ON THE DEVELOPMENT OF HAMAMELIS VIRGINIANA.

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(WITH PLATES VI AND VII)

INTRODUCTION.

THIS work on Hamamelis was undertaken on account of its peculiar habit of flowering. It is one of the few angiosperms that flowers in the fall and matures fruit the following year. This peculiarity made it seem worth while to investigate its entire embryological history, and especially the behavior of the pollen tube and the time and manner of fertilization. The subject was suggested by Dr. D. S. JOHNSON, to whom I wish to express my gratitude for sympathetic guidance and instruction during its prosecution.

The literature of the family is not extensive, and none of it has to do with the embryology of any of its forms. The most complete working out of the anatomy and affinities is by REINSCH (12). BAILLON (3) carefully described the organogeny of the flower in *Hamamelis virginiana* and *Fothergilla Gardeni*. VAN TIEGHEM (14) worked on the secretory canals of Liquidambar and Altingia. THOUVENIN (13) described the structure of the root, stem, and leaves of various members of the family. The account given by NIEDENZU in Engler and Prantl's *Natürlichen Pflanzenfamilien* is the most complete I have seen.

In some American oaks, which require two years to mature seed, it has been found that fertilization takes place about a year after pollination. The statement is made by GOEBEL (15, p. 392) that a period of rest occurs after the pollen tube has reached the embryo sac in Ulmus, Quercus, Fagus, Juglans, Citrus, Aesculus, Acer, Cornus, and Robinia. As Miss BENSON (4) points out, this statement is erroneous in the case of British Amentiferae. It is not true of Hamamelis.

In *Colchicum autumnale*, according to HOFMEISTER (7), the pollen

tube reaches the embryo sac at the latest by the beginning of November, and it is not until May of the next year that the embryo begins to form. This plant and the autumn-flowering species of Crocus, like *Hamamelis virginiana*, bloom and shed their pollen in the fall. HOFMEISTER'S account of Colchicum shows that it differs essentially from Hamamelis in the behavior of the pollen tube and the time of fertilization. The other genera named as having a longer or shorter period between pollination and the beginning of embryonic growth are all pollinated in the spring, so that the resting period for most of them does not extend through the winter.

The family of the Hamamelidaceae comprises some fifty species, in eighteen genera, of which eight are monotypic. North America has three representative genera: Hamamelis, Liquidambar, and Fothergilla. The first is found nearly always in such sheltered places as harbor *Polystichum acrostichoides*, from Labrador to Florida and west to the Mississippi River. The second is found on the coastal plain and on bottom lands from New York to Florida, thence to Central America, and up the Mississippi River to southern Indiana. The third is found from Virginia to Florida, east of the Appalachian Mountains. Hamamelis has two more species, one in southern China and one in Japan; Liquidambar is also represented by two species which occur in southern Asia from Asia Minor to Formosa; and Fothergilla is represented by one other species in Persia. The remaining genera are confined to southern and eastern Asia and Malaysia, with the exception of three genera found in Madagascar and southern Africa. Thus the whole family, with the exception of three genera, is confined to the eastern and southern parts of North America and Asia. This peculiar and as yet unexplained distribution occurs in a large number of genera, but this family is one of the most pronounced cases found. It should also be said that Hamamelites and Parrotia are found in the Dakota group of the Cretaceous (9), and that Hamamelis (5) and Hamamelidanthium are found in Eocene strata in northern Europe, so that the family had formerly a much more extended range than at present.

I have usually found Hamamelis on rather steep hillsides with a northern exposure, or more rarely on low ground along streams. It is said to grow abundantly on mountain tops in Pennsylvania

and western Maryland, and COWLES (6) reports it as growing on sand dunes near Chicago. From his description, however, it is doubtful if the seed had germinated in these dry localities. It has thus a very restricted range, and seems to grow only in positions in which moisture is abundant and transpiration slow. Liquidambar is confined to alluvial soils and moist situations, and so follows river valleys and coast lines; its northern limit is the valley of the Hudson River in New York. Fothergilla grows in moist places.

The material of *Hamamelis virginiana* and *Liquidambar* for the present work was obtained from the region about Baltimore, from northern Virginia, from Long Island, and from southwestern Ohio. I am indebted for exotic forms to the kindness of Mr. GEO. V. NASH, of the New York Botanical Gardens, and to Mr. J. G. JACK of the Arnold Arboretum, Jamaica Plain, Mass. The long period of development made it necessary to collect nearly every week of the year for Hamamelis. Material of Fothergilla was collected in central South Carolina.

Killing and fixing was mostly done with a sublimate-acetic mixture made by adding 5 per cent. glacial acetic acid to a saturated aqueous solution of corrosive sublimate; this was often used hot. The material was cleared in xylol and imbedded in paraffin; and sections were cut 5 to 15 μ in thickness. For staining, a combination of haem-alum and Bismarck brown was tried, but Flemming's triple stain was found more satisfactory and was used almost exclusively. It was necessary before the young carpels could be sectioned in paraffin to remove carefully and laboriously the hairs from their bases, as on account of their thick walls the hairs could not be cut in paraffin, invariably tearing the sections. To avoid this process celloidin imbedding was used in a few instances, but it was impossible to get the sections thin enough for most purposes.

ORGANOGENY OF THE FLOWER.

