On the Ovary and Ovule Development in *Nicotiana tabacum* induced by α-Naphthaleneacetic acid.[1) 2)]

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Few reports have been published concerning the effect of chemicals in stimulating the development of ovule and embryo. Yasuda (1940) reported that by injection of a growth promoting substance into the ovary of *Petunia*, when the stimulated cells were embryonic, cell division took place, while if the cells were fully developed, the growth of the cell-membrane alone was stimulated. Overbeek, Conklin, and Blakeslee (1941), testing many chemicals, in a wide range of concentrations, for their effect upon ovule development, reported that auxins injected into the ovaries of *Datura* induced the development of parthenocarpic fruits with enlarged ovules which contained pseudoembryos originated from the integuments. They suggested that other plants might be found in which similar treatments would stimulate the formation of true embryos.

In order to examine the possibilities of artificial parthenogenesis suggested by the reports mentioned above, the present writer has carried out a few experiments in this line. The present report deals with injections of a growth hormone into the ovaries of *Nicotiana tabacum* L.

MATERIAL AND METHODS

Field-grown Bright Yellow variety of tobacco was used as material.

Flowers which would open one day later were emasculated and bagged and on the next day 0.1 per cent aqueous solution of sodium salt of α-naphthaleneacetic acid (NAA) was injected into the ovaries. The concentration of NAA was based on Overbeek's data (Overbeek et alii 1941). The injection was made through the ovary wall by means of a small hypodermic syringe. Care was taken to prevent injuries of the ovary tissue as far as possible and to fill the entire space of the loculi with the solution. Then, for microscopic examination ovaries were collected at regular intervals and fixed in Carnoy's solution. Sections were cut 10–14 μ in thickness and stained with Delafield's haematoxylin. For comparative observations, ovaries from self-pollinated flowers, from emasculated but not pollinated flowers and also ovaries injected with distilled water instead of NAA were investigated.

OBSERVATHIONS AND DISCUSSION

Ovary development.——90 to 100 per cent of ovaries treated with NAA developed into parthenocarpic fruits. Comparative growth rates of these fruits and those obtained by pollination are as follows (Table 1):

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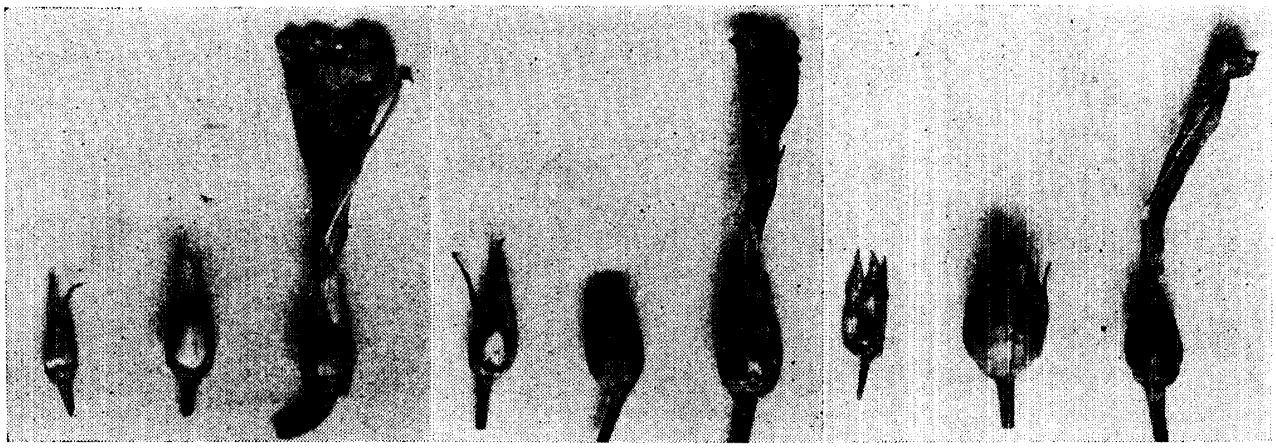
Table 1. Comparative growth rates of tobacco capsules obtained by NAA-injection and by self-pollination.

	Treated with NAA			Pollinated		
Age in days	3	5	9	3	5	9
Length × width in mm.*	8.8×5.6	11.0×8.1	11.3×8.0	8.0×4.9	10.6×7.5	17.6×10.4

* Average dimensions of 20 ovaries measured at point of greatest length and greatest width.

As shown in Table 1, NAA-treated ovaries grow faster than the pollinated ones during the first few days of development, but in the interval between the 5th and the 9th day the pollinated ones start to grow more rapidly (Figs. 1–3). These results are in accord with those obtained by GUSTAFSON



Figs. 1–3. Ovary development in tobacco. a, no pollination and injection after emasculation. b, development after pollination. c, development after NAA-injection. ca. ¾. Fig. 1, three days after treatment. Fig. 2, five days after treatment. Fig. 3, nine days after treament.

