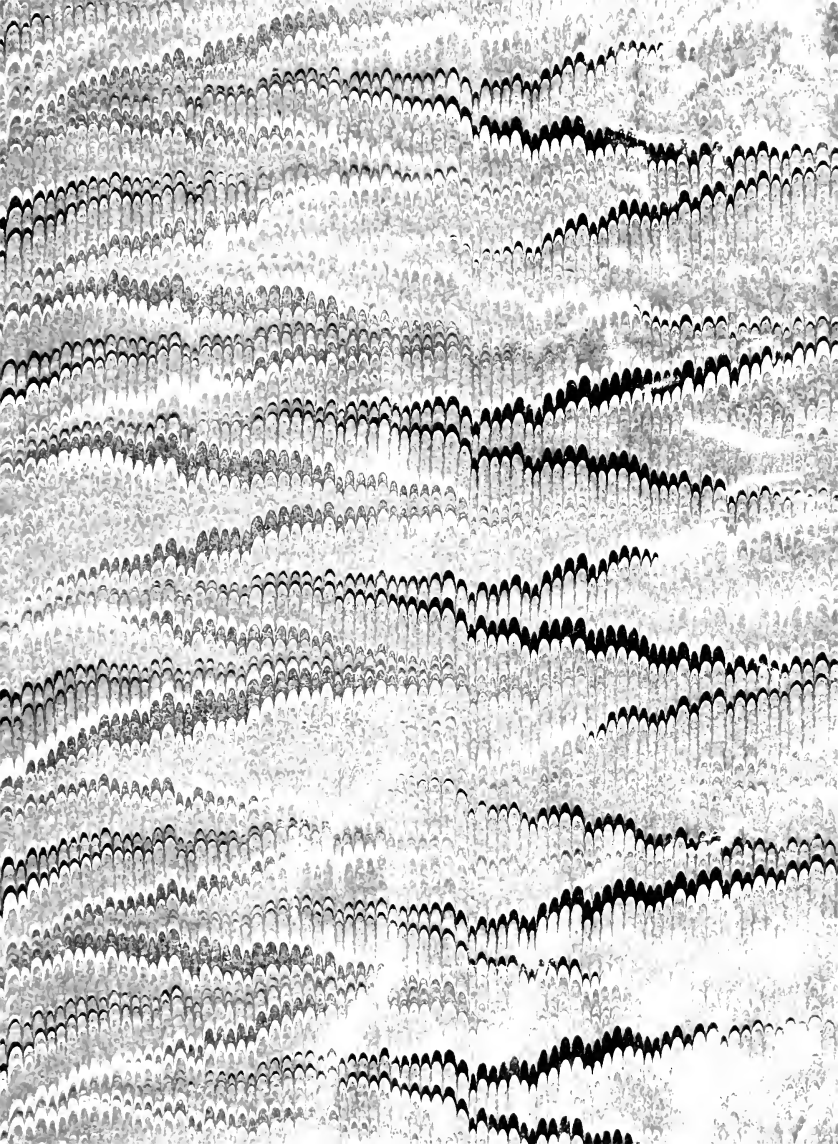

ON THE DEVELOPMENT OF HAMAMELIS VIRGINIANA L.

A DISSERTATION

Submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirements for the Degree of Doctor of Philosophy,

by

Daniel Naylor Shoemaker.

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Contents.

Acknowledgment.

This work was undertaken at the suggestion of Dr. D. S. Johnson and was carried out with his guidance. The author wishes to express his sincere thanks to him for sympathetic guidance and instruction. His thanks are also due to Professor Brooks for much kindly instruction, help and advice. He is also indebted to Professor Howell, Dr. Andrews, Dr. Dreyer, and Dr. Drew, for advice and instruction. He wishes moreover to express the sense of personal loss he feels from the death of Dr. Humphrey, under whom his botanical work began.

ON THE DEVELOPMENT OF HAMAMELIS VIRGINIANA L.

Introduction.

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This work on Hamamelis was undertaken on account of its peculiar habit of flowering. It is one of the few angiosperms whose flowers open in the fall, and which 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 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 Fleteren (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 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(10) on page 392, that this 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 misleading in the case of British Amentiferae. It does not fit 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 the May of the next year that the embryo begins to form. Colchicum autumnale and the autumnal species

of Crocus have the same flowering habits as Hamamelis virginiana, but if Hofmeister's account of the first should also hold for the second, they differ essentially from Hamamelis in the behavior of the pollen tube. The other genera named as having a longer or shortened period are all pollinated in the spring so that their resting period 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 Aspidium 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, and thence to Central America, and up the Mississippi river to southern Indiana. The third is found from Virginia to Flor-

ida, 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. Fothergilla is representes 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 Par-

rotia are found in the Dakota Group of the Cretaceous (9) and that Hamamelis 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 thus has a very restricted range and seems to seek the best positions in which to procure moisture and prevent transpiration.

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, Md., from northern Virginia, from Long Island, and from south-western Ohio. I am indebted for exotic forms to the kindness of Mr. George 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 subli-

mate-acetic mixture made by adding five per cent. glacial acetic to a saturated aqueous solution of $HgCl_2$. This was often used hot. The material was cleared in xylol and imbedded in parafine. Sec - tions were cut from five to fifteen mikrons in thickness. For staining a combination of Haemalun and Bismark Brown was tried, but Flemming's Triple was found to be more satisfactory and was used al- most exclusively. It was necessary before the young carpels could be sectioned in parafine to carefully and laboriously remove the hairs from their bases, on account of their thick walls which could not be cut in parafine, but would invariably tear the sections. To avoid this necessity Celloidin imbedding was us- ed in a few instances, but it was impossible to get the sections thin enough for most purposes by this

method.

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 (Figs 2a-2b). These hairs develope from a single epidermal cell, which protrudes from the surface and is cut by anticlinal walls into from four to twenty cells, each of which sends out a long process (Figs. 1a-1b) making a many-armed star. They are often raised on a slight multicellular papilla.

Each bud produces a head of from 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 unfold the other two bracts come in 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 develope. The outer cycle opposite to the lobes of the calyx, becomes stamens, the inner sterile staminodes.

The torus has by this time, by unequal growth, become concave, and on its floor are developed two horse-shoe shaped ridges, one anterior and one pos-

terior. 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 developed 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. In the course of some hundreds of carpels investigated I have found but a single one with two ovules. Baillon (17) also speaks of Hamamelis as being polygamous; of this I have seen no evidence in my materi-

al. It is possible that these conditions may occur more frequently in places from which I have no material, or under 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 hair. Fig. 16 shows how the rudiments of the growing flowers 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 is best performed by the hairs on the tips of the sepals and bracts, and the bases of the carpels. These young hair cells are long and tortuous, and wind among the growing rudiments. Their cavity is full of sap, and they have very active nuclei. (Fig.3) They also remain in this active stage

longer than hairs on the other parts of the plant. As they grow older all the hairs acquire thick cell-walls and each hair cell tends to straighten. In their mature state they function 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 early begins to form two pollen-sacs. There is apparently no evidence of the presence at any stage of the other two microsporangia which are usually found in Angiosperms. The sterile rudiments which develope into nectaries do not make a beginning at pollen formation.

The first evidence of the formation of arche-

sporium is found about the middle of June. The sub-epidermal layer divides at the place where the two pollen sacs are to be formed by periclinal walls.(Fig. 4) 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 these a moderately well-defined layer of tapetum, and the outside wall of the pollen-sac is three or four cell layers in thickness (Fig.5). 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 archesporium and tapetum. 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. (Fig. 6). The nuclei of the pollen mother-cells increase in size both absolutely, and as compared with the size of the mother-cell. The nucleoli also increase notably, and the contents of the cell become more largely vacuolated (Fig. 7). The pollen mother-cells thicken their walls, and soon float freely in the cavity of the anther. The two tetrad divisions occur in very quick succession, almost simultaneously (Fig.8). In this division the nuclear processes do not show at all clearly, though there is evidence of striae connecting the nuclei. The walls of the separate pollen grains soon develope, and the grains are released by the using up of the mother-cell walls. The mature grain was described by Von Mohl in 1833 where he gives it a very short characterization. It is shaped as an oblate spheroid, with three meridional furrows (Fig. 9)between these furrows the

surface is covered by a fine reticulation (Fig. 10). 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. 11, 12).

Soon after the pollen grain is freed from the mother-cell its nucleus divides, and the smaller nucleus which is here called the generative nucleus in accordance with other Angiosperms, 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. 11). 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. 12).

Of the four layers in the pollen sac wall only the subepidermal layer has any part in the opening of the anther. This layer is made into the fibrous layer. It covers the whole introrse face of the anther, and over the whole surface of the pollen sacs. The first, and for a long time the only evidence of the formation of this layer is the radial lengthening of the cells. At this stage it is possibly not inappropriate to digress for a single 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 cut off short secondary epiderm-

al cells at their outer ends, as shown in Fig. 13). 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 fibres at the proper time. Here then the epidermis seems to have been more plastic than the tissue under the injured spot, and whatever influences are at work on the subepidermal layer are also exerted on any cells which occupy this position.

The fibres are developed in this layer shortly before the time that the anther is to open. They are developed on the side and bottom of each cell. Around the top, posterior and bottom of the pollen sac, there is a groove (Figs. 14, 15). In the bottom of this groove no fibres are developed, and the cells moreover become quite thin-walled, so that they readily break, and thus form the line of dehiscence of the anther. As Leclerc du Sablon (8) has shown for anthers in gen-

eral, 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 liquified fibres. By this means the whole outer covering is bent on itself and points directly toward the carpels 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-fertilization, but no well-developed mechanism to prevent self-fertilization. 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 weath-

er, 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 be developed, but it is very difficult to distinguish archesporial cells. There seems to be several of these, however. They each cut off a tapetal cell above, 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 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 from three to five macrospars 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. 17). The antipodals very early disappear so that they are hard to find at the time of fertilization. The endosperm nucleus is found at about the middle of the sac.

The tissue of the nucellus surrounding the mature embryo-sac is much disintegrated (Fig.17). Around the chalazal end of the sac the tissue is always very deeply staining and there is a quite evident strand of conducting tissue from the tip of the fibro-vascular bundle at the chalaza to the lower end of the embryo-sac (Fig. 17). 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. 18. 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,

and then leave a wide open micropyle (Fig. 19). This is finally closed into 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 its thickness. 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 sixteen per cent. sugar solution, made with tap water, in which one and one-half per cent. gelatine was dissolved. The pollen grains first be-

came spherical by the filling out of the furroughs, thus using the masses of intine on the inner sides of the furroughs (Fig. 20). Tubes sprout out very soon after being placed in the nutrient gelatine, in from 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 moulds, 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. No more than two nuclei were ever found in a tube. The tubes showed a marked tendency toward the formation of cellulose plugs as shown in figure 21. 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. 12) 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 was withdrawn including one or both nuclei, when a wall was formed behind. This completely closed 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 the same 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-tem-

perature whenever the flowers opened. A collection made in Ohio early in January after the unopened buds had endured a week of very cold weather, with the mercury as low as - 15° Fahr., sprouted in a seemingly normal way, though not so vigorously as earlier. I am inclined to think that pollen shed so late never fertilizes, however.

Conductive Tissue of the Style and Ovary.-

By the folding of the carpels each style has a groove formed between the edges of the folded carpel. This groove leads from the stigma to the ovary, it is open at the top, where its sides are slightly separated and its inner surface thus exposed, bears the loosely arranged pappilose cells of the stigma. The epidermis of this groove, and two subepidermic layers continue this stigmatic tissue down to the base of the fun-

iculus, the strand of conducting cells getting gradually deeper and deeper in the tissue of the style. The cell-walls of this strand are thickened, partly gelatinized and the contents of the cells are dense. Fig. 23 shows its appearance and position about midway in the height of the style. When the flower first opens the epidermis of the funiculus is not yet differentiated (Fig. 24), but as winter approaches, the base of the funiculus becomes glandular. The epidermal cells enlarge, the walls thicken and the contents become vacuolated.(Fig. 25). 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 mucous which fills the small remaining cavity around the base of the funiculus (Fig. 17).