The flower buds arise from axils of the leaves of the current year, or from latent buds of the two preceding years, and appear early in May. They, as well as the leaf buds and the young twigs, are covered by a dense coating of tufted hairs. Each hair develops from a single epidermal cell, which protrudes from the surface and is cut by anti-

clinal walls into four to twenty cells, each of which sends out a long process, making a many-rayed star. They are often raised on a low multicellular papilla.

Each flower bud produces a head of two to four flowers, and there are often as many as three buds from an axil. At first the tip of each bud is protected by three or four alternate bracts, which are soon left below on the stem of the flower head and finally fall off. The first floral organ to appear is the outside bract of each flower. As the buds grow the other two bracts arise successively, one on each side of the flower. The sepals appear in pairs, the first pair being anterior and posterior. The petals then arise in one cycle of four rudiments, inside which two successive alternating cycles of four rudiments each develop. The outer cycle, opposite to the lobes of the calyx, becomes stamens, the inner sterile staminodes.

The torus by this time, through unequal growth, has become concave, and on its floor are developed two horseshoe-shaped ridges, one anterior and one posterior. These grow together on the median line, and this line of fusion is carried upward by growth, so that there is a solid wall between the cavities of the carpels for a short distance. The carpels have separate styles and stigmas, but are united throughout their hollow portions. In each ovary there is found one ovule, which is suspended from the margin of the carpel. This development of the flower is essentially as described by BAILLON (3), except that he describes the ovary as originally having two ovules, one of which nearly always atrophies. LE MAOUT and DECAISNE (16) also figure a cross-section of the fruit showing two mature seeds in each carpel. Although I have examined several hundred carpels I have found but a single one with two ovules. BAILLON (17) also speaks of Hamamelis as being polygamous, but of this I have seen no evidence in my material. It is possible that these conditions may occur more frequently in places from which I have no material, or in other surroundings, yet the form has been very constant from all my collecting points.

In the mature flower the temporary parts, the stamens, petals, and nectaries, are smooth. The outside of the sepals and bracts, and the bases of the carpels are thickly covered with hairs. *Figs. 8* and *9* show how the rudiments of the growing flower fit together, and

how the bud is protected by its hairy covering. Most of these hairs have a double function. While young they act as slime cells in keeping the growing point and growing tissues moist, this function being best performed by the hairs on the tips of the sepals and bracts, and the bases of the carpels. These young hairs are long and tortuous, and wind among the growing rudiments (*fig. 9*). Their cavities are full of sap, and their nuclei take a very dark stain; they remain in this active stage longer than hairs on other parts of the plant. As they grow older all the hairs acquire thick cell walls, and tend to straighten. In their mature state they act as a protection against moisture. This function they perform by means of a waxy covering which repels water, so that it is very difficult to moisten a young flower bud, or a growing twig, or fruit; but if these hair-covered parts be soaked a short time in strong alcohol, and allowed to become dry again, they may be very readily moistened.

POLLEN SACS AND POLLEN.

Each fertile stamen rudiment begins early to form two microsporangia. There is apparently no evidence of the presence, at any stage, of the other two microsporangia that are usually found in angiosperms. No indication of pollen-formation is ever seen in the sterile rudiments.

The first evidence of the formation of the archesporium is found about the middle of June. The subepidermal layer divides by periclinal walls at the place where the microsporangia are to be formed (*fig. 1*). The exact derivation of layers is hard to trace, but it is quite certain that it is from the inner layer thus formed that all the archesporial tissue comes. By the middle of July the archesporium is well blocked out, and shortly after the spore mother-cells are formed. At this stage there is about them a moderately well-defined tapetum, and the outside wall of the microsporangium is three or four cell layers in thickness (*fig. 2*). Here the pollen mother-cells are only noticeable by their slightly larger nuclei and more deeply staining contents.

The further growth of the microsporangium is brought about by the increase in size of its cells, both sporogenous and tapetal. Before the tetrad division, the nuclei of the tapetum divide without

the formation of cell walls, and the tapetal cells have two or three nuclei each; the nucleoli also increase notably, and the contents of the cell become more largely vacuolate (*fig. 3*). The pollen mother-cells thicken their walls, and soon float freely in the cavity of the anther; increasing further in size, with a much larger nucleus and nucleolus (*fig. 29*). The two tetrad divisions occur almost simultaneously, although stages are found showing two nuclei (*fig. 30*). In the tetrad division the nuclear processes do not show at all clearly, though there seem to be fibers connecting the nuclei in one stage observed (*fig. 31*). The pollen grains have cell walls and but one nucleus when the mother-cell wall is broken down (*fig. 32*) and the grains released into the cavity of the microsporangium. The mature grain was described by VON MOHL, very briefly, in 1835. It has the shape of an oblate spheroid, with three meridional furrows (*fig. 33*); between these furrows the surface is covered by a fine reticulation. An equatorial section shows that the intine is strongly developed under the furrows, which gives the section of the interior a decidedly three-lobed appearance (*figs. 33* and *34*).

Soon after the pollen grain is freed from the mother-cell, its nucleus divides, and the smaller nucleus, probably the generative, retires into the extremity of one of the lobes. Here it becomes closely applied to the intine and is cut off from the larger cell by a very noticeable wall, which is probably of cellulose (*fig. 33*). Shortly before the pollen is shed this wall disappears, and the two nuclei then lie free in the cavity of the grain. The larger of the two, the tube nucleus, is loosely vesicular, while the structure of the generative nucleus is dense and deeply staining (*fig. 34*).

Of the four layers in the microsporangium wall only the subepidermal layer has any part in the opening of the anther, becoming the fibrous layer and covering the inner and lateral faces of the anther (*figs. 5* and *6*). The first and for a long time the only evidence of the differentiation of this layer is the radial lengthening of the cells. At this stage it is possibly not inappropriate to mention an instance of regeneration observed in this fibrous layer. From some unknown cause, the first subepidermal layer had been destroyed over a small area. The remaining part of that layer had developed normally. Into this gap tissue had grown from both sides, but that which came

from below was slight in amount, and had retained its original character; while the epidermal cells had greatly elongated and had divided into epidermal and secondary fibrous layer cells (*fig. 4*). These secondary fibrous layer cells resembled very closely at this stage those of the primary fibrous layer, and I see no reason to suppose that they would not have developed fibers at the proper time. Here then the epidermis seems to have been more plastic under the injured spot than the deeper lying tissues.

The fibers are developed in this layer shortly before the time the anther is to open, appearing on the side and bottom of each cell. Around the top, posterior, and bottom of the microsporangium there is a groove (*figs. 5* and *7*), and in the bottom of it the cells have no fibers and are quite thin-walled, so that they readily break, thus forming the line of dehiscence of the anther. As LECLERC DU SABLON (8) has shown for anthers in general, the opening is due to unequal shrinkage of the two walls of the fibrous layer. The outer wall being of cellulose, shrinks more on drying than the inner wall, which is strengthened by its lignified fibers. By this means the whole outer covering is bent upon itself and points directly toward the carpels (*fig. 7*); nearly all of the pollen adheres to these wings, and so is placed in the way of any insect that comes to visit the nectaries. The stigmas are ripe for pollination at the same time that the anthers open. Any insect visiting many flowers in succession must scatter pollen promiscuously, so that there is sufficient adaptation to insure cross-pollination, but no well-developed mechanism to prevent self-pollination. Pollen is ripe and begins to be shed in the latter part of October, and is shed from that time on into the winter, as the flowers keep opening with each return of warm weather, even as late as January.

OVULES AND EMBRYO SAC.

The ovules show specialization of archesporial tissue at the tip of the nucellus before the integuments have begun to develop, but it is very difficult to distinguish archesporial cells, although there seem to be several of these, each one cutting off a tapetal cell which divides repeatedly. The cells of this tissue then elongate in the direction of the long axis of the nucellus, and bury the mother-cells by some eight layers of cells in the nucellus.

It is also probable that this part of the nucellus forms the extremity of the conducting tissue for the pollen tube. In early stages three to five macrospores can be seen, but only one germinates. This goes through the usual stages, the resulting nuclei being arranged in the order most often found among angiosperms (*fig. 10*). The antipodals very early disappear, so that they are hard to find at the time of fertilization. The endosperm nucleus is found about the middle of the sac.

The tissue surrounding the mature embryo sac is much disintegrated (*fig. 10*). Around the chalazal end of the sac the tissue always stains deeply, and there is a quite evident strand of conductive tissue from the tip of the fibrovascular bundle at the chalaza to the lower end of the embryo sac (*fig. 10*). The base of the nucellus shows by its smaller cells that it is the most rapidly growing part. The development which the ovule has attained at the beginning of winter is shown in *fig. 11*. The integuments up to the early part of April are still behind the nucellus in growth; in spring their growth is hastened and they soon project beyond the nucellus, leaving a wide open micropyle (*fig. 21*). This is finally closed to a slit-like fissure between the edges of the outer integument.

The outer integument is now quite thick, formed of about eight layers of cells, and is uniform in structure throughout. The inner integument is made up of three layers of cells which are very much alike. The epidermal layer of the nucellus has already become slightly different from the underlying tissue.

POLLEN TUBES ARTIFICIALLY GROWN.

Pollen taken from open anthers was very readily sprouted in a 16 per cent. sugar solution made with tap water, in which 1.5 per cent. gelatin was dissolved. The pollen grains first became spherical by the filling out of the furrows, thus using the masses of intine on the inner sides of the furrows. Tubes sprout from the pollen grains when placed in the nutrient gelatin in one to three hours, and always arise from the smooth bands. The cultures were kept at room temperature and growth was more luxuriant in the dark. The growth of molds, etc., usually disturbed the cultures at the end of a few days.

The behavior of the nuclei was not readily observed. Methyl-green-acetic-acid was used to kill the pollen tubes, and no more

than two nuclei were ever found in a tube. The tubes showed a marked tendency toward the formation of cellulose plugs (*fig. 12*). It rarely occurred that part of the contents of the tube was in this way shut off, as the spaces walled off by plugs were mostly empty.

In the course of about three days' growth the pollen tube frequently "encysted" (*fig. 35*), that is a spherical swelling developed at the tip or near the tip of the tube into which nearly all the contents of the tube were withdrawn, including one or both nuclei. A wall was then formed completely closing off the swelling, which was often as large as the original grain. I have not determined the exact conditions which called forth this action, neither have I found such things in the style in normally grown pollen tubes. Miss BENSON (4) reports a case of somewhat similar character as occurring in Carpinus, though the spherical character and the separating wall were not nearly so pronounced. She suggests that this may be of use in the short resting period of this form, but was not able to find such appearances in the style.

The pollen showed ability to sprout at room temperature whenever the flowers opened. A collection made in Ohio early in January, after the unopened buds had endured a week of very cold weather, when the temperature had been as cold as $-15°$ F., sprouted in a seemingly normal way, though not so vigorously as earlier. I am inclined to think, however, that pollen shed so late never functions.

CONDUCTIVE TISSUE OF THE STYLE AND OVARY.

By the folding of the carpels each style has a groove formed, which leads from the stigma to the ovary. It is open at the top, and where its sides are slightly separated and its inner surface thus exposed, it bears the loosely arranged papillose cells of the stigma. The epidermis of the groove and two subepidermal layers continue this stigmatic tissue down to the base of the funiculus, the strand of conducting cells getting gradually deeper and deeper in the tissue of the style. The cell walls of the strand are thickened, partly gelatinized, and the contents of the cells are dense. *Fig. 13* shows its appearance and position about midway in the height of the style, and its course in longitudinal section is shown in *fig. 18*. When the flower first opens, the epidermis of the funiculus is not yet differentiated,

but as winter approaches the base of the funiculus becomes glandular. The epidermal cells enlarge, the walls thicken, and the contents become vacuolated. In the spring this process is carried still farther, and as the ovule occupies more and more of the cavity of the ovary, these cells secrete a mucus which fills the small remaining cavity around the base of the funiculus (*fig. 10*).

THE DESCENT OF THE POLLEN TUBE.

The pollen grains begin growth very shortly after falling on the stigma, and the growth is at first comparatively rapid. Its course is readily traced in the conducting tissue of the style, which is greatly disorganized by the growth of the large number of tubes usually present. The course is between the cells rather than through them. By the time that winter sets in the living part of one or more tubes is to be found in the neighborhood of the base of the funiculus (*fig. 11*). There are usually several living tubes at varying heights in the style at this time (*figs. 17* and *18*), and evidence of many more which have been stranded above. The unprotected tip of the style is dead and withered, while that part which is clothed with hairs is alive. It is in this protected part of the carpels that the pollen tubes hibernate. Soon after pollination the flower head twists on its stalk so as to invert the blossoms. The inverted calyx then very effectually protects the carpels from rain, sleet, and snow.

The pollen tubes found at this time are usually of greater diameter than at the beginning of growth. Tubes grown in sugar-gelatin solutions are 5 to 8 μ in diameter, and those which sprout on the stigma are at first about the same size; but those found during the resting stage are 12 to 15 μ in cross-section, and the wall is thicker than at first (*fig. 14*). The nuclei found did not exceed two in any tube.

When growth is renewed in the spring, the area of conducting tissue in the funiculus being increased, the pollen tube is soon seen in the cavity of the ovary. More than one tube may reach the ovary. At first the ovule is by no means ready for fertilization, and the integuments have not yet closed up the micropyle (*fig. 21*). The tubes do not appear at this time to have any definite direction of growth, but grow down beside the ovule or into the wide open

micropyle, or between the integuments. The course to the egg cell is through the micropyle and into the tip of the nucellus through the tissue derived from the tapetal cells, which stains deeply at the time and is probably conductive tissue. Thus it is seen that the tube grows just about as fast as the conductive tissue is prepared for it, and stops in the fall when it reaches the end of the mature conductive strand. The transference of the male nucleus has not been observed, but fertilization takes place about the middle of May, which is from five to seven months after pollination.

ENDOSPERM.

The antipodals very early disappear. The first result of fertilization is apparent in the action of the endosperm nucleus, which immediately begins to divide. The stage of free endosperm nuclei is very short, as cell walls have appeared in the twelve-nucleate stage. These walls first arise in the bottom of the embryo sac. Both endosperm and nucellus grow rapidly from this time forward. The endosperm early disintegrates the neighboring nucellar tissue except at two points, the tapetal strand of tissue leading down from the micropyle and bearing the fertilized egg at its lower end, and the pit at the chalazal end of the embryo sac which earlier held the antipodals. This and the deeply staining tissue surrounding it resist the action of the endosperm for some time, and by the growth of the base of the nucellus are pushed into a position on the side of the growing endosperm (*fig. 23*). This antipodal pit is finally absorbed.

The nucellus keeps pace with the growing endosperm, its epidermal layer being changed to make part of the inner seed coat. The differentiation of this layer is shown mainly by the larger size of its cells, especially at the tip of the nucellus, and by the crowded cell contents which take up blue stains very readily. This layer is the only part of the nucellus that permanently resists the action of the endosperm, and it is completed across the region of the chalaza so that it entirely surrounds the endosperm. Its nuclei are usually applied to the outer cell wall, and help doubtless in making the clear membrane which surrounds the nucellus in the mature seed. It is thrown into folds shortly before the ripening of the seed (*fig. 20*). The endosperm

is finally stored with food in the form of proteid grains, which shortly before ripening show numerous globoids (*fig. 22*) that disappear later. The ripe endosperm contains in its cells much oil along with proteid material, and in the cell walls there are imbedded numerous crystals of calcium oxalate (*fig. 15*).

THE EMBRYO.