(1938) with K-indole acetate but do not agree with the results of his experiments with indoleacetic acid. From his results with K-indole acetate injection, GUSTAFSON concluded that ovaries produced by injection had at first more available growth promoting material which later became depleted, while in the pollinated flowers there was a continuous renewal of the growth substance. The same may be said of the results of NAA-injection. If the parthenocarpically produced fruits were supplied continuously with growth substances, they might grow as rapidly as they did from the very beginning.

When the ovaries were treated with NAA, the capsules were often distorted in shape. Dissection of these ovaries showed an uneven swelling of the placentae or carpels, perhaps owing to uneven penetration of the solution into the ovaries.

The fruits produced by the injection contained a considerable number of enlarged ovules, which sometimes had seed-coats. They looked somewhat like small seeds, but failed to germinate.

The dropping of the corolla was in the NAA-treated flower more delayed than in the untreated or pollinated one. A part of the solution, overflowing from the injected ovary, might have influenced the tissue of the basal part of the corolla, preventing abscission.

Ovule development. — Microscopic examination of the ovules of NAA-treated ovaries showed that they have enlarged considerably, the nucellar and integumental cells increasing in size, approximately to the same extent as in the normally fertilized ovules. Nucellar cells, however, acquired an irregular polygonal shape and were irregularly arranged (Figs. 5–6), while in the fertilized ovules shape and arrangement of the nucellar cells were regular.

In the embryo sac of either the NAA-treated or the untreated ovary, an egg cell, two synergids

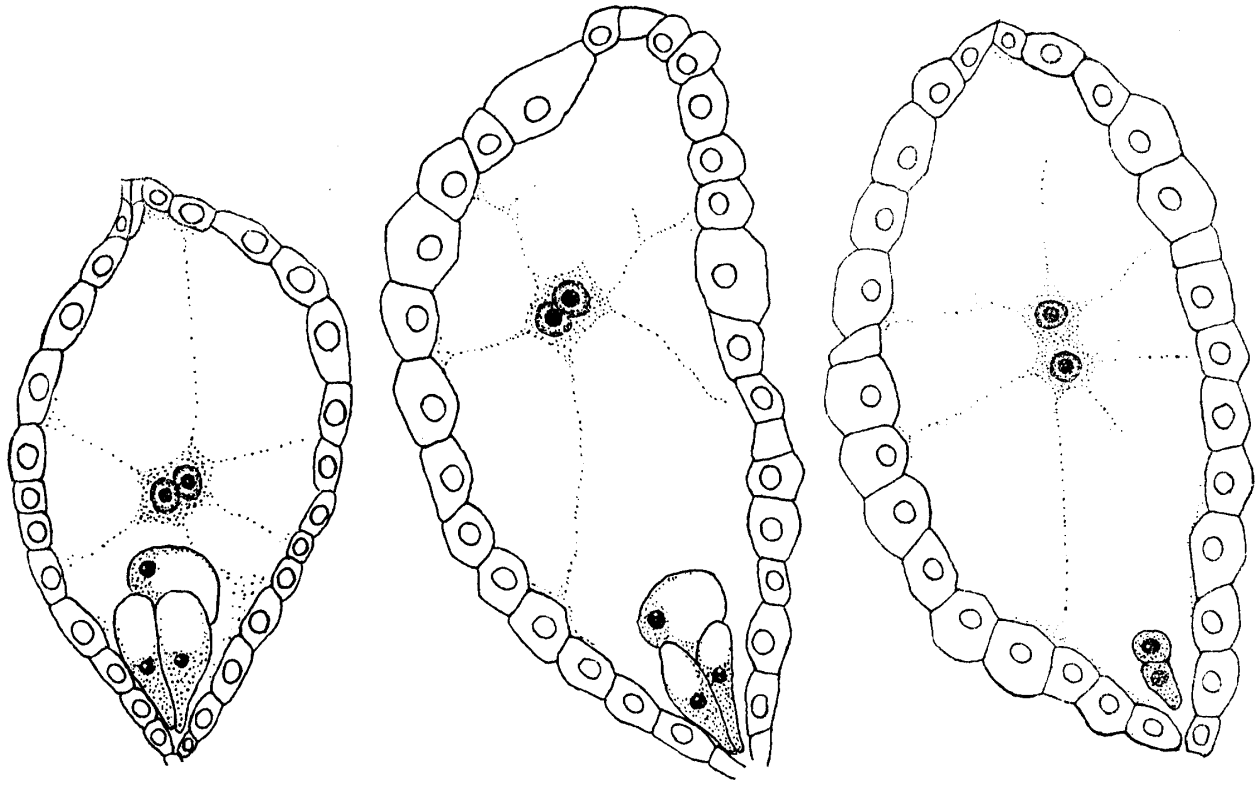


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Fig. 4. Embryo sac of tobacco left for three days without pollnation and injection after emasculation. ×ca.600. Figs. 5–6. Embryo sacs of tobacco injected with NAA, three days after treatment. ×ca.600. Fig. 5, shows an enlargement of nucellar cells. Fig. 6, embryo sac with a two-celled embryo.

