THE ROLE OF PLANT HORMONES IN FRUIT DEVELOPMENT

Thesis by

Jean Paul Nitsch

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1951

ACKNOWLEDGEMENTS.

The writer wishes to take this opportunity to express his sincere appreciation and deepest gratitude to Professor F. W. Went for his help and suggestions throughout the course of this work. Professor Went's enthusiasm in plant science was most stimulating, and his kindness and optimism were a constant encouragement. The writer is especially indebted to Professor S G. Wildman who gave many of his evenings to criticise and correct the manuscript of this thesis. To Professor F. G. Gustafson, of the University of Michigan, under whom the first investigations on the tryptophane-converting enzyme in pollen and the action of seeds on fruit growth were started, go many sincere acknowledgements. The writer also recalls many interesting discussions with Professor James Bonner, Professor A. J. Haagen-Smit, and Dr. A. Galston, and wishes to thank them as well as many of his colleagues who were very generous with their help during various phases of this work, especially Drs. G. Camus, T. Nelson, J. Henderson, and Messrs. J. Liverman and E. Kurtz.

Finally the writer expresses his indebtedness to the Institute for the grants which allowed him to continue his work, as well as to the "Direction Générale des Relations Culturelles" in Paris which initially made this study possible by granting the first scholarship.

ABSTRACT.

Starting with the initiation of the ovary primordium, a coordinate study of the hormonal relationships in fruit growth has been attempted with fruits attached on the plants and also by the new technique of in vitro culture. Several phases were recognized in the ontogeny of a fruit. To be able to initiate flower primordia the plant has first to enter a reproductive condition. The formation of ovary primordia was studied in some cucurbits, in which it was found that the environment profoundly influences the apparition of female vs. male flowers. It has been concluded that these environmental factors regulate the length of successive phases in cucurbit flowering. Once initiated, the ovary enlarges regularly, mainly by cell division until anthesis. If pollination is prevented, ovary growth ceases at this stage, and there is indication that the auxin level of the flower decreases. The ovary then shrinks or drops off the plant. Flower abscission is prevented and growth is stimulated by the pollen which performs these effects mainly by increasing the auxin level of the ovary, partly at least through an enzymatic mechanism. After fertilization fruit growth is controlled by the developing seeds which release large quantities of auxin, the latter apparently manufactured in the endosperm. Thus, auxin has been found to affect any stage of fruit development.

TABLE OF CONTENTS

GLOSSARY OF AUXINIC TERMS AND ABBREVIATIONS.

IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
NAA	naphthalene-1-acetic acid
NOA	naphthoxy-3-acetic acid
P-CPA	para-chlorophenoxyacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
auxin	organic substance active at low concentrations in the standard Avena test
free auxin	auxin readily extractable from plant tissues with organic solvents
diffusible auxin	auxin which diffuses out of fresh plant tissue when placed on materials such as agar blocks
bound auxin	auxin extractable with organic solvents only after liberation from plant tissues by alkaline or enzymatic digestion
total auxin	the sum of free and bound auxin of a tissue
growth substance or	growth regulator has been used interchangeably in this thesis to designate organic substances, generally synthetic, which control growth in a manner similar to auxin

INTRODUCTION

THE PROBLEM FOR INVESTIGATION

Since the first apple in the Paradise gardens, men have been interested in fruits. Horticulturists have tried to grow them large, geneticists to breed good quality, food engineers to devise methods to store them, etc. Plant physiologists also have been attracted by these structures and have studied their origin in the flowering process, or their responses to growth substances, their maturation, etc. Much progress has been made in furthering our knowledge of fruit growth, but, in this age of specialization, some aspects have been worked out more than others. For example, a large amount of effort has been centered around the initiation of flower primordia, while little attention has been paid to the development of these primordia into various organs like sepals, petals, stamens or carpels. Again, much work has been devoted to the action of pollen on fruit growth, which, although somewhat inconclusive, has overshadowed the effect of the young seeds. Finally, after the discovery that synthetic growth regulators can induce the setting of fruit, the field of fruit physiology has been dominated by the chemicals which one gets out of a bottle, and the study of the physiology of the normal fruit has been left aside.

A somewhat coordinated study of fruit growth has therefore been attempted in this thesis, so that the investigations include many different aspects of fruit development starting with ovary differentiation.

In addition, a new method of approach to problems of fruit physiology has been devised through realization of the culture of excised ovaries and fruits on artificial nutrient solutions.