The embryo begins growth comparatively late, so that the endosperm has already acquired some size before the first division of the egg occurs. The egg after fertilization becomes slightly imbedded in the tissue of the tapetal strand, and enlarges greatly. The first division is transverse and cuts off a small cell below and a large one above. By this continued cross division the suspensor may have five or six cells (*fig. 23*). The first division of the embryo is longitudinal. The embryo dissolves the endosperm in much the same way as the endosperm dissolves the nucellus, and lies free in a cavity formed by the disintegration of the endosperm. At maturity there is a straight axial embryo which extends from end to end of the seed and is richly stored with oil and proteid material in all its cells. The upper side of the cotyledons has already a well-developed palisade layer (*fig. 24*).

INTEGUMENTS.

The inner integument is at the beginning three cell layers in thickness, the innermost layer taking part in the formation of the inner seed coat. It early becomes filled with dense contents that stain blue readily (*fig. 20*), and finally shrink and become applied to the inner cell wall. The remaining layers are crumpled up so that they can only be made out with difficulty.

The outer integument thickens greatly, and its cells elongate, taking a curved oblong shape. The cell walls then begin to thicken and the whole integument forms the outer seed coat which is moderately hard, black, and very resistant to water. The outer integument is very smooth over the whole surface, except at the place of attachment of the funiculus, where there is a white saddle-shaped scar. The seed is ovoid, but very decidedly pointed at the lower end. The shape of the seed is a very important part of the discharging mechanism.

THE CARPELS.

At blooming time the carpels are very slightly imbedded in the tissue of the torus (*fig. 17*). There is a very short calyx tube, however, shown in the figure below the attachment of the anther. As the fruit matures, the calyx tube lengthens proportionately more than the carpels, and this gives the fruit the appearance of being half buried in the torus (*fig. 38*). A longitudinal section (*fig. 39*) shows that this is only apparent and that the fruit is only slightly buried.

The substance of the carpels develops into two tissues, the outer one becoming fleshy with numerous roundish stone cells, the inner one forming part of the mechanism for expelling the seed. In each carpel this inner layer is formed in two halves, which are not closed at the top and are higher toward the posterior of each carpel, as shown in side view in *fig. 42*. These halves are never closely joined on the inner sides of the carpels, and there is provision for a split along the dorsal line also. The cells are developed into fibers diagonally from the inner edge to the dorsal line of each carpel, parallel to the top of the layer. In opening, this layer splits down the midrib of the carpel and in front. It then opens at the top and each half below begins to contract in a transverse direction. The cross-section of the opening layer is shown before contraction in *fig. 40*, after the opening of the fruit in *fig. 41*. The pressure exerted comes gradually on the seed, and it is thrown out, not by a sudden movement of the capsule, as in many such contrivances, but by being pinched on the smooth pointed lower end. The great smoothness of the seed and of the inside of the capsule assists greatly in the process. The seeds are often thrown to a distance of twenty feet. This movement is caused by drying, as can be proved by placing an opened capsule in water, when after some hours it will close entirely, and will open again on being dried.

GERMINATION.

The seed thus distributed lies on the ground for two winters according to BAILEY (1), sprouting the second year. Under trees which fruited abundantly in the fall of 1901, but where the crop was a failure in 1900, it was not possible to find young seedlings in May 1902, though many seeds were found. Under trees which fruited

in 1900 it was easy to get young seedlings in various stages. The cotyledons remain in the seed coats until they have absorbed the stored up nourishment of the endosperm (*fig. 16*); they are then freed and exposed as green assimilative leaves. Attempts at sprouting the seed in damp sphagnum were made in the laboratory. The seed was planted in September of 1900, and by May of 1902 had just begun to protrude the tips of the radicles. They had been in the temperature of an unheated room constantly, but had not been subject to frost, and had never been allowed to dry out.

HAMAMELIS ARBOREA.

I procured one stage of the Japanese species *H. arborea* in the latter end of October. This differs from *H. virginiana* in its time of flowering, which is in very early spring. A variety, *H. arborea zuccariniana*, flowers as early as February, and thus approaches the flowering time of the American species. The flowers are in about the same condition in October as those of *H. virginiana*, except that the stamens are rather backward. The pollen grains are free and have each two free nuclei, and evidently pass the winter in that stage (*fig. 36*). As the pollen is shed in March at the latest, it probably must rest about two months before fertilization occurs. The petals are coiled involutely in the bud as in *H. virginiana*, but instead of being entirely smooth have a tuft of hairs on the tips. In other respects the two genera are much alike in their development so far as studied.

FOTHERGILLA GARDENI.

An incomplete series of stages of this species was studied. Its flowers appear in the spring along with the leaves. It lacks a corolla, and its calyx tube is much longer than that of Hamamelis, having five to seven very small teeth. The development of the stamens is described by BAILLON (3). They arise first as five single rudiments, which are followed by other rudiments on each side, so that there are finally five groups of five or six stamens, those of each group being of different ages and different heights. They pass the winter in the pollen mother-cell stage. At the time of flowering the ovules are not yet ready for fertilization, so that the pollen must have a resting period of nearly a week. The anthers have four microsporangia and

open by two valves instead of one, very much as is common in most angiosperm stamens which open by slits. The structure of the seed and fruit is like that of Hamamelis, except that the seed is smaller.

CORYLOPSIS PAUCIFLORA.

Only one stage of this species was examined. It was obtained in the spring before the flowers open,which occurs before the leaves appear. They are borne in drooping racemes, with many bracts, which are smooth outside, but covered by silky hairs within. The structure of these hairs is much like that in Hamamelis, but they are not rigid. The stamens, which have four microsporangia, pass the winter containing nearly mature pollen grains (*fig. 37*), with two free nuclei. The ovule passes the winter in the same stage as does that of Hamamelis (*fig. 25*). It is difficult to determine whether there is present a definitive macrospore or a macrospore mother-cell; however, there is no evidence of the presence of more than one macrospore mother-cell. There must also be some time here between pollination and fertilization.

LIQUIDAMBAR STYRACIFLUA.

Liquidambar is not so closely related to Hamamelis as the other genera studied. The buds in this species pass the winter with the merest rudiments of the floral organs present. The stamens are only small protuberances which do not show any archesporium. The mature anthers have four microsporangia and open by slits (*fig. 26*). The fibrous layer is very slightly developed as compared with Hamamelis. The flowers are imperfect, with rudiments of stamens appearing as nectaries among the flowers in the female heads. These were formerly thought to be both petals and stamens; abortive pollen is sometimes developed in them, which is evidence of their staminal nature (*fig. 27*). The carpels, which occur in pairs as in Hamamelis, are collected into large heads containing thirty-five to fifty flowers each. Each carpel has a double row of ovules developed on marginal placentae, and a broadly expanded stigmatic surface. With very rare exceptions, only one of these many ovules is fertilized, and this one is near or at the bottom of the cavity. There is a week or ten days between pollination and fertilization in this case. The developing seed shows the same resistant tissue at the antipodal

end of the embryo sac as is found in Hamamelis, but here it persists into the ripe seed. The epidermal layer of the integument is not used up, and around the chalaza there is a small fragment of the nucellus left in the ripe seed. This is never stored with food materials, and so cannot be called perisperm (*fig. 28*). The macrospore is buried about as deeply as in Hamamelis. It germinates only in the lower ovules, the upper ones never showing typical embryo sacs and being less developed progressively toward the top of the ovary. In the sterile ovules the cells of the outer integument become very much enlarged and at last empty. The substance of the nucellus is absorbed, and the ovules become polygonal bodies, resembling sawdust, which fill the upper part of the ovary. The outer integument of the fertile ovule (*fig. 28*) grows into a wing. The embryo is straight, and in bulk bears about the same relation to the endosperm as in Hamamelis.

SUMMARY AND CONCLUSION.

1. In Hamamelis the anthers have two microsporangia from the beginning.

2. The generative cell in the pollen grain has a cell wall developed which is afterward dissolved.

3. The pollen tube when grown artificially shows a marked tendency to form cellulose plugs, and also forms spheres into which the contents of the tube are withdrawn. Thus far these phenomena have not been observed in normal growth.

4. The development of the pollen tube in the style may be divided into three periods: first period of growth, hibernation, and second period of growth. During hibernation the walls are thickened and the diameter of the tube enlarged, in the next stage having a smaller size and thinner walls.

5. There are several macrospores developed, only one of which becomes functional. It is deeply buried in the nucellus by the growth of tapetal tissue.

6. The germinating macrospore is nourished through a strand of conductive tissue from the chalaza.

7. The antipodals are sunk in the tapering lower end of the embryo sac. This tip is surrounded by deeply staining tissue which for a time resists the dissolving action of the endosperm.

8. The epidermis of the nucellus is the only part not used up by the endosperm. Its walls are thickened and it helps to form the inner seed coat.

9. Fertilization takes place in May, five to seven months after pollination.

10. The embryo is slow to begin growth and has a short suspensor.

11. The seeds sprout normally after lying on the ground for two winters.

12. The hairs serve a twofold function while young, to keep the growing tissues moist, and when mature to keep off moisture.

13. One case of the regeneration of the fibrous layer of the anther wall by the epidermal layer was observed.

14. The other investigated genera of the family all have a resting stage of the pollen, although it is much shorter.

15. The other genera of the family all have anthers with four microsporangia.

In comparing *Hamamelis virginiana* with its relatives, it seems certain that it was once a spring-flowering plant, whose blossoming has worked backward through the winter. It differs from *H. arborea* essentially in the way its pollen passes the winter, for the development of each is much the same in October.

Most of the plants showing long resting periods in pollen growth belong low in the system, in the Amentiferae; but with the exception of some oaks Hamamelis has the longest resting period known. It seems probable, therefore, that this resting period can not be regarded as a primitive character in any case.

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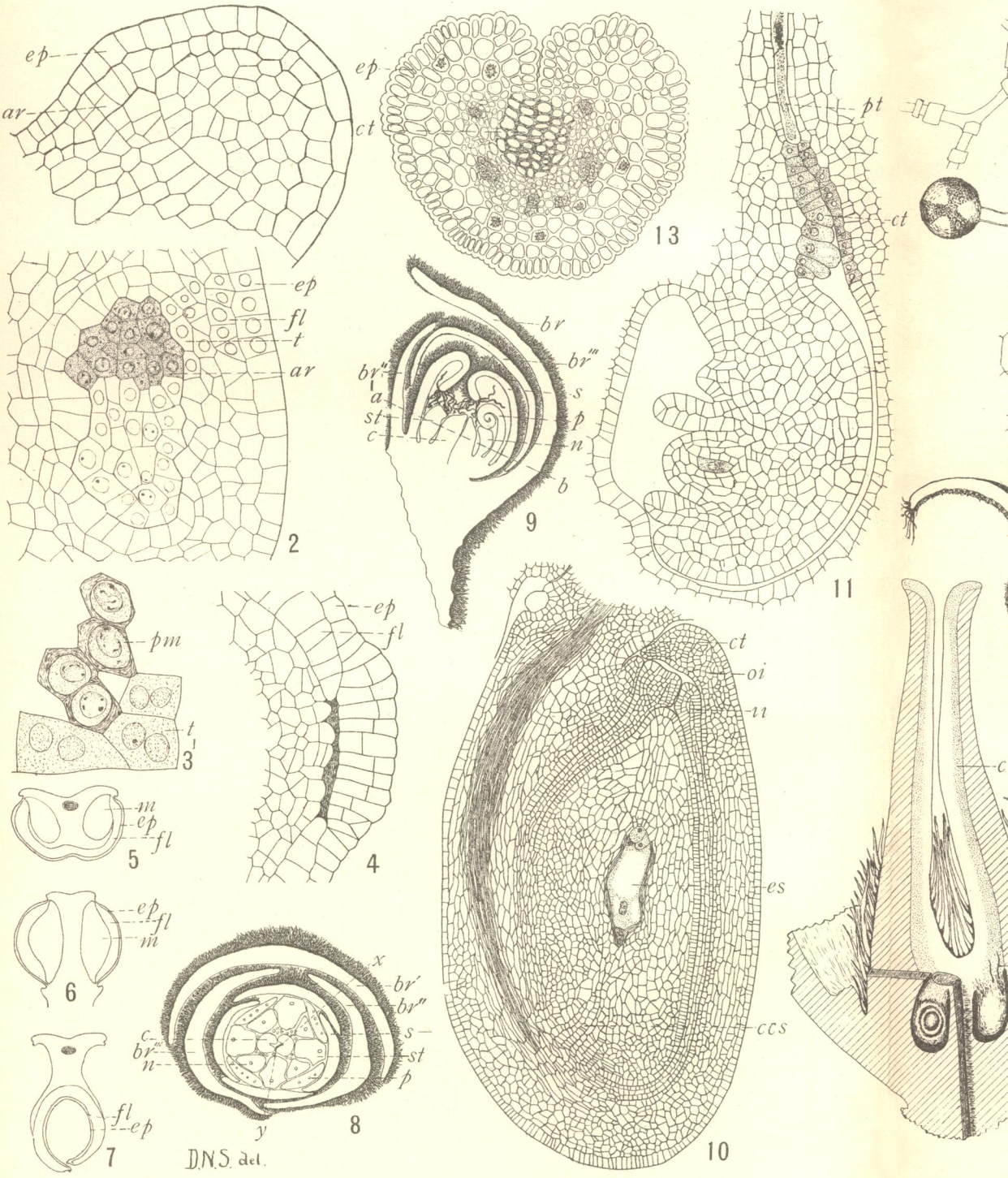

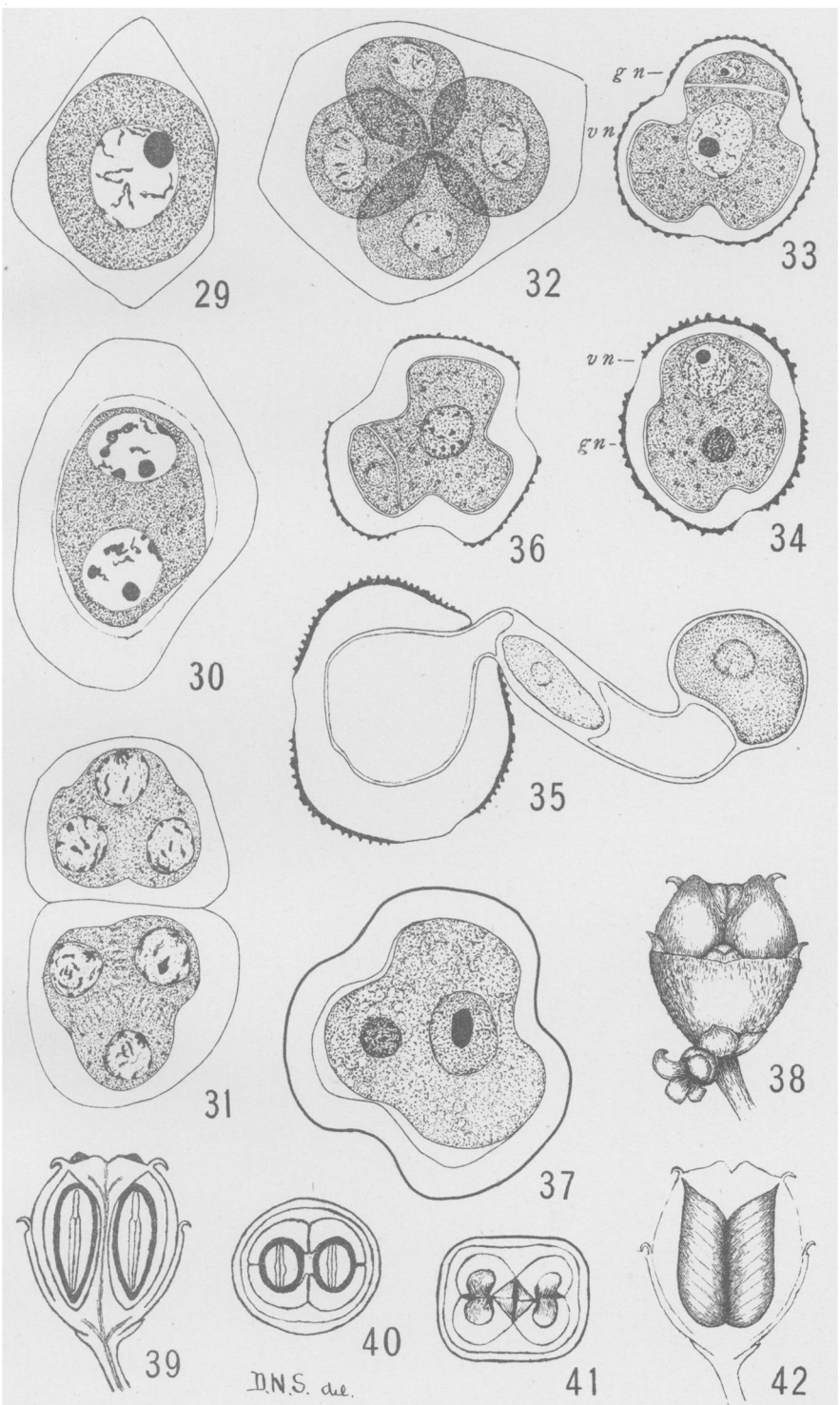

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EXPLANATION OF PLATES VI AND VII.

All figures were drawn with a Zeiss camera lucida, and are of *Hamamelis virginiana* unless otherwise stated. The magnifications indicated are those of the reduced plates; those of *Plate VI* having been reduced one-half, and those of *Plate VII* one-fifth. The abbreviations are as follows. *ar*, archesporium; br^1, br^2, br^3, first, second, and third bracts; *c*, carpels; *ccs*, chalazal conducting strand; *ct*, conductive tissue; *em*, embryo; *en*, endosperm; *ep*, epidermis; *es*, embryo sac; *f*, funiculus; *fl*, fibrous layer; *gn*, generative nucleus; *ii*, inner integument; *m*, microsporangium; *n*, nectaries; *nu*, nucellus; *ot*, outer integument; *ov*, ovules; *p*, petals; *pm*, pollen mother-cells; *pt*, pollen tube; *s*, sepals; *st*, stamens; *t*, tapetum; *vb*, vascular bundle; *vn*, vegetative nucleus.

FIG. 1. Growing stamen; longitudinal section. × 550.
FIG. 2. Growing stamen; transverse section. × 550
FIG. 3. Tapetum and pollen mother-cells. × 550.
FIG. 4. Anther wall showing regeneration. × 220.
FIG. 5. Mature anther; diagrammatic transverse section. × 35.
FIG. 6. Mature anther; diagrammatic longitudinal section. × 35.
FIG. 7. Mature anther, open; diagrammatic transverse section. × 35.

FIG. 8. Growing flower bud; transverse section in plane *ab* in *fig. 9*. × 22.

FIG. 9. Growing flower bud; longitudinal section along line *xy* in *fig. 8*. × 22.

FIG. 10. Ovule; longitudinal section. × 97.

FIG. 11. Ovule at beginning of winter. × 220.

FIG. 12. Pollen tube with cellulose plugs, grown in gelatin. × 330.

FIG. 13. Style; transverse section. × 220.

FIG. 14. Hibernating pollen tube. × 550.

FIG. 15. Endosperm cell walls with crystals. × 550.

FIG. 16. Seedling in seed coats. × 1.

FIG. 17. Flower in winter; longitudinal section. × 9.

FIG. 18. Carpels in winter; diagrammatic longitudinal section. × 20.

FIG. 19. Ovule; longitudinal sections. × 35.

FIG. 20. Seed coats. × 175.

FIG. 21. Ovule in spring; longitudinal section. × 80.

FIG. 22. Endosperm cell stored with food. × 550.

FIG. 23. Embryo and suspensor. × 550.

FIG. 24. Cotyledon in seed. × 220.

FIG. 25. Nucellus of *Corylopsis pauciflora* in spring. × 550.

FIG. 26. Mature anther of *Liquidambar styraciflua;* transverse section. × 35.

FIG. 27. Nectary of the same; transverse section. × 35.

FIG. 28. Seed of the same, nearly mature. × 5.

FIG. 29. Pollen mother-cell. × 1745.

FIG. 30. First division of pollen mother-cell. × 1745.

FIG. 31. Second division of pollen mother-cells. × 1745.

FIG. 32. Pollen grain, in pollen mother-cell. × 1745.

FIG. 33. Pollen grain. × 1745.

FIG. 34. Pollen grain. × 1745.

FIG. 35. Pollen grain sprouted in gelatin. × 1745.

FIG. 36. Pollen grain, winter condition; *Hamamelis arborea*. × 1745.

FIG. 37. Pollen grain, winter condition; *Corylopsis pauciflora*. × 1745.

FIG. 38. Ripe fruit. × 1.6.

FIG. 39. Fruit; longitudinal section. × 1.6.

FIG. 40. Fruit; transverse section. × 1.6.

FIG. 41. Fruit, after discharge of seed; transverse section. × 1.6.

FIG. 42. Opening layer of capsule; side view. × 1.6.