- The Descent of the Pollen Tube.

The pollen grains begin growth very shortly after be-

ing shed on the stigma, and the growth is at first comparatively rapid. Its course is readily traced in the conductive tissue of the style, which is greatly disorganized from 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 live part of one or more tubes is to be found in the neighborhood of the base of the funiculus (Fig. 18). There are usually several live tubes at varying heights in the style at this time,(Figs. 26,27) 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 each blossom. The inverted calyx then very effectually protects the

carpels from rain and other forms of moisture.

The pollen tubes found at this time are usually of greater diameter than at the beginning of growth. Tubes grown in sugar-gelatine solutions are from five to eight mikrons in diameter, and those which sprout on the stigma are at first approximately of the same size. But those found during the resting stage are from twelve to fifteen mikrons in cross-section. The wall is also thicker than at first (fig.28). The nuclei found have not exceeded two in any tube. There seems some evidence from figure 18 that the division of the generative nucleus has taken place at this stage

When growth is renewed in the spring the area of conductuve tissue on the funicules 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. 19). 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 down the tip of the nucellus through the tissue derived from the tapetal cells, which stains deeply at the time and is probably conductive tissue. The transferrence 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. This begins immediately to di-

vide. 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 endoderm and nucellus grow rapidly from this time forward. The endosperm early disintegrates the neighboring nucellar tissue except in 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. 19). It is finally absorbed, however.

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 takes up blue stains very readily. This layer is the only part of the nucellus that permanently resists the action of the endosperm (Fig. 30), 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 (Fig. 30). It is thrown into folds shortly before the ripening of the seed.(Fig.31) The endosperm is finally stored with food in the form of proteid grains. These, shortly before ripening show numerous globoids (Fig. 32) which disappear later. The ripe endosperm contains in its cells much oil along with proteid material.In the cell walls are imbedded

numerous crystals of calcium oxalate (Fig. 33).

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. (Fig. 34) The egg-cell 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 cut off (Fig. 35). 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 swung from the suspensor in a disintegrated mass, which fills a

cavity in the central part of the developing seed. At maturity there is a straight axial embryo which extends from end to end of the seed and which is richly stored with oil and proteid material in all its cells The upper side of the cotyledons has already a well-developed palli sade layer. (Fig. 36).

Integuments -

The inner integument is at the beginning three cell layers in thickness, the inner of these three layers takes part in the formation of the inner seed coat. It early becomes filled with dense contents which stain blue readily (Fig. 31). This finally shrinks and becomes applied to the inner cell wall. Fig. 30). 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 begin then to thicken and the whole integument forms the outer seed coat which is moderately hard, black, and very resistant to water. This 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 ovate in shape but very decidedly pointed at the lower end. This sloping of the lower end is of use in discharging the seed.

The Carpels -

At blooming time the carpels are very slightly imbedded in the tissue of the torus (Fig. 26). There is a very short calyx tube, however, shown in this figure

below the attachment of the anther. As the fruit matures this calyx tube lengthens proportionally more than the carpels, and this gives the fruit the appearance of being half buried in the torus (Fig. 37). A longitudinal section (Fig. 38) shows that this is only apparent and that the fruit is only very slightly buried.

The substance of the carpels develops into two kinds of tissue; the outer half becomes fleshy with numerous roundish stone cells. The inner layer of each carpel is developed into an apparatus for expelling the seed. In each carpel this layer is formed in two halves. These are not closed at the top, and are higher toward the posterior of each carpel. This is shown in side view in figure 4s. These halves are never closely joined on the inner sides of the carpels, and there is provision for a split in the region of the

midrib also. The cells are developed into fibres 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 figure 39 after opening of the fruit in figure 40. 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 assist greatly in the process. The seeds are often thrown to a distance of twenty feet. This movement is caused by drying, as

can be proven 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 lie on the ground for two winters, according to Baily (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. 41), they are then freed

and exposed as green assimulative 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 have been in the temperature of an unheated room constantly, but had not been subject to frost. They had never been allowed to dry out.