This thesis will therefore include two parts. The first part contains a study of the factors governing the development of the ovary while it is attached to the plant. The second part will deal with the growth of the ovary when it is cut from the plant and grown on artificial media.

PART I

IN VIVO EXPERIMENTS

CHAPTER I

INITIATION OF THE OVARY PRIMORDIUM

CONTENTS:

I. Floral initiation.

The first step in the development of a flower ovary lies in floral initiation. It is thought that the change of a growing point from a vegetative to a flowering condition is under hormonal control. While the evidence is still scanty, many workers consider that a particular substance, called florigen, is responsible for the induction of floral primordia. It is not intended to give here a detailed account of the phenomena

which lead to the transformation of a vegetative plant into a flowering one, instead a few points only will be stressed.

To be able to be induced to flower, a plant has generally to reach a certain stage which may be determined by age, nutritional status, or other internal factors, and which is called "ripeness-to-flower". When the ripeness-to-flower stage is reached, then the plant may flower, either without further external stimuli ("indeterminate" type: tomato) or after a suitable thermo- and/or photo-treatment (the beet, and Xanthium).

As an example, let us summarize what is known to date about one plant in which floral initiation has been intensively studied, namely Xanthium pennsylvanicum. Xanthium is a short day plant that needs nights longer than 8.5 hours to flower. A prerequisite of the action of the dark period is that the plant should contain a good supply of products from photosynthesis, especially sucrose (Bonner (1948) unpublished, Liverman and Bonner (1950) unpublished). The dark period does not directly cause the plant to manufacture any florigenic hormone, but only seems to condition the mature leaves in such a way that they will become able to produce a florigen later on. This conditioning seems to have some relationship with a drop in the auxin level of the leaf since:

a) auxins applied during the dark period inhibit flowering (Bonner and Thurlow, 1949),

b) auxin antagonists can undo the inhibition of flowering produced by a flash of light in the middle of the dark period (Liverman

and Bonner (1950), unpublished).

After proper treatment, the leaf is ready to produce a florigenic stimulus. If we subject a cocklebur plant to one short day, put it back under long days and immediately remove the leaves, the plant will not produce flowers. However, if we delay removing the leaves for 4 days after they have been exposed to one short day, the plant will flower. Evidently the leaf has manufactured something which has been transported out of the leaf to the buds to cause flower formation.

This stimulus, which must be transported from the leaf to the primordia, seems to be a very elusive one since it has not been defined chemically despite the numerous attempts of many workers. Some of the properties of the floral substance, however, have been defined. It is readily transmitted by grafting, although it has not been extracted and reintroduced again into the plant with any success, and it moves through the phloem with the photosynthates so that its movement is stopped by girdling. All that is possible to state at the present time is that the floral hormone, if it is a definite chemical entity, is very different from other plant growth factors such as auxins or vitamins.

II. Ovary differentiation.

The first step in ovary development lies in the shifting of the plant from a vegetative to a reproductive metabolism, and it appears to be an all-or-none process which results in the formation of flower primordia. After induction, however, these primordia may develop

in various ways depending upon the environmental conditions to which they are subjected. Thus, flowers without stamens, or with extra-numerous petals, etc., result from abnormal environmental conditions.

1) Previous work.

Blaauw et al. (1932) showed that the number of the flower parts in the tulip may vary from 16 to 22 according to the temperature at which the bulbs are stored, the higher numbers being correlated with low temperatures (9°C.), the lower numbers with high temperatures (28°C.). The ratio $\frac{\text{stamens}}{\text{carpels}}$ seems to remain about constant, however. This is not the case in many other plants. Howlett (1939) showed that winter days favor the development of the pistil in the tomato, whereas summer days favor the production of large stamens. He correlates stamen development with high carbohydrate content of the plants (1936). In the strawberry it can be observed that the first flowers which open very early in the spring do not have stamens. We see, therefore, that a hermaphroditic flower can be shifted by appropriate environmental conditions toward maleness or femaleness.

Such an effect as just described is even more pronounced in monoecious plants. In corn, Shaffner (1930) showed that short winter days can completely inhibit the formation of male flowers. In Xanthium, male flowers appear after one short night, but female flowers develop only if more photoinductive

cycles are given. In Ambrosia trifida, Mann (1942) observed that the proportion of female flowers increases when an increasing number of short days (6 hours) are given in order to induce flowering.

In cucumber, Tiedjens (1928) showed that light duration more than the application of nitrates to the soil is effective in changing the ratio of male to female flowers. He found that with the decrease in light duration and intensity in autumn, the percentage of female flowers increased. He also noticed that, in December, some parthenocarpic fruits were formed. The effect of the season on the ratio male/female flowers in cucumber was again stressed by Edmond (1930), while Currence (1932) showed that the percentage of female flowers increases as the vine lengthens. This last report, however, is subject to criticism since the plants were grown from June 12th to September 19th and the decrease in day length was not taken into account.

2) Original experiments; ovary differentiation in cucurbits.

a) Observations on the small gherkin (Cucumis anguria L.)

The following experiments are concerned with the effect of day length and temperature on the ratio of staminate to pistillate flowers in the small gherkin. Previously, Danielson (1944) found that day length influenced the number of flowers formed in the gherkin, but his experiments were designed in such a manner

that he could not investigate the ratio of staminate to pistillate flowers. Hall (1949) found that the ratio male/female flowers was almost the same over a period of about 100 days, regardless of whether the day length was 8 or 16 hours. His experiments, however, were done only under one set of temperatures, namely 26.7°C. during the day and 21°C. at night. The results of both of these workers may lead to the belief that environment has little influence on the formation of male vs. female flowers in the gherkin. This would be rather surprising since, as it has been shown in the previous paragraph, cucurbits are known to modify their flowering habit with the time of the year.

An experiment was started in the Phytotron (Earhart Laboratory) where gherkins were sown in gravel and placed under the various combinations of day and night temperatures and daylengths which are described in Table 1. The plants were watered twice daily with Hoagland's solution. A natural daylight was used for the 8 and 10 hour photoperiods, and the 16 hour period was composed of 8 hours of daylight supplemented with 8 hours of artificial light having an intensity of 800 to 1,000 foot-candles. The experiment was conducted from August to October. Finally, the flowers were protected from pollination to avoid any interference in the growth of the plants from developing fruits.

TABLE I

THE EFFECT OF ENVIRONMENT ON THE FLOWERING OF THE SMALL GHERKIN (CUCUMIS ANGURIA L.)

Flower types found on the vines 83 days after sowing.

Temperature		Length of day	Type of open flowers
Day	Night		
30°C.	30°C.	16 hours	no flowers
30°C.	30°C.	10	no flowers
30°C.	30°C.	8	no flowers
30°C.	26°C.	8	no flowers
30°C.	23°C.	~~8~~ 16	male
26°C.	23°C.	16	male
26°C.	23°C.	8	male + female
26°C.	20°C.	10	male + female
26°C.	20°C.	8	male + female
23°C.	23°C.	8	male + female
23°C.	17°C.	10	female
23°C.	17°C.	8	female (1)

(1) Some occasional male flowers opened in this series.

Table I gives the flowering status of the plants 83 days after sowing. At high day and night temperatures (30°C.) flower primordia were present, but no flowers ever opened. The primordia grew for a while, then dried out when the flower buds were still small (fig. 1). When the day temperature was maintained at 30°C. but the night temperature was lowered to 23°C., the daylength being 16 hours, male flowers of normal appearance were produced. This was also the case for the plants grown at 26°C. during the day and 23°C. at night, providing the daylength was long enough (16 hours). Female flowers, together with male flowers, developed when the days were shorter (10 or 8 hours). When the temperature was decreased even more (23°C. day and 17°C. at night) and the plants were grown under short days, a great majority of the flowers were female, although a few male flowers developed occasionally.

Thus, in contrast to previous work, these experiments show that environment has a profound effect not only on the number but also on the type of flowers produced. High temperatures and long days favor the production of male flowers, while low temperatures and short days favor the appearance of female flowers. Intermediate temperatures lead to the formation of both male and female flowers.

b) Sequence in flower bud development in the Acorn squash (Cucurbita pepo L.)

In the gherkin the male flowers appear in clusters at the

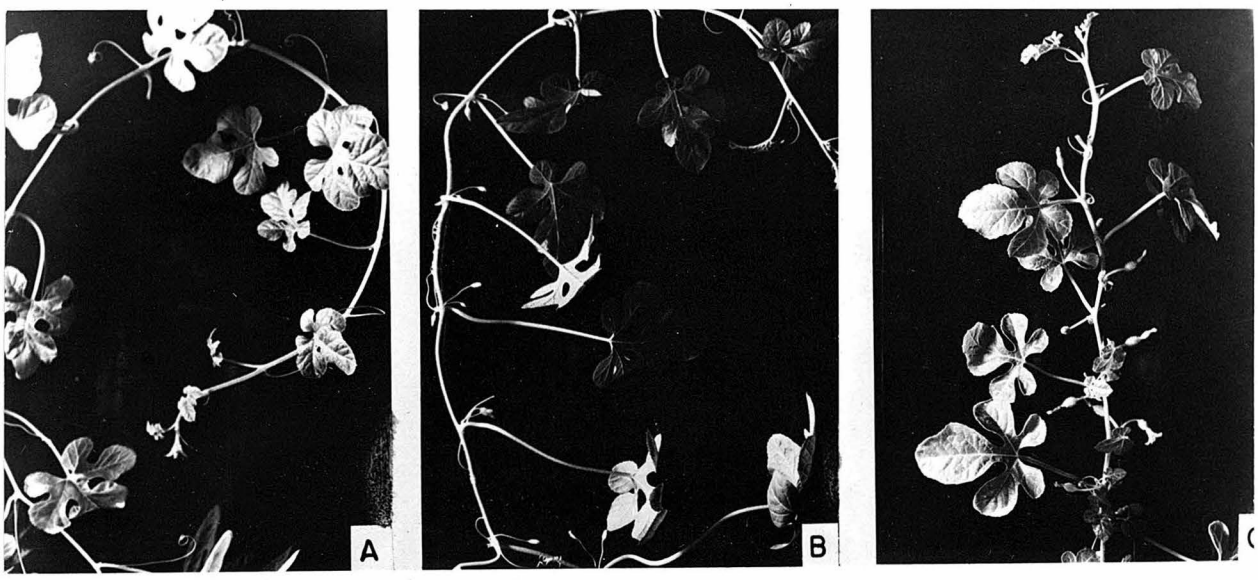


Fig. 1. Flowering of the small gherkin:
A. Plant grown at 30°C. (day and night): no developed flowers.
B. Plant grown at 26°C. (day) and 23°C. (night) and under 8 hours of sunlight: male and female flowers.
C. Plant grown at 23°C. (day), 17°C. (night) and 8 hours of sunlight: predominance of female flowers.

axil of a leaf, whereas female flowers are formed not on the main stem but rather at the axil of the first leaf of an axillary shoot. It might, therefore, not be correct to evaluate flowers which do not arise at comparable sites on the same basis. A more suitable material was found in the Acorn squash in which both male and female flowers can appear on the main stem.

Fundamentally, this variety bears a flower bud at each node, starting with the first true leaf, as illustrated in fig. 2, although, as the vine lengthens, the type of

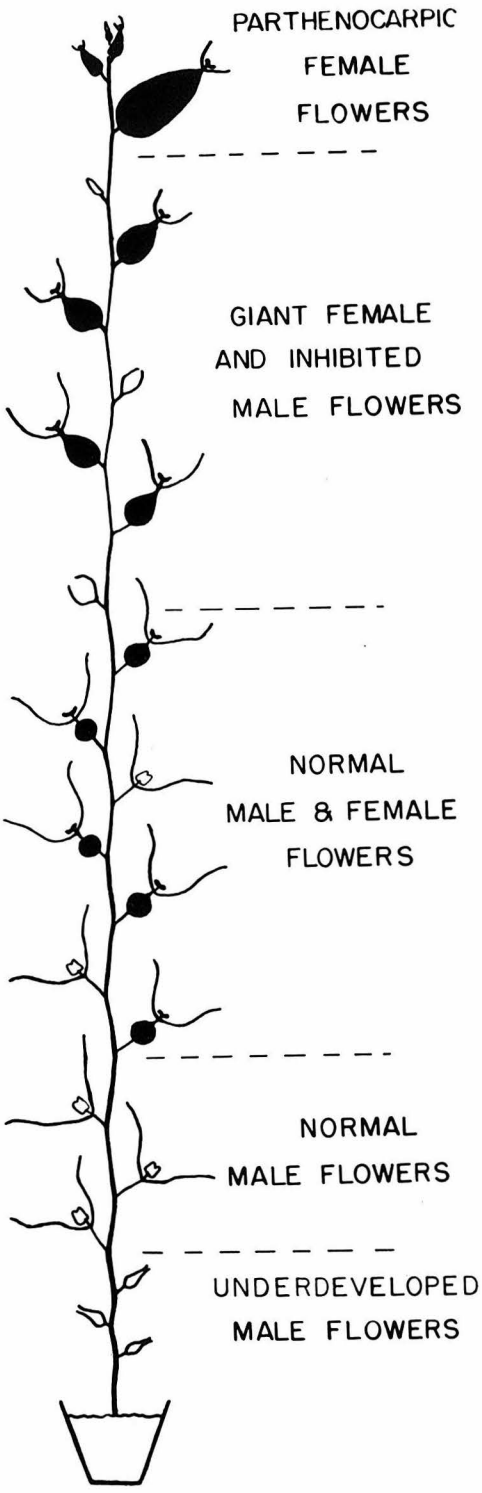
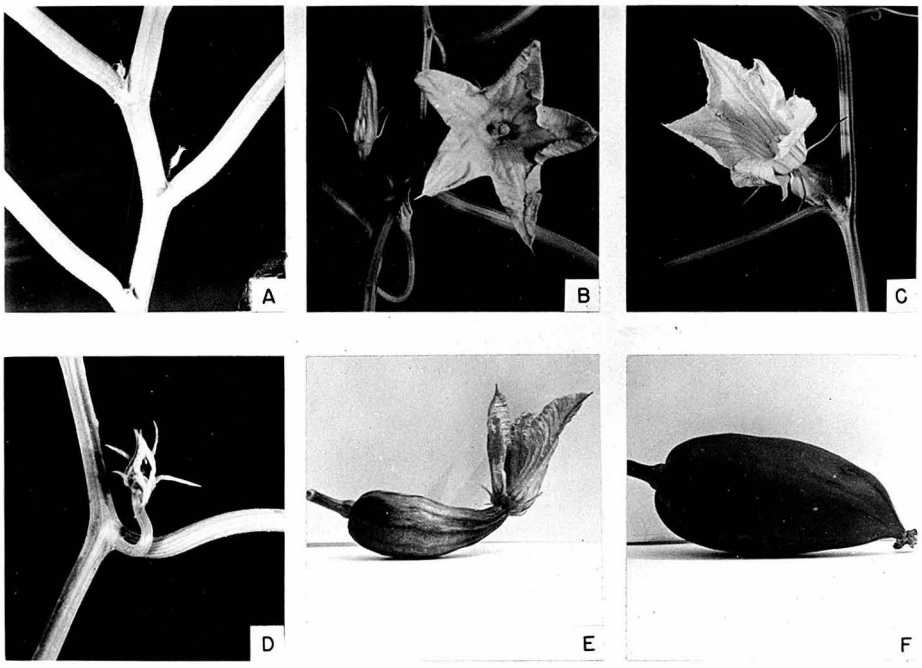


FIG. 2

flower is changed. If the plant is grown at 26°C. (day) and 20°C. (night) with 8 hours of sunlight the following sequence in flower types occurs. At the axil of the first leaves male flowers are generally found which do not develop but do dry out. Higher on the vine an area appears which contains only normal male flowers, followed by an area containing both male and female flowers. As the vine lengthens further, peculiar male flowers appear which have greenish petals instead of bright yellow ones and the anthers bear no pollen. We will call these flowers "inhibited males" together with male flowers still more reduced in size and in development and which will not even open any more. As the male flowers become more and more reduced, the female flowers, on the contrary, increase in size, especially the ovary, while the petals may also become reduced. An extreme case of this ovary enlargement is found when the temperature is lowered to 14°C. (day and night) for in this case the ovary of a flower high in the vine will finally develop to such an extent that it will form a fruit of normal length, although the diameter may be smaller, because of the absence of seeds. Such a fruit, produced without pollination, is called a parthenocarpic fruit. The photographs of fig. 3 illustrate the different types of flowers just described.

Fig. 3. Flower types in the Acorn squash: A. Underdeveloped male flowers of the lower nodes.
B. Normal male flowers.
C. A normal female flower.
D. An inhibited male flower.
E. A female flower with giant ovary.
D. A parthenocarpic fruit.



Actually, the description of flower sequence in Acorn squash given above is somewhat simplified since, especially toward the base of the plant, two flowers are frequently found at one node. The two flowers are not equivalent, however, and can be distinguished from one another. One of them directly arises from the leaf axil and may be called a "primary flower". The other generally bears a small leaf or a reduced leaf vestigium which indicates that this flower may be a part of a rudimentary lateral shoot. Combinations of a primary male plus a secondary male, of a primary male plus a secondary female, and of a primary female plus a secondary female flower can be found, as shown in fig. 4, but their order of appearance follows the pattern described for the primary flowers on the main shoot, i.e. the male flowers precede the female flowers. Finally, an occasional axillary shoot may develop, especially at the axil of the lower leaves. It might be stated, however, that the general evolution of the laterals is similar to the evolution of the main stem, as shown by Currence (1932), with the exception that the first flower on a lateral shoot has a greater tendence to be female.

In summary, it appears that the flowers of squash plants gradually change from a condition of maleness to a condition of femaleness, as the vine lengthens. This change is both quantitative and qualitative; quantitative because the percentage of female flowers increases with time (fig. 5), qualitative, because male flowers become more and more reduced while female flowers develop larger and larger ovaries. However, no flower of a hermaphroditic character has been found, indicating that there is no gradual transition between a male and a female flower, so that the onset of female flowers appears as an all-or-none effect.

c) Influence of environment on flower development in Acorn squash.

The situation just described can be greatly modified by

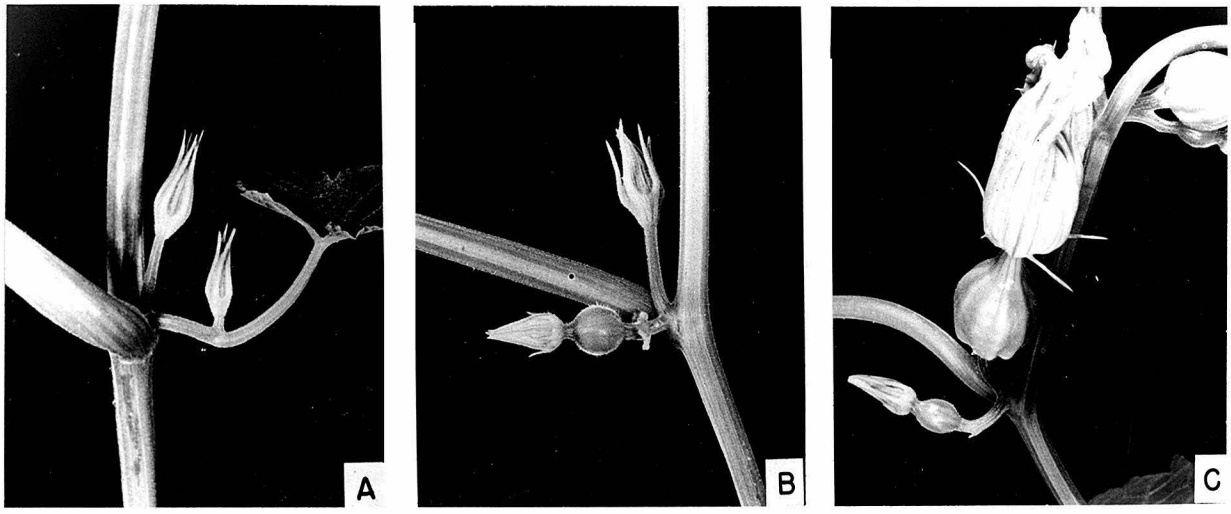


Fig. 4 Combinations of primary and secondary flowers in the Acorn squash:
A. A primary male plus a secondary male.
B. A primary male plus a secondary female.
C. A primary female plus a secondary female.

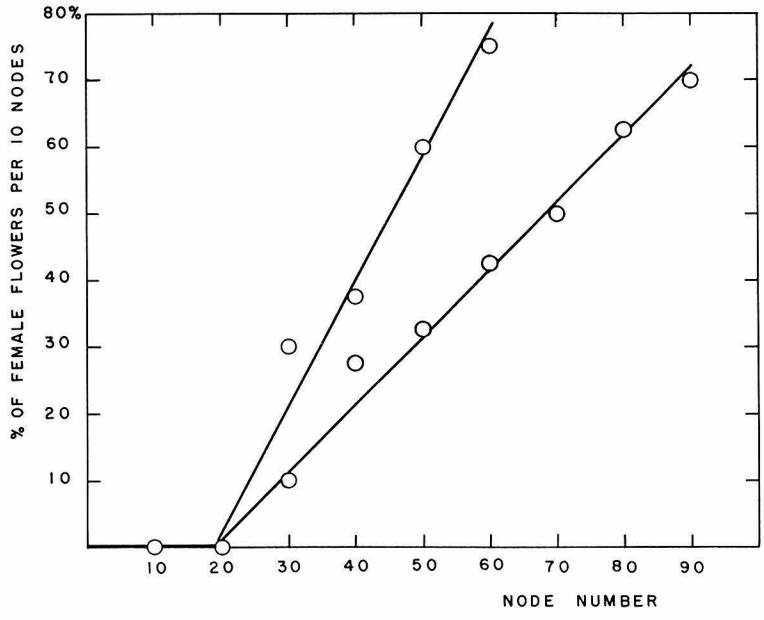


Fig. 5. Increase in the number of female flowers in Acorn squash grown at 26°C. Lower curve: night temperature: 26°C.
Upper " : " " : 20°C.

environment, as shown by the following experiments which will give the flowering patterns of squash plants subjected to different conditions of light and temperature. These experiments can be summarized as follows:

1) Plants grown at 30°C. (day), 30°C. (night), and 24 hours of light produce only male flowers which will dry out before opening. In other words, these male flowers remain in an underdeveloped phase, which we will call the first phase.

2) Plants grown at 30°C. (day), 30°C. (night), and 16 hours of light produce male flowers which do not open, followed by a zone of normal male flowers, but they never produce female flowers. Thus, the second phase appears to be the formation of normal male flowers.

3) Plants grown at 26°C. (day), 26°C. (night), and 8 hours of light produce a few underdeveloped male flowers, then only normal males, then a mixture of male and female flowers. These plants reach a third phase characterized by the formation of female flowers.

4) Plants grown at 26°C. (day), 20°C. (night), and 8 hours of light give one or two underdeveloped male flowers, then normal males, then alternating males and females, finally alternating inhibited males and large females. A fourth phase thus appears, in which maximal production of female flowers seems to inhibit the development of male flowers.

5) Plants grown at 14°C. (day and night) and 8 hours of light reach a fifth phase in which female flowers with giant ovaries are produced, one of which will eventually develop into a true parthenocarpic fruit which appears to inhibit the subsequent development of the vine.

d) A general theory of the phasic development of flowering in cucurbits and its variations with environment.

In view of the experiments described in the preceding paragraph, we may advance two conclusions:

1) In its flower sequence, the Acorn squash passes through a succession of phases, the male flowers preceding the female flowers.

2) The length, but not the order, of each phase is determined by environment. In other words, all plants can potentially go through the complete development, but the speed at which this happens varies with environment.

Fig. 6 is a diagrammatic evaluation of the effect of a light and temperature climatic complex in controlling the phasic development of squash flowers. It shows that plants grown under long days and at high temperatures will need an infinite time to reach the female state, whereas under 8 hours of sunlight the first primary female flower will appear around the 28th node at 26°C. (day and night), or around the 15th node at 20°C. (day) and 10°C. (night).

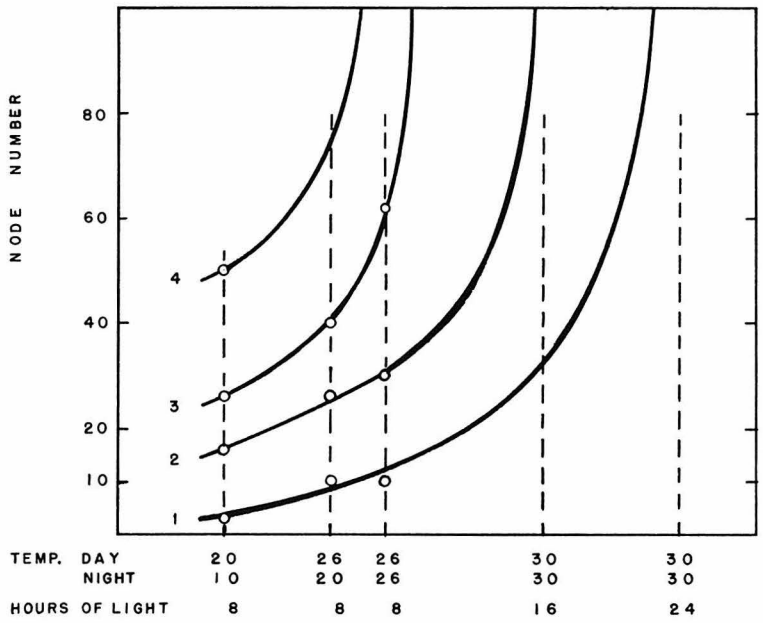


Fig. 6. A diagrammatic representation of the effect of environment on the flowering of the Acorn squash:
Curve 1: position of the first normal male flower.
Curve 2: " " " female "
Curve 3: " " inhibited male flower.
Curve 4: " " parthenocarpic female flower.

The data on the flowering of the small gherkin present a pattern similar to the results obtained with the Acorn squash. In fact, experiments of the same type have been performed with a cucumber (Cucumis sativus L. var. Boston pickling), in which comparable phases could be distinguished during flowering, which were also regulated by environment. It should then be possible to construct for each variety of cucurbit a graph of the type shown in fig. 6 which would give the characteristics of the variety in regard to flowering. In a given climate, one could then find from such a graph when male and female flowers will be present together on the vine in order to perform pollinations, or on the contrary when complete flowers of only one type will be present.

3) Possible role of auxin in ovary differentiation.

From the experiments performed on flower differentiation in cucurbits, it appears probable that different factors govern the formation of the ovary, the stamens, the petals, etc. These unknown factors are controlled in some way by environment. Although experiments are needed to define these factors more exactly, we can already formulate some suggestions.

One of these suggestions concerns the possible role of auxin in ovary differentiation. Such a role seems likely for the following reasons. First, we have seen that parthenocarpic

fruits may develop under suitable conditions of environment. These parthenocarpic fruits represent the ultimate degree of femaleness because they are preceded by an increasing number of female flowers and because the ovaries of these female flowers become larger and larger as the parthenocarpic phase comes closer. On the other hand, it is well known from the work of Gustafson and others that high levels of auxin are responsible for the production of parthenocarpic fruits. Second, van Overbeek could induce flowering in pineapple by the use of cold treatments (1948) instead of auxins (1946). Finally, in a recent note, Laibach and Kribben (1950) reported that the number of female flowers could be markedly increased in cucumber by spraying the tops of young plants with NAA[1]. Thus, one effect of the environment could be to regulate the auxin level in the cucurbits, either by increasing auxin production, or by decreasing auxin destruction, or else by rendering the tissues more sensitive to auxin.

III. Summary and conclusions.

In this chapter we have seen that, in order to produce flowers and fruits, plants have first to be shifted from a vegetative to a reproductive condition. This change is believed to be under hormonal control, at least in some plants such as the cocklebur. In addition, auxin seems to influence one or more steps in the initiation of flower primordia.

[1] A list of abbreviations may be found at the beginning of this thesis.

In these primordia, ovaries may or may not start to differentiate, according to the environmental conditions which influence the internal metabolism of the plant. Here again auxin might play a role in enhancing the differentiation of ovaries preferentially to stamens.

We will now study the growth of these ovary primordia and their development into fruits. This investigation will be divided into several chapters according to the consecutive phases which can be distinguished in the development of a fruit, namely: the growth of the ovary until full bloom, the pollination of this ovary, and finally the growth of the ovary after pollination.

CHAPTER II

THE GROWTH OF THE OVARY FROM ITS INITIATION UNTIL POLLINATION

CONTENTS:

1) Growth characteristics of the ovary.

The growth of the ovary has been extensively studied in cucurbits by Sinnott (1939, 1945 a and b). He concluded that growth proceeds at a constant exponential rate before and after full bloom and slows down only when fruits approach maturity. He found that the increase in ovary size is chiefly due to cell multiplication before full bloom, but that after pollination growth of the ovary is achieved mainly through cell enlargement. Finally he was able to correlate the difference in fruit size observed in different varieties of cucurbits with duration of cell multiplication, cell division continuing longer after full bloom in large fruited varieties than in small fruited ones. In the tomato, Houghtaling (1935) showed that early growth of the ovary is largely the result of cell multiplication. Shortly before flowering the cells begin to enlarge, all later growth being associated with cell enlargement. In tomatoes also, the ultimate size of the fruit is generally determined

very early by the amount of cell multiplication in the floral primordium. In pome- and stone-fruits, the same picture seems to prevail. For example, Tukey (1939), in a study of the development of the sour cherry, concluded that before fertilization practically all ovary growth is a result of cell multiplication, but that after full bloom ovary growth mostly results from cell enlargement. In the apple, a similar status was described by MacArthur and Wetmore (1941), by Tukey and Young (1942), and confirmed by the recent work of Smith (1950).

Thus, in all cases investigated, it seems that two processes regulate the growth of an ovary: cell division and cell enlargement. Mitotic activity usually ceases shortly after the full bloom stage. When, however, cell multiplication continues after pollination, the fruits obtained become larger as a consequence of the increased number of cells.

2) The cessation of growth in unpollinated ovaries at the wilting of the flower.

In contrast to the continued growth of the vegetative primordium, one of the most striking facts about the development of the ovary is that it grows regularly until the flower opens and then abruptly ceases to enlarge, unless fertilization of the ovules or initiation of a parthenocarpic fruit takes place. In most plants, if pollination is prevented, the whole flower drops off. One of the reasons, therefore, for failure of the ovary to grow after anthesis may be this abscission. Such a phenomenon prevents us from knowing what the fate of the ovary would have been had it remained attached to the plant. Fortunately, gherkin ovaries are unusