometimes spherical, but more often amoeboid (*fig. 12*).

Early in April the binucleate pollen grains, rich in starch, were found resting on the nucellus (*fig. 19*); and at the end of June micro-pyles were found full of pollen grains, most of which had sent out no tubes, but were still full of starch, nucleate, and apparently alive.

As fertilization occurs about the middle of August, active pollen tubes occupy the sterile cap of the nucellus about four months, and their behavior is exceedingly variable. They may advance towards the embryo sac rapidly, reaching it at a very early stage, as early as June 21, when the endosperm consists of only sixteen to sixty-four parietally placed free nuclei (*figs. 14* and *15*); or they may advance slowly, being found at all stages of progress at the same date. They may advance so directly that the tube resembles a straight cleft through the nucellar tissue to the sac, or they may pursue a remarkably devious course. In one case a tube passed directly half-way

down the nucellar cap, then advanced spirally downward and outward until it reached the peripheral cells of the nucellus, and after destroying several of these it turned abruptly inward, penetrated the nucellus at the level of the archegonium, crossed the top of the endosperm, and discharged its contents into the archegonium on the farther side. In another instance, such a pollen tube had entered the inner integument and destroyed some of its cells before turning back into the nucellus. It is very common for the pollen tube to push into the sac at its free nuclear stage, making a deep invagination, often to the middle; from this pocket the tube turns back again into the nucellus. It is of interest to note in this connection that the usually solitary archegonium is never centrally placed in the endosperm, and the pollen tube enters the sac to one side of it (*fig. 16*).

In all the wanderings of the pollen tube, the body cell, stalk nucleus, and tube nucleus are conspicuous. The division of the generative cell was not observed, but that it occurs very early in the development of the tube is evident. The body cell is relatively very large, with a conspicuous nucleus and investing cytoplasm, and was always found consorting closely with the stalk and tube nuclei (*figs. 13 and 14*).

The division of the body cell just before fertilization results in unequal male cells (*fig. 23*), almost exactly resembling those of *Taxus*. It is not a case of the extrusion of one nucleus from the common cytoplasm, as observed by COKER (2) in *Podocarpus*, but the cytoplasm is unequally divided, so that there are two distinct and naked male cells very different in size. The whole cavity of the pollen tube surrounding the cells and nuclei is rich in starch and other food materials (*fig. 13*).

A consideration of the time involved in these various events shows that a period of about fifteen months elapses between the first appearance of the microsporangiate strobilus and fertilization, divided as follows: June, first appearance of strobilus; August, first appearance of staminal primordia; September, distinct differentiation of sporangia; April, shedding of spores; August, fertilization. The strobilus was first observed in July, but in such a condition that it must have been evident in June, if not earlier. In comparing this schedule with that given by Miss ROBERTSON (8) for *T. californica*, it is evident

that the sporangium in both species passes the winter in the mother-cell stage, but that the subsequent stages appear earlier in *T. taxifolia* growing in its natural habitat than in *T. californica* growing in England. For example, in the latter case the reduction division takes place about the time that *T. taxifolia* sheds its pollen, and pollination in *T. californica* does not seem to occur until late in May or early in June.

#### OÖGENESIS.

**STROBILI.**—The ovulate strobili are borne in the axils of the lower leaves of short young shoots (*fig. 2*). They usually occur in pairs upon a very short axillary branch, usually one pair, frequently two pairs, very rarely three pairs appearing upon a single shoot. A cluster of two to six strobili, therefore, appears near the base of the strobilus-bearing shoot, the upper pairs never maturing, usually one and sometimes both strobili of the lowest pair producing the large plum-like seeds (*fig. 3*). The strobilus is a very simple one, consisting of four enveloping bracts and a single terminal ovule with two integuments (*figs. 17* and *18*), the outer one often called an arillus because it ripens fleshy. The whole structure resembles a simple ovulate flower with a perianth of four bracts, which perhaps deserve to be called a perianth as much as the so-called perianth of Gnetales; but it is none the less evident that they are the sterile bracts of a much reduced strobilus.

The strobili were first seen July 26, at which time the growing point, enclosed by the bracts, was composed of entirely homogeneous tissue, showing no differentiation as an ovule (*fig. 17*). No subsequent stages were found until April 7 (*fig. 18*), when the mother-cells were in synapsis (*fig. 20*); and it seems probable that the winter was passed in the mother-cell stage. At this time the integuments are entirely free from the nucellus, appearing to arise from the base of the ovule. Soon, however, extensive intercalary growth below the mother-cell becomes evident, the ovule being greatly elongated and broadened below, the original free nucellus with its integuments forming only the tip. This growth continues throughout the season of fertilization and the following one; and OLIVER (6), who has described this intercalary growth in *T. nucifera*, estimates that in that species in the maturing seed the original nucellus with its free

integuments represents only one-twentieth of the entire length of the seed. The characteristic structures of the integuments are continued as two distinct peripheral layers of this large mass of additional tissue, and the tissue within these layers may be taken to represent in a similar way the downward extension of the nucellus. It is this additional nucellar tissue that the endosperm chiefly invades, giving rise to the phenomenon of "rumination," to be described later. OLIVER (7) has also described fully the course of the vascular strands in the ovule of certain species of *Torreya*, a description which seems to serve as well for *T. taxifolia*.

FEMALE GAMETOPHYTE.—The mother-cell is solitary and with no differentiation of a nutritive mechanism about it, such as appears in connection with the "spongy tissue" of *Pinus*, and in all the Pinaceae investigated. In *Torreya* it is directly in contact with the cells that are resorbed, without any intervening digestive layer (*fig. 20*). Since this is true also of *Podocarpus* (2) and *Taxus* (10), it suggests the possibility that Taxaceae in general may be characterized by the absence of a special digestive layer about the mother-cell.

The reduction division was not seen, but a more or less complete tetrad is formed, as observed by Miss ROBERTSON (8) also in *T. californica*.

The germination of the megaspore begins with the usual free nuclear division, the nuclei being in the parietal position when only sixteen to thirty-two in number (*figs. 14 and 15*). The interior of the sac contains cytoplasmic material, much less dense than the cytoplasm of the parietal layer, and also some reserve food. In this early few-nucleate stage of the endosperm there is always an appearance suggesting that the sac has sent a beak-like projection, containing a nucleus, upwards into the nucellar tissue (*fig. 15*). After a careful comparison of the position of this apparent projection in reference to the surrounding parts with that of the megaspore, it seems that the "projection" is the original site of the megaspore, and that the appearance of a projection is due to the fact that the sac has encroached almost exclusively upon the chalazal tissue. This conspicuous beak, containing one of the parietal nuclei, often appears close to the tip of an advancing pollen tube, and suggests a possible explanation of the peculiar behavior ascribed to the archegonium

initial of Tumboa. Inasmuch as the archegonium initial of *Torreya* often occupies this beak, the suggestion becomes still more pertinent (*fig. 21*).

The formation of walls in the endosperm was not observed before July 1, and in several instances they did not appear for a month later. When wall-formation began, repeated countings showed 256 free nuclei, which seems to be a very common limit of free nuclear division among gymnosperms.

Since fertilization was observed August 12, it is evident that the archegonium is developed very early in the history of the gametophyte. In fact, as soon as the very small sac is filled with extremely delicate tissue the archegonium initial becomes evident. It does not seem possible for archegonia to appear any earlier, for the initials are organized as soon as the free nuclear stage has passed. In *Torreya*, therefore, nearly all of the endosperm, which becomes an extensive tissue, develops after fertilization.

The single archegonium initial is always to one side of the central axis (*fig. 16*), often occupying the "beak" referred to above, and so projecting above the endosperm (*fig. 21*). A neck cell is cut off and divides anticlinally, forming a two-celled neck (*fig. 22*), the usual limit of neck-formation among gymnosperms. In fact, it is only among Podocarpeae and Abietae that a more extensive neck is usually formed, consisting of more than one tier of cells, unless the somewhat anomalous neck of *Ephedra* be included. The fact that there is variation in the number of neck cells in the same form (two to twenty-five in *Podocarpus*), and that as a rule necks are destroyed as soon as formed by the growth of the central cell and pollen tube (*fig. 23*), suggests that their extent depends somewhat upon the approach of the pollen tube, which usually checks neck formation early, but sometimes permits it to become more extensive. In *T. californica* Miss ROBERTSON (9) has found that the archegonia are usually three in number, ranging from two to five, and that the necks consist of four or six cells.

The central cell enlarges rapidly, no jacket-layer being evident until after fertilization, and even then it is weakly organized (*fig. 25*). The nucleus is spherical and lies near but not against the neck cells, more nearly resembling an egg nucleus than in any gymnosperm



we have observed (*fig. 22*). We could not detect the formation of a ventral canal cell or nucleus, or anything that stood for such a structure at later stages. A ventral nucleus was expected, for a distinct ventral canal cell among Coniferales seems to be restricted to the Abietae and does not always occur in them, and there seemed to be no excuse in our preparations for missing it. We are fully aware that all previous negative evidence as to the occurrence of at least a ventral nucleus in archegonium-forming gymnosperms has proved to be deceptive, but a study of the behavior of the central cell of *Torreya*, from the formation of the neck cell to fertilization, not only failed to show any indication of division but suggested that it may not occur. In *T. californica* a spindle seen twice in the central cell was interpreted by Miss ROBERTSON (9) as representing the "cutting off" of a ventral nucleus, but no other traces of it could be found.

In the single case in which two archegonia were observed, they were at opposite sides of the gametophyte, with the tip of a pollen tube between them.

At the time of fertilization the gametophyte contains 400–800 cells, with extremely thin walls and scanty cytoplasm (*fig. 23*). The only differentiation observable is the abundant accumulation of reserve food in the peripheral cells of the antipodal region. The whole mass of endosperm at this period usually measures 20 by 30 $\mu$ ; while in the mature seed the endosperm mass is ordinarily about 20<sup>mm</sup> long by 14<sup>mm</sup> at its widest part, and all of it surcharged with starch and other food materials. The food material is particularly conspicuous in a broad central band extending from the advancing tip of the embryo and widening to the antipodal end of the sac. *Fig. 5* shows the longitudinal extent of the band, and *fig. 4* its cross-section. The peculiar behavior of the endosperm after fertilization will be considered under the discussion of the maturing of the seed.

#### FERTILIZATION.

The forcible discharge of the contents of the tube may be inferred from the vacuole-like appearance in the center of the egg, produced by the inrush (*figs. 24* and *25*). The male nucleus in its cytoplasmic sheath passes through the cytoplasm of the egg and comes in contact with the egg nucleus. The male cytoplasm becomes closely appressed

to the surface of the female nucleus, slips from its own nucleus, and was observed extending over fully two-thirds of the female nucleus (*fig. 24*). This behavior of the male cytoplasm has been observed by COKER (3) in *Taxodium* and by Miss ROBERTSON (9) in *Torreya californica*. The male cytoplasm of *Torreya taxifolia* is sharply differentiated by staining from the cytoplasm of the egg, and undoubtedly completely invests the fusion nucleus. The appearance of a similar mass of cytoplasm investing the free nuclei of the first division (*fig. 25*), and continued in the second division in connection with wall-formation (*fig. 26*) suggests the possibility that the male cytoplasm may remain differentiated through more than one cell generation. Near the neck end of the archegonium nuclei are evident, which seem to be the stalk and tube nuclei and the other male nucleus with its investing cytoplasm (*fig. 24*).

#### EMBRYO.

Soon after fertilization the first division of the egg nucleus was observed (*fig. 25*), and almost immediately the second division follows, giving rise to four large free nuclei almost filling the egg (*fig. 26*), one nucleus in the base of the egg, the other three in a plane above. At this time wall-formation occurs, the cytoplasmic radiations which precede it being very evident (*fig. 26*). Two weeks later the egg is completely filled with a proembryo consisting of twelve to eighteen cells (*fig. 27*). This complete filling of the egg by the proembryo is remarkable among Coniferales, having recently been observed also by LAWSON (4) in *Sequoia*, but as yet not recorded in other genera. In *Torreya*, at least, this fact seems to be related to the relatively small size of the egg, the very large nuclei, and the early appearance of walls.

The cells of the proembryo at this early stage are distinctly in three tiers; that nearest the neck of the archegonium comprising five or six cells and forming the primary suspensor tier; the middle tier, comprising five or six cells and forming the secondary suspensor tier; and the lowest consisting of a single cell which ultimately contributes to suspensor-formation and forms the embryo. The inequality in the number of cells entering into the tiers seems to be characteristic of Taxaceae. In *Podocarpus* COKER (2) found the three tiers made up

of eleven cells in each of the upper two and one in the lowest; and a similar but less striking inequality is to be observed in *Taxus*. Occasionally in *Torreya* other divisions may occur, giving rise to approximately four tiers and a proembryo of about eighteen cells; but no other division occurs until the following spring, the winter condition being a proembryo of three or four tiers of cells as described above.

In the following season the suspensor develops and the embryo is formed, along with the characteristic "rumination" of the endosperm and the development of the testa. The first indication of change from the winter proembryonic condition is the elongation of the primary suspensor cells (*fig. 29*); a little later this is shared by the secondary suspensor cells; and this is followed by the elongation of the third tier, if four tiers are formed. In the meantime the terminal cell has begun a series of rapid divisions, resulting in a cylindrical mass of meristematic tissue, much as LYON (5) has described in the case of the embryo of *Ginkgo*. This meristematic cylinder advances gradually into the endosperm, its basal tiers of cells successively contributing to the suspensor elongation. Thus in the formation of the suspensor there seems to be developed what may be called a wave of elongation, beginning with the uppermost tier of the proembryo and extending gradually downward, tier after tier, until it includes the upper region of the meristematic cylinder formed by the terminal cell. This same phenomenon is very evident also in *Thuja*.

After the meristematic cylinder has advanced into the endosperm and has become prominent, the growing points are organized; the two cotyledons presently becoming beautifully crescentic in outline and completely surrounding the stem-tip; and the root-tip being organized deep within the meristematic cylinder.

In several instances a number of small embryos were observed imbedded in the endosperm about the suspensor region of the normal embryo. Our material did not permit any determination of their origin, but they resemble the proembryo of the normal embryo, and are developed while the latter is in its second season's growth. After the pollen tube has reached the archegonium, the endosperm grows up around it (*figs. 23 and 24*), so that the tube lies in a cup-shaped depression. After fertilization, the rim of this endosperm cup con-

tinues growth and gradually incloses the fertilized egg, in most cases forming quite an elongated beak above the embryo. Later many of these cells round off, forming a loose tissue, and it is among these rounded-off cells that the feeble accessory embryos are produced. Whether these have been developed apogamously from the endosperm cells, or have budded from the suspensor cells can only be conjectured. In any event, they might develop further if there was any failure in the development of the normal embryo.

The time involved in the series described above, that is from the first appearance of the megasporangiate strobilus to the maturity of the seed, is about thirty months, distributed as follows: June (?), first appearance of the strobilus; April, mother-cells in synapsis; August, fertilization; October, proembryo of 12 to 18 cells (winter condition); following season, development of embryo, "rumination" of endosperm, and development of testa; October, fall of seed.

#### MATURING OF SEED.

The outer integument and its histological continuation about the ovule develops a thick fleshy coat containing very numerous large resin cavities, and completely inclosing the structures within (*figs. 4 and 5*). This fleshy coat gives to the mature seed the appearance of a plum (*fig. 3*), as in the seeds of Cycads and Ginkgo. Within the broad band of resin cavities, near the inner limit of the integument, two conspicuous vascular strands occur, directly opposite one another (*fig. 4*). These are the main strands of the very characteristic vascular system of the ovule described by OLIVER (7).

The inner integument early differentiates into two distinct layers, a differentiation just as evident in Cycads and Ginkgo. The outer layer forms the stony coat, and the transformation from soft to very hard tissue begins after the embryo and endosperm have completed their development. The hardening begins at the apex of the ovule, and on account of resistance to stains appears under low power as a clear band (black in *fig. 5*). The hardening band gradually extends downwards through the relatively very short integument, and differentiating as a distinct layer in the much larger mass of tissue below completely invests the ovule within the fleshy coat. Protoplasmic connections between the cells of the stony coat and striations in the cell walls are unusually clear.

The inner layer of the inner integument comprises several layers of thin-walled cells, but beyond the integuments it is not histologically differentiated as a layer distinct from the tissue within. Accordingly, through the great bulk of the seed this layer may be neglected, and the whole mass of tissue within the stony layer and outside of the embryo sac will be spoken of as nuclear tissue or perisperm.

The behavior of the endosperm is peculiar, resulting in what is called "ruminated endosperm," a phenomenon peculiar to *Torreya* among gymnosperms, and commonly illustrated by the nutmeg "Rumination" of endosperm proves to be a misnomer, for the endosperm is always the successfully aggressive tissue in developing this condition. The perisperm continues to grow throughout the maturing of the seed, and the final condition results from what might be called the struggle of two growing tissues that have been abutting upon one another through their whole period of growth.

In ordinary seeds the endosperm invades the surrounding tissue more or less uniformly; in the case of Cycads and Ginkgo, for example, obliterating most of the perisperm. In *Torreya*, on the other hand, the invasion by the endosperm is irregular in the extreme. It is in the season after the proembryo has been formed that the active invasion of the perisperm begins. The extension of the endosperm into the tip of the nucellus above the sac proceeds in the usual way, obliterating all of it except a few peripheral layers of cells. This uniform invasion seems to be due to the fact that in this apical region (the original nucellus) the perisperm is not growing actively if at all. Below this small region at the tip, however, the perisperm is very active and evidently resists disintegration much more at certain points than at others. As a consequence, the perisperm becomes eroded by the irregularly advancing endosperm, and is left in the condition of a much dissected coast-line (*figs. 4 and 5*). To the casual observer this results in an appearance suggesting that the endosperm is being invaded by plates of perisperm, but this is no more true than that the promontories of a dissected coast-line are advancing into the sea. The suggestion of an invading perisperm is further strengthened by the fact that within the perisperm bordering the endosperm a dark brown and finally black band of cells is developed, due to abundant food storage (*figs. 4, 30, 31*), but this really recedes as the endosperm advances.

A cross-section of the mature seed always shows a definite and deep constriction of the endosperm in the center, exactly opposite the two opposed vascular strands that run up on each side of the seed through the inner part of the outer integument (*fig. 4*). This constriction is the cross-section of two opposite and deep longitudinal furrows in the endosperm, and it means that in this longitudinal plane the endosperm encounters the greatest resistance in invading the perisperm. This most resistant perisperm certainly seems to hold a very definite topographical relation to the principal vascular strands, and this relation may explain the resistance.

That endosperm is the aggressive tissue at every point, even at the region of most resistant perisperm, is evident for several reasons. In no case were the peripheral cells of the endosperm broken down; and in no case did there fail to appear one or two layers of disorganized cells of the perisperm in contact with the endosperm (*figs. 30* and *31*). In every case, also, the peripheral cells of the endosperm appeared active and very vigorous, and their different appearance in regions of more and less active encroachment is striking. In regions of active invasion the endosperm cells are radially elongated, and many of them are binucleate (*fig. 30*); while in regions of less active invasion the cells are more nearly isodiametric and rarely binucleate (*fig. 31*).

Another proof that endosperm is the encroaching tissue may be obtained from comparative measurements. At the times of fertilization the gametophyte usually measures 20 by 30 $\mu$ . In the mature seed the ordinary length of the gametophyte is 20<sup>mm</sup>, the greatest width being 14<sup>mm</sup>, and the least (at the deep constriction opposite the vascular strands) 1.5<sup>mm</sup>. At this most resistant region of the perisperm, therefore, where it is hard to escape the conviction that the perisperm plate has cut the endosperm nearly in two, the endosperm has increased its diameter against the perisperm seventy-five times.

The best reason, however, for concluding that the endosperm is the invading tissue in this case is that this is always the behavior of endosperm; and it is singular that the old explanation of "rumination" was ever suggested. An examination of the nutmeg, the classic illustration of "ruminated endosperm," and of *Asimina triloba*,

showed that precisely the same explanation applies to them that we have given in the case of *Torreya*.

#### SUMMARY.

The staminate strobilus consists of a series of closely overlapping sterile bracts, in four vertical rows, completely enveloping the tip of the axis bearing numerous stamens. The large adaxial resin cavity that occurs in the stamen occupies the site of three abortive sporangia.

The male gametophyte has no prothallial cell, and the male cells are very unequal, resembling those of *Taxus*. The pollen tube is exceedingly variable in the rate and direction of its advance through the nucellar cap, sometimes pushing in the embryo sac while it is in an early free-nucleate stage.

The ovulate strobilus consists of four enveloping bracts and a single terminal ovule with two integuments. Extensive intercalary growth below the mother-cell forms the bulk of the mature ovule and seed. There is no organization of a special digestive layer around the mother-cell.

The solitary archegonium initial appears as soon as walls are formed, is always at one side of the central axis of the gametophyte, and forms a two-celled neck. The nucleus of the central cell was not observed to divide, nor could any trace of a ventral nucleus be found.

In fertilization the male cytoplasm invests the fusion nucleus, and seems to remain distinct until wall-formation at the four-nucleate stage of the proembryo.

In the development of the proembryo, four free nuclei appear before wall-formation, and the proembryo completely fills the egg, having no "open cells." A proembryo of twelve to eighteen cells is the winter stage. In the spring the suspensor is formed by what may be called a wave of elongation, beginning with the uppermost tier of the proembryo, and extending gradually downward, tier after tier, until it includes the upper region of the meristematic cylinder formed by the terminal cell.

Small embryos are formed during the second season in the suspensor region of the normal embryo; but whether they arise from prothallial or suspensor cells was not determined.

The "rumination" of endosperm, peculiar to *Torreya* among gymnosperms, arises from the extremely irregular encroachment of the endosperm upon the perisperm, the endosperm being resisted much more at certain points than at others. The same was found to be true of other "ruminated" seeds.

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#### EXPLANATION OF PLATES I-IV.

With the exception of *Plate I*, all figures were drawn with the aid of an Abbé camera lucida and reduced one-half in reproduction. Abbreviations are as follows: *br*, bract; *a*, primordium of stamen; *oi*, outer integument; *ii*, inner integument; *r*, resin cavity; *n*, nucellus; *pc*, pollen chamber; *s*, sporogenous cells; *tn*, tube nucleus; *g*, primary spermatogenous cell; *stn*, nucleus of stalk cell; *b*, body cell; *m*<sub>1</sub> functional male cell; *m*<sub>2</sub> functionless male cell; *hr*, haustorial region of female gametophyte; *nc*, nucleus of neck cell; *o*, egg; *end*, endosperm; *per*, perisperm.



## PLATE A.

FIG. 1. Staminate branch; October 21, 1904.  $\times 1$ .

FIG. 2. Ovulate branch; April 7, 1904.  $\times 1$ .

FIG. 3. Mature seed; October 21, 1904.  $\times 1$ .

FIG. 4. Cross-section of seed showing ruminated endosperm with storage region in center, seed coats, stored food in the perisperm, and resin ducts.  $\times 3$ .

FIG. 5. Longitudinal median section through seed showing embryo, ruminated endosperm, and seed coats.  $\times 3$ .

## PLATE I.

FIG. 6. Transverse section through a staminate strobilus showing bracts and primordia of stamens; August 12, 1904.  $\times 24$ .

FIG. 7. Longitudinal section through a staminate strobilus showing enveloping bracts, primordia of stamens, and storage region; the position of the bundles is shown by the dotted lines; August 12, 1904.  $\times 24$ .

FIG. 8. Primordium of a stamen; August 12, 1904.  $\times 460$ .

FIG. 9. Median section through an adaxial sporangium showing the disorganizing primary sporogenous cell and two wall cells; October 21, 1904.  $\times 460$ .

FIG. 10. Cross-section of a sporophyll showing the three abortive adaxial and the three functional abaxial sporangia; October 21, 1904.  $\times 47$ .

FIG. 11. Longitudinal section through a stamen showing early stage of the resin cavity above and a functioning sporangium below; October 21, 1904.  $\times 255$ .

FIG. 12. Pollen grain showing primary spermatogenous cell and tube cell; April 7, 1904.  $\times 1250$ .

FIG. 13. Tip of a pollen tube which has penetrated about half way through the nucellus; June 10, 1904.  $\times 460$ .

FIG. 14. Tip of pollen tube in contact with the embryo sac; the female gametophyte is in the free nuclear stage and the nuclei are placed parietally; June 21, 1904.  $\times 460$ .

FIG. 15. Embryo sac in free nuclear stage showing one of the nuclei occupying the place of the megaspore mother-cell; June 10, 1904.  $\times 185$ .

FIG. 16. Pollen tube in contact with the endosperm; an archegonium is at the left of the tube; July 26, 1904.  $\times 255$ .

## PLATE II.

FIG. 17. Young ovulate strobilus showing enveloping bracts, inner integument, and nucellus; July 26, 1904.  $\times 47$ .

FIG. 18. Ovulate strobilus showing bracts, integuments, nucellus with rudimentary pollen chamber, and megaspore mother-cell; April 7, 1904.  $\times 24$ .

FIG. 19. Tip of nucellus with pollen chamber containing a microspore; April 8, 1904.  $\times 220$ .

FIG. 20. Megaspore mother-cell in synapsis; April 7, 1904.  $\times 460$ .

FIG. 21. Micropylar end of female gametophyte with archegonium initial projecting into the space formerly occupied by the megaspore mother-cell; July 26, 1904.  $\times 460$ .

FIG. 22. Archegonium consisting of two neck cells and central cell; the nucleus of the central cell has rounded out, and is passing downward to the center of the cell and taking on the appearance of an egg nucleus; July 26, 1904.  $\times 460$ .

FIG. 23. Median longitudinal section of female gametophyte showing egg, remains of a neck cell, and antipodal haustorial cells; the tip of the pollen tube in contact with the egg contains stalk and tube nuclei, functional and functionless male cells; the upward growth of the endosperm cells forms a sheath around the pollen tube; August 12, 1904.  $\times 460$ .

PLATE III.

FIG. 24. Fertilization; the male nucleus is in contact with the egg nucleus; the cytoplasm of the male cell is closely applied to the egg nucleus; the functionless male cell, and tube and stalk nuclei are in the upper part of the egg cytoplasm; the cavity in the egg cytoplasm is caused by the inrush of the contents of the pollen tube; August 12, 1904.  $\times 460$ .

FIG. 25. Two-celled proembryo; the dense cytoplasmic mass surrounding the nuclei is probably in greater part derived from the male cytoplasm; August 27, 1904.  $\times 460$ .

FIG. 26. Four-celled proembryo; walls coming in; August 12, 1904.  $\times 460$ .

FIG. 27. Proembryo shortly after walls are laid down; the proembryo passes the winter in this stage; August 27, 1904.  $\times 460$ .

FIG. 28. Cross-section through suspensor cells; September 12, 1904.  $\times 460$ .

FIG. 29. Proembryo showing elongation of suspensor cells; April 7, 1904.  $\times 460$ .

FIG. 30. Endosperm cells encroaching on perisperm; August 12, 1904.  $\times 640$ .

FIG. 31. Endosperm cells which have ceased to encroach on perisperm; August 12, 1904.  $\times 460$ .