and two polar nuclei were found (Figs. 4–5). The antipodal cells had degenerated by the time the embryo sac was mature. The degeneration of the embryo sac occurred about five days after the injection like in the controls left without pollination.

Three days after the NAA-treatment, almost all of the embryo sacs in the treated ovaries remained unchanged except for the enlargement of the nucellar cells and a slight disintegration of egg cells and synergids. In a few ovules, however, namely in three among about 2000 examined, embryos were found in two-celled stage (Fig. 6). Their shape and location resembled closely that of a true embryo developing after normal fertilization, though the latter would have reached the three- or four-celled stage by this time. It is unknown, whether the two-celled embryos had the reduced or the unreduced number of chromosomes, as no nuclear division could be observed.

Though no evidence was found that the two-celled embryos were derived from the division of an egg cell or one of the synergids, it may be assumed that either the former or one of the latter responded to the stimulation by NAA. Because the shape of the embryos resembled rather that of an egg cell than of a nucellar cell, and also because in the ovaries left untreated after emasculation such embryos were never found, although more than 2000 ovules have been examined.

Although great care regarding emasculation and other treatments was taken, yet there might have been experimental errors such as self-contamination resulting from incomplete bagging. However, considering the facts that neither a trace of a pollen tube nor a division of the polar nuclei was found in the embryo sacs in which the two-celled embryos were observed, it seems likely that they were originated without fertilization. In *Petunia* Yasuda (1940) found that when heteroauxin was injected into the ovaries, divisions of egg cells took place and multicellular embryos developed. The two-celled embryos in the present experiment seem to degenerate soon after, since five days and seven days after the treatment not a single ovule did contain such an embryo. In these ovules

when the embryo sac had degenerated to a considerable degree, the nucellus surrounding the embryo sac was also disintegrated.

FERGUSON (1927) described that the polar nuclei in *Petunia* divide before fertilization. KOSTOFF (1930) observed that, when *Nicotiana Langsdorffii* was pollinated with pollen of *Petunia violacea*, *Petunia* pollen tubes induced the development of diploid endosperm. OVERBEEK et alii (1941) stated that division of polar nuclei was not necessarily connected with fertilization. Therefore it seems possible that some chemicals may stimulate endosperm development without fertilization. So far as the present investigation is concerned, no endosperm development was observed in the ovules of the NAA-treated ovaries.

KOSTOFF (1930) and COOPER and BRINK (1940) reported proliferation of nucellar cells (endothelial cells, according to Schnarf) in interspecies crosses in *Nicotiana* and in an attempted cross *Nicotiana rustica* × *Petunia violacea*. In *Datura* OVERBEEK et alii (1941) found pseudo-embryos originated by proliferation of endothelial cells in the ovaries injected with naphthaleneacetic acid or indolebutyric acid. Such proliferation of nucellar cells failed to occur in the present experiment. 0.1 per cent aqueous solution of sodium salt of α-naphthaleneacetic acid seems not to induce either endosperm development without fertilization or proliferation of nucellar cells when injected into tobacco ovaries.

In the NAA-treated ovaries, the ovules showed no enlargement or persistence of antipodal cells such as observed by YASUDA (1940) in *Petunia* ovaries injected with heteroauxin and by *Guignard* (1902) in *Nicotiana*.

SUMMARY

0.1% aqueous solution of sodium salt of α-naphthaleneacetic acid was injected into the young ovaries of tobacco after emasculation. Parthenocarpically produced fruits grew during the first few days faster than those following pollination.

Three days after the treatment, in three among about 2000 ovules examined, two celled embryos were found. Although no figures of nuclear division could be observed, it may be assumed that the embryos were originated from egg cells or synergids, considering the facts that their shape and location were similar to those of a normal embryo developing after fertilization, and that no pollen tube and no division of polar nuclei were observed in the embryo sacs in which those embryos were found. They did not grow further and seemed to degenerate soon after.

In the ovules of the treated ovaries, endosperm development without fertilization, proliferation of nucellar cells and enlargement or persistence of antipodal cells were not observed.

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