Hamamelis arbora.

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 Zuccariniano, flowers as early as in February, and thus approaches the flowering time of the American species.

The flowers are in about the same condition in October as in H. virginiana, except that the stamens were rather backward. The pollen grains were free and had each two free nuclei, and evidently pass the winter in that stage (Fig. 42). 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 had a tuft of hairs on the tips.

In other respects the two genera were much alike in their development so far as studied.

Fothergilla Gardeni -

An incomplete series of stages of this was studied. Its flowers appear in the spring, along with the

leaves. It lacks a corolla; its calyx tube is much longer than that of Hamamelis. It has from five to seven very small calyx teeth. The development of the stamens is as described by Baillon (3). They arise first as five single rudiments, which are followed by other rudiments on either 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 are quadrilocular 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

Stadler.

CORYLOPSIS PAUCIFLORA.-

Only one stage of this was examined. It was obtained in the spring before the flowers open. Its flowers open before the leaves appear in the spring. The flower has five sepals, five petals, five stamens, and two carpels. They are borne on drooping racemes, with many bracts. These bracts are smooth on the outside but covered by silky hairs within. The structure of these hairs is much like that in Hamamelis, except in stiffness.

The stamens, which are quadrilocular pass the winter containing nearly mature pollen grains, (Fig.43) with two free nuclei. The ovule is in the stage in which Hamamelis passes the winter (Fig. 44). It is

difficult to determine whether there is present a definitive macrospore or a macrospore mother-cell. There is no evidence of the presence of more than one macrospore mother-cell however. 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 here 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 are quadrilocular and open by slits,(Fig. 40). 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 surmised to be both petals and stamens. Pollen is sometimes developed in them which is evidence of their staminal nature. (Fig. 46)

The carpels, which occur in pairs as in Hamamelis, are collected into large heads containing from 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 can not be called perisperm (Fig. 47). The macrospore is buried about as deeply as in Hamamelis. It germinates only in the lower ovules, the upper ones never getting 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. 47) grows into a wing. The embryo is straight, and bears about the same relation to the amount of endosperm as in Hamamelis.

Summary and Conclusion.

1. In Hamamelis the anthers are bilocular 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, a smaller size and thinner walls appearing in the next stage.

5. There are several macrospores developed, only one

of which becomes functional. It is [illegible] buried in the nucellus by the growth of tapital tissue.

6. The terminating macrospore is nourished through a strand of conducting 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 resists the dissolving action of the endosperm for a time.

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 is furnished with a short suspensor.

11. The seeds normally sprout after lying on the ground

for two winters.

12. The hairs serve a two-fold function, while young to keep the growing tissues moist, and when grown 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 species of the family investigated all have a resting stage of the pollen, though it is much shorter.

15. The other genera of the family all have granular anthers.

In comparing Hamamelis virginiana with its relatives, it seems certain that it was once a spring flowering plant, and that it has worked backward through the winter. It differs from H. arborea essentially in

t[illegible] pollen stops in the winter, but 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 that that this resting period can not be recorded as a primitive character in any case. I have no suggestion to offer as to the use of this resting period to the plant.

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Explanation of Figures.

All Figures were drawn with Zeiss Camera Lucida, and are of Hamamelis virginiana, unless otherwise stated.

Fig. 1a - 1d. Development of epidermal hair taken from sepal x 440

Fig. 2a - Mature hair in cross-section x 440.

Fig. 2b - Mature hair in Longitudinal section x140

Fig. 3. - Hair from tip of sepal X440.

Fig. 4. - Growing stamen. Longitudinal section x1090, beginning of archesporial development.

Fig. 5. - Growing stamen cross-section x1090. Archesporium. Tapetal layer. Thickness of anther wall.

Fig. 6. - Anther wall x1090

Tapetal layer. Glandular layer. Tetrads.

Fig. 7. - Anther x1090

Tapetum whose nuclei have dissolved. Pollen mother-cells.

Fig. 8. - Tetrad division x1090

Fig. 9. Mature pollen grain x1090

Fig. 10. - Reticulation on surface of Pollen grain x1000.

Fig. 11. - Pollen grain. Equatorial section. x1090

Fig. 12. - Pollen grain. Equatorial section x1090

Generative nucleus free in cavity.

Fig. 13. - Anther wall showing regeneration of Fibrous layer x440.

Fig. 14. - Mature anther. Longitudinal vertical section. x40. Fibrous layer and line of dehiscence.

Fig. 15. - Mature anther, cross-section x140. Fibrous layer and line of dehiscence.

Fig. 16. - Growing flower bud. x 44. Showing manner of packing rudiments, and protective coating of hairs.

Fig. 17. - Longitudinal section of ovule x 194. Mature embryo-sac, and chalazal conducting strand.

Fig. 18. - Ovule at beginning of winter and hibernating pollen tube x 440.

Fig. 19. - Ovule in spring. Growing pollen tubes x 440

Fig. 20. - Pollen grain swollen by water x 440

Fig. 21. - Pollen tubes with cellulose pulse x 440

Fig. 22. - Spherical protuberance on pollen tube x 1090

Fig. 23. - Section of style showing conductive tissue x 440.

Fig. 24. - Section of base of funicules at time flower opens x 440

Fig. 25. - Same section at beginning of winter x 440.

Fig. 26. - Section of winter condition of flower, x 18.

Fig. 27. - Section of flower, [illegible] path. x 40.

Fig. 28. - Hibernating pollen tube x 1090.

Fig. 29. - Embryo-sac. Endosperm. Pit at side. x 70.

Fig. 30. - Section through seed coats. x 440.

Fig. 31. - Section of nearly ripe seed coat. x 440 also epidermis of nucellus.

Fig. 32. - Endosperm cell stored with food. x1090

Fig. 33. - Endosperm cell walls with crystals. x 1090.

Fig. 34. - Embryo-sac at first division of [illegible]. x 70.

Fig. 35. - Embryo and suspensor. x 1090.

Fig. 36. - Cotyledons showing palisade. x 140.

Fig. 37. - Ripe fruit. x2.

Fig. 38. - Ripe fruit. Longitudinal section. x 2.

Fig. 39. - Cross-section opening layer of capsule. x 4.

Fig. 40. - Same after opening. X 4.

Fig. 41. - Growing seedling in seed coats. x 1.

Fig. 42. - Pollen grains, H. arborea x 1090, winter condition.

Fig. 43. - Pollen of Corylopsis pauciflora Section . Winter condition. x 1090.

Fig. 44. - Ovule of Corylopsis pauciflora in spring x 1090.

Fig. 45. - Cross-section mature anther. Liquidambar styraciflua. x 70.

Fig. 46. - Nectaries Liquidambar with pollen.
x 70.

Fig. 47. - Seed of Liquidambar. x 9. Nearly ripe.

Fig. 48. - Side view of opening layer of carpels
x 2. Hamamelis virginiana.

VITA.

Daniel Taylor Shoemaker was born [illegible] March 2, 1871, daughter of Daniel Kinley, near Fair Haven, Ohio, on the 15th of November, 1869.

He was taught by public school teachers of his home district and entered Earlham College, Richmond, Indiana, as a preparatory student in the fall of 1889. Graduated from thence with the degree of Bachelor of Science in 1894, having been absent from college for one year. Attended Johns Hopkins University as a graduate student during the year 1895-96. Was superintendand of the High School of his native place from 1896 to 1899. Has since been a graduate student in Botany at Johns Hopkins University.

Was Assistant in Cryptogamic Botany at the Cold Spring Harbor Summer Laboratory in 1900. Assistant in Biology, Johns Hopkins University, 1900-1901. Fellow in Biology 1901-1902. His subordinate subjects are Zoology and Physiology.