# PARTHENOCARPY INDUCED BY POLLEN EXTRACTS[1]

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PARTHENOCARPY, unlike parthenogenesis, is a term infrequently used; yet the former phenomenon is perhaps the more prevalent of the two. Bananas, seedless grapes, grapefruit, oranges, and cucumbers may be cited as common illustrations of parthenocarpy. But these are not the parthenocarpic fruits in which the writer is interested. From numerous genetical studies we are familiar with another type of parthenocarpy. Sometimes when cross pollinations are made between species or genera, no seeds are produced, but the ovary develops into a fruit. What causes these fruits to develop? Usually it is considered, perhaps without much thought, that the growth of the ovary into a fruit is the result of the development of an embryo and the resulting seed. But obviously that cannot be the explanation of the parthenocarpially formed fruits.

Since 1902, when Massart placed dead pollen on the stigma of an orchid and observed a slight enlargement of the ovary, attempts have been made to discover what causes the ovary to develop into a fruit when no embryos are formed.

In 1909 Fitting experimented extensively with tropical orchids at the Botanical Gardens at Buitenzorg. He made detailed observations on the normal responses of the orchid flowers to pollination and compared with them the responses produced when dead or foreign pollen or pollen extracts were placed on the stigma of a flower. The normal responses were a closing of the stigma, a shortening of the life of the flower, an enlargement of the gynostemium, an enlargement of the ovary, and sometimes a change in the color of the corolla. He found that mechanical injury shortened the life of the flower to about the same extent as pollination or the placing of dead pollinia on the stigma. Dead or ungerminated pollen on the stigma caused it to close, and usually a swelling of the gynostemium also took place, but neither brought about the swelling of the ovary. He made cold and hot water extracts of pollinia and found that, when placed on the stigmatic surface, extracts from some species caused a slight swelling of the ovary, but extracts from other species had no effect upon the ovary. Some of these extracts also caused color changes in the corolla. He further found that absolute alcohol extracts were as effective as water extracts. He found active substance only in the viscid material holding the individual pollen grains together and thought it might also be produced in the pollen tubes; he considered it to be absent from the pollen grains. Pollen masses of *Hibiscus Rosa sinensis* were also effective in closing the stigma and shortening the life of the blossom. In later work (1910) he found that in some orchids pollination caused a lengthening of the life of the blossom. This work led him to believe that the active substance was soluble in water and absolute alcohol but not in petroleum ether, sulfuric ether, or chloroform. He believed further that it contained no nitrogen and that it was not an enzyme.

Morita (1918) found that pollen from several orchids extracted with hot or cold water, absolute alcohol, or ether gave a substance which caused a closing of the stigma and a slight enlargement of the gynostemium, but which had no effect upon the ovary. The ether and alcohol extracts were less effective than the water extracts. Glycerine was not a solvent for the active substance.

Laibach (1932) has repeated some of Fitting's work and obtained essentially the same results.

Yasuda (1934a) found that water extract of *Petunia* pollen injected into the ovaries of egg plants caused them to grow from 4.9 × 5.6 mm. to 41.1 × 73.0 mm. Three out of 37 ovaries treated grew. He also found (1934b) that water extract of *Petunia* pollen injected into the ovaries of tobacco flowers caused them to grow from a size of 2.8 × 4.66 mm. to 3.68 × 5.68 mm. Two ovaries out of 20 injected grew this much; others showed no increase. In still further work (1935) he found that the cucumber develops seedless fruits when pollinated with its own old or very young pollen and with pollen from other cucurbitaceous plants or the sunflower. In cases where fruit developed, the pollen tubes grew into the style but did not reach the ovary. Water extracts of cucumber pollen injected into the cucumber ovaries caused 3 fruits to be produced out of 50 injected ovaries. These fruits were 4.3 cm. in diameter and 20.3 cm. long. No seeds were produced.

Recently the writer (Gustafson, 1936) caused fruits to develop parthenocarpically in several species of plants by treating the pistils with known chemical compounds. Normal-sized fruits developed in tomato, *Petunia*, and *Salpiglossus* when the pistils were treated with indole-3n-propionic acid, but no seeds were produced. Similar treatment initiated growth in the snapdragon ovaries, but did not produce mature fruits. Phenylacetic acid caused seedless tomato fruits to develop and slight growth in snapdragons, but none in tobacco. Indole-acetic acid caused seedless fruits to be formed in the tomato, *Salpiglossus, Petunia, Begonia*, pepper, and eggplant. The same substance initiated ovary growth in the snapdragon, *Zephyranthes carinata, Agapanthus umbellatus*, crookneck summer squash, and Hubbard squash, but not in cucumber and watermelon. Indole-butyric acid caused seedless fruits to be formed in tomato, *Salpiglossus, Petunia*, crookneck summer

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squash, *Begonia*, pepper, and eggplant, but produced only slight growth in the ovaries of Hubbard squash, cucumber, and watermelon.

It is thus evident from the literature that hot and cold water, ether, and absolute alcohol extracts of pollen from various plants contain a substance or substances which, when placed on the stigma of a pistil or injected into the ovary, causes some of the changes to take place that normally occur when pollination and fertilization are consummated. It is further evident that indole-3n-propionic, indole-acetic, and indole-butyric acids and phenyl-acetic acid when applied to the pistils of flowers in many cases cause seedless fruits to be formed, and in other instances cause only a slight growth.

PROCEDURE.—The writer has been very much interested in extending this work to other plants and in obtaining a greater development of the ovary. Thimmann (1934) found that growth hormones are more readily extracted with freshly distilled chloroform than with water. His method of extraction was therefore followed by the author. Injecting the extract into the ovary as practiced by Yasuda was tried but given up, because it seemed that the injury caused was too great. In most cases the ovary decayed around the wound. Instead, the chloroform was evaporated, and the residue mixed with lanolin according to the method used by Laibach (1933). Just enough lanolin was used to make a paste with the residue, which in some instances was quite hard and brittle, in others soft, in which latter case only a minute quantity of lanolin was needed. This paste was placed on the stigma or on the cut surface of the style just above the ovary, the upper part of the style being removed to reduce the distance the substance would have to diffuse, except when the style was short, in which case it was left intact and the paste was placed on the stigma. Only flower buds that had not opened and in which the anthers had not dehisced were used. Two kinds of controls were used. In one the style was cut and no further treatment carried out; in the other the cut surface of the style was smeared with pure lanolin. Some flowers were pollinated to note the rate of growth, size, and other characteristics of the normal fruits.

The treated flowers were not covered with bags to prevent pollination because in the greenhouse there was, of course, no need for such precautions. In some carefully conducted experiments in which pollen was placed on the cut surface of the style there was no development of the ovary to indicate that fertilization had taken place. In all of the plants used in this experiment, except in the peppers, the style was cut. In treating the peppers, the petals were removed, so that no insects would be attracted to the flowers. No seeds were found in the fruits developed by the treatments. From all of these facts it seems certain that accidental pollinations could not have taken place.

In these experiments pollen was used from *Corylus americana, Salix sp., Thalictrum dioicum, Petunia hybrida, Zea mays, Pinus ponderosa, Althaea rosea* (hollyhock), *Cucurbita maxima* (Hubbard squash), and *C. moshata* (crookneck summer squash), and the plants used for the ovary development were *Salpiglossus variabilis, Petunia hybrida, Nicotiana Tabacum* (Maryland Mammoth variety), *N. Tabacum* × *N. glaucum*, hybrid, *Cucurbita maxima* (Hubbard squash), *C. moschata* (crookneck squash), *Solanum melongena* var. *esculentum* (eggplant), *Capsicum fruticosum* (pepper), *Citrullus vulgaris* (watermelon), *Lycopersicum esculentum* (tomato), *Antirrhinum majus*, and *Cucumis sativus* (cucumber). These plants were chosen either because the flowers were unisexual or the stamens were large and could easily be removed. Many of these plants were grown in the field, but others were grown in the greenhouse.

RESULTS. — No results were obtained with the pollen of *Corylus* and *Salix*. They were the first ones tried, and the technique may have been faulty.

*Thalictrum dioicum.* — The pollen was collected May 13 and dried. Some was extracted at once and used on tomato, snapdragon, and one tobacco flower. The results were all negative. The rest of the pollen was stored in a desiccator in the dark at a temperature of 15°C. until the following November. The extract prepared then was used exclusively on Maryland Mammoth tobacco. The results are somewhat in doubt. At the time the tobacco plants were treated with *Thalictrum* pollen extract, the ovaries treated with pure lanolin also showed a slight enlargement. The growth of the ovaries treated with *Thalictrum* pollen extract was on the whole somewhat greater, yet there was little growth in either. It is exceedingly difficult, therefore, to estimate the amount of enlargement in these plants. The ovary did not grow uniformly, a swelling appearing where growth took place, which resulted in a much wrinkled ovary (fig. 1, 4). At no other time did ovaries treated with pure lanolin show any increase in size. The only explanation that can be offered is that the lanolin had been contaminated with some substance which acted as a growth promoter.

*Petunia hybrida.* — In a preliminary experiment *Petunia* pollen was used in several cross pollinations. Only with a hybrid of *Nicotiana tabacum* × *N. glaucum* was there any result. With this plant a high percentage of flowers pollinated produced fruits which matured and dehisced but had no seeds.

A rather small quantity of *Petunia* pollen was obtained in August from plants grown in the field. Pollen from several cultures, genetically different, was secured and kept separate. Two of these cultures were treated with their own pollen extract, but no growth resulted. On August 11, 110 flower buds of the pepper were treated with *Petunia* extract. Of these 110 flowers one developed into a fruit that was normal except for its lack of seeds and poorly developed placenta (fig. 2). During the whole summer not one control developed into a fruit. Out of 15 eggplant blossoms treated with *Petunia* pollen extract one developed into a fruit, which was 7.0

cm. in diameter at the time of harvesting in late September. Several other ovaries grew to a size of about 2 cm. in diameter. These fruits were all growing at the time of harvesting. No controls grew.

*Zea Mays.*—Pollen was collected during the summer and stored in a desiccator at 15°C. until the middle of October, when an extract was made. Forty-five *Salpiglossus* flowers were treated with maize pollen extract on October 12. Of these at least 50 per cent showed some growth and about 10 per cent grew to about half the size of the normal fruits.

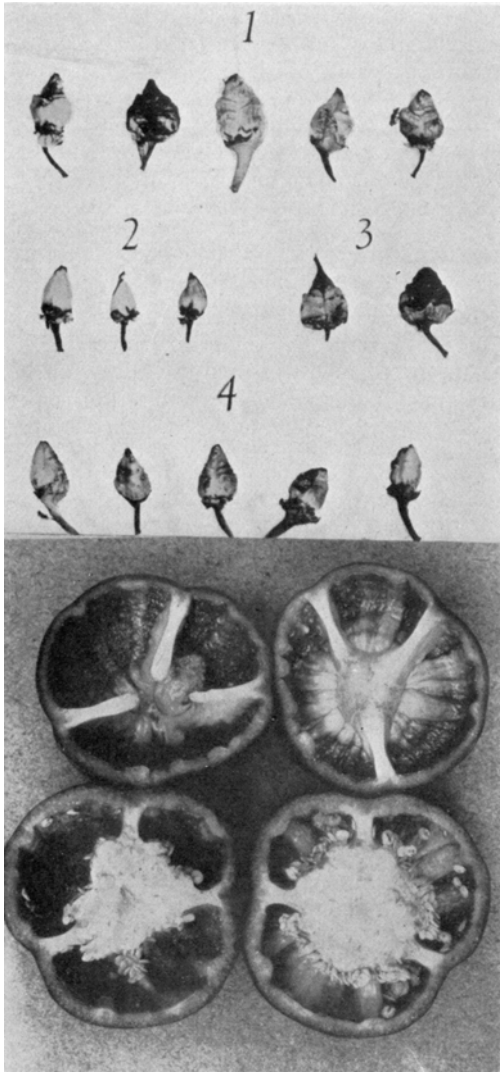


Fig. 1. Photograph of ovaries of Maryland Mammoth tobacco treated (1) with Hollyhock pollen extract, (2) with pure lanolin, (3) with *Pinus ponderosa* pollen extract, and (4) with *Thalictrum dioicum* pollen extract.

Fig. 2. Photograph of the cross section of pepper fruits, about half natural size. The fruit without seeds was produced parthenocarpically by placing lanolin containing an extract of *Petunia* pollen on the stigma of the flower. The fruit with the seeds is a normally produced fruit.

At first the treated ovaries grew as rapidly as those of the pollinated flowers, but after a week or two they slowed down and soon ceased growing entirely. It is possible that the supply of growth substance was exhausted and none was being produced. More *Salpiglossus* flowers were treated, and for the most part they increased to about one-third the size of the normal fruits and were all wrinkled like those produced earlier by indole compounds (Gustafson, 1936). Controls with pure lanolin did not grow.

Some pepper and tobacco flowers were also treated with maize pollen extract, but all the flowers dropped off within a few days without any sign of enlargement of the ovary.

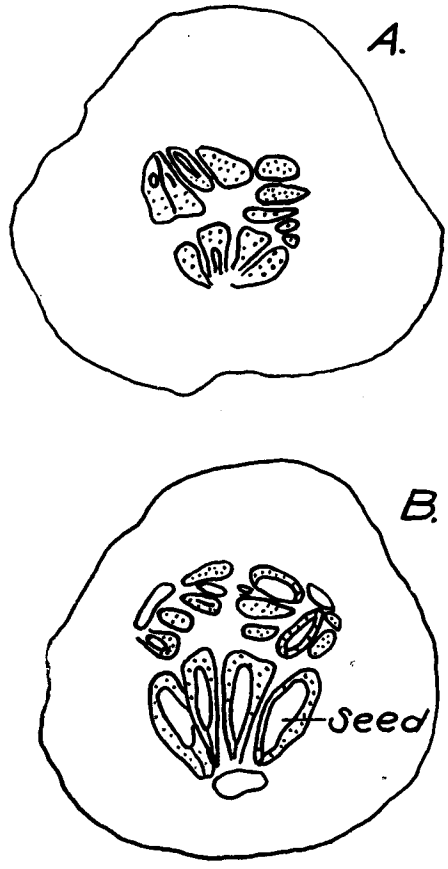


Fig. 3. (A) A parthenocarpic cucumber fruit produced by treating the ovary with pollen extract from Hubbard squash. (B) Normal fruit. Both are natural size.

*Pinus ponderosa.*—A large quantity of *Pinus ponderosa* pollen was collected in May and stored in a desiccator at 15°C. until October. Several extracts were made, but only the last extract seemed to give any results. Thirty-five flowers of *Salpiglossus* were treated with extracts of pine pollen, and when examined a month later, all but one ovary had grown considerably. A large number of tobacco flowers were also treated with this extract, resulting, as illustrated by figure 1, 3, in some growth of the ovaries as compared with the lanolin treated controls (fig. 1, 2). Indifferent results were obtained with *Petunia*, and

results very definitely indicating no growth were obtained with peppers.

*Althea rosea* (Hollyhock).—Considerable hollyhock pollen was collected during the summer. Part of this was used at once and part stored in a desiccator at 15°C. until the fall. *Petunia* flowers treated with chloroform extract of hollyhocks during the summer showed a decided ovary growth, their ovaries remaining green much longer than the untreated ones and enlarging considerably. Only four blossoms of *Salpiglossus* were treated with hollyhock extract, and they all grew slightly. A large number of tobacco flowers were also treated with this extract, most of them showing some growth (fig. 1, 1).

*Curcurbita maxima* (Hubbard Squash).—Only a small amount of Hubbard squash pollen was obtained. Twelve cucumber flowers were treated with this extract, and one externally normal but seedless fruit (21 cm. long and 5 cm. in diameter) was obtained (fig. 3), as well as a few that grew only slightly. The average size of 19 ovaries of crookneck squash treated with Hubbard squash pollen extract was a little greater than the average of the control ovaries (table 1).

*Cucurbita moschata* (Crookneck Summer Squash).—Considerable pollen was obtained from these plants, and several extractions were made. For the most part, the Hubbard squash flowers treated with this extract showed no growth of the ovary, but there were a few exceptions to this rule in which the ovary about doubled in size. Fifty-eight cucumber blossoms were treated with pollen extract of crookneck squash. Of these, one increased to 6 cm. in length and 2.2 cm. in diameter, and many others grew to about double the size of the original ovary, but none to full size. The ovary of the crookneck summer squash used in these experiments frequently grows considerably even when no fertilization takes place. Therefore, in order to obtain accurate results, it was necessary to measure ovaries from unpollinated flowers at the time they were ready to drop off, and the same was done with the treated flowers. Table 1 gives these results in centimeters.

While the average increase in the experimental ovaries over the controls is not great, it is, nevertheless, large enough to be significant in showing that extract from the pollen of crookneck summer squash causes enlargement of the ovary of the same species. This statement will be further strengthened when it is mentioned that one fruit was 44.0 cm. long, the basal diameter 6.7 cm., and the apical diameter 5.0 cm. This was practically the size of a small normal fruit except that the apical diameter was not more than half as large as that of a normal fruit. One hundred and sixty-four *Petunia* blossoms were treated with the extract from the crookneck squashes, but none of the ovaries showed any increase in size.

Table 1. *Growth of ovaries of crookneck summer squash.*

| | Length | Basal diameter | Apical diameter |
|---|---|---|---|
| Control — 37 plants | 14.20 ∓ .74 | 2.10 ∓ .16 | 2.00 ∓ .09 |
| Crookneck squash extract | | | |
| No. 1 — 49 plants | 15.10 ∓ .59 | 2.64 ∓ .15 | 2.05 ∓ .25 |
| No. 2 — 16 plants | 19.01 ∓ 2.11 | 2.80 ∓ .09 | 2.21 ∓ .25 |
| Hubbard squash extract | | | |
| 19 plants | 15.4 ∓ 1.22 | 2.30 ∓ .19 | 1.92 ∓ .15 |

Discussion.—These experiments supply indubitable evidence that there is present in the pollen of some plants a substance (or substances) which initiates and sometimes causes a continuation of the growth of ovaries into fruits. The experimental evidence indicates that this substance (or substances) is present in much greater amount in some pollen than in others. Another interesting thing is that the ovaries of some plants are much more readily acted upon than others. Does this indicate a certain amount of specificity? Or is it merely a matter of employing the proper technique? The matters of diffusion and penetration are undoubtedly factors of considerable importance. Injecting the extract into an ovary would seem to be a very simple method of getting the material in place to act, but the injury to the plant in the writer's experiments appeared to be quite great. On the other hand, placing the active substance mixed with lanolin on the stigma or on the cut style seems to be much more natural. The substance has a chance to diffuse basipetally into the ovary in a way not dissimilar to that in pollination. If a sufficient concentration is obtained, it seems that there should be no reason why growth should not be initiated. If once initiated, why does growth stop? Does this mean that normally the developing ovules continue to supply the growth substance? Dollfus (1936) answers this question in the affirmative. He has found that the ovary wall of several plants which he studied contained very little growth hormones, while the ovules and presumably the placentae contained considerable quantities. Further experiments showed that when the interior—i.e., the ovules and placentae—was removed from an ovary it stopped growing. When, on the other hand, the cavity in such an ovary was filled with lanolin paste to which either indole-acetic acid or orchid pollinia had been added, the ovary made nearly normal growth. From these experiments Dollful concludes that the ovules produce growth substances which cause the ovary to enlarge and grow into a fruit. The writer had a similar idea, and he made a chloroform extract of the interior of crookneck squash and injected an aqueous

TABLE 2. *Summary of results.*

| Pollen extract of | Pollen, fresh or dried (age if dried) | Species to which extract was applied | Number of ovaries treated | Results: negative or positive | Comments |
|---|---|---|---|---|---|
| *Corylus* | fresh | snapdragon | about 100 | — | Both chloroform and hot water extracts injected into the ovaries. |
| | | tobacco | about 20 | — | |
| *Salix* | fresh | snapdragon | ? | — | Chloroform extract injected. |
| | | tobacco | ? | — | |
| *Thalictrum* | fresh | tomato | only a few | — | Chloroform extract in lanolin. |
| | | snapdragon | about 50 | — | |
| | | tobacco | 1 | — | |
| | dried for six months | tobacco | 72 | doubtful | Chloroform extract in lanolin. |
| *Petunia* | fresh | pepper | 110 | 1 fruit | Chloroform extract in lanolin. |
| | | eggplant | 15 | 1–7 cm. diam. Several 2 cm. | At the time of harvesting these were growing nicely. |
| | | *Petunia* | 185 | — | |
| *Zea Mays* | stored about two months | *Salpiglossus* | 49 | + | About 50% showed definite increase in size; about 10% grew to half size of normal fruit. |
| | | peppers | 25 | — | |
| | | tobacco | ? | — | |
| *Pinus ponderosa* | dried for about five months | *Salpiglossus* | 35 | + | Considerable growth. |
| | | tobacco | 156 | + | Some growth. |
| | | *Petunia* | 136 | ? | It seemed that some *Petunia* ovaries remained green longer than controls and were somewhat larger. |
| | | pepper | 131 | — | |
| Hollyhock | fresh | *Petunia* | 36 | + | Some growth. |
| | | tomato | 22 | — | |
| | dried four months old | *Salpiglossus* | 4 | + | Some growth. |
| | | tobacco | 101 | + | Some growth. |
| Hubbard squash | fresh | cucumber | 12 | + | 1 large fruit, several others grew a few cm. in length. |
| | | Crookneck squash | 17 | ? | |
| Crookneck summer squash | fresh | Hubbard squash | 19 | ? | Some showed considerable increase, others less. |
| | | Crookneck squash | 65 | + | |
| | | *Petunia* | 164 | — | |

solution of this into ovaries of the same plant one day after these ovaries had been treated with indolebutyric acid lanolin paste, but ovaries so treated made no more growth than the ones not injected. This was only one experiment involving about a dozen ovaries, and one cannot give too much weight to it. However, if the ovules produce the growth substance which causes an ovary to develop into a fruit, where does the growth substance come from which causes the ovary to develop into a fruit when there are no developing ovules (parthenocarpy)? It is possible that under these conditions the ovary wall itself or else the placentae, which are sometimes well developed in these fruits, may produce such substances. As pointed out in a former paper (Gustafson, 1936), the placentae are normally quite well developed in the fruits produced parthenocarpically by the indole compounds employed in that investigation. The question raised as to why some ovaries continue to grow and others grow only for a short time and then stop entirely has not been answered. As mentioned under the discussion of the effect of maize pollen extract upon *Salpiglossus*, the treated ovaries made as rapid initial growth as the pollinated

ones, but after a week or two they ceased growing entirely. Evidently there was eventually a lack of growth hormones.

The nature of the substance obtained from the pollinia of orchid flowers has been investigated by Laibach (1932). He found that hot water extract which caused swellings in the gynostemium of orchids also caused a stretching in oat coleoptiles. Extracts from organs of animals which caused elongation in oat coleoptiles also caused enlargement of orchid gynostemium. Laibach is therefore of the opinion that the hormone from pollen and growth substances such as auxins are either identical chemically or at least closely related. The indole compounds of acetic, propionic, and butyric acids and phenylacetic acid which the writer has used cause the production of normal-sized fruits parthenocarpically in tomato, *Petunia, Salpiglossus*, pepper, and eggplant, and initiate such development in several other plants. These compounds have many times been shown to be growth promoters. Indole-acetic acid, or heteroauxin, has been extracted from some plants, and while the others have not as yet been extracted from plants, it is very likely they may also be present. Their action on the above mentioned plants is identical. The writer agrees with Laibach that if the hormone or hormones in the pollen are not identical with some of the growth hormones, they are at least closely related to them.

## SUMMARY

Chloroform extracts of pollen have been used to stimulate ovary growth in a number of plants, and the results are summarized in table 2. The table shows that in the pollen of some plants is a chloroform-soluble material which initiates growth of the ovary and in some instances causes seedless fruits to be formed.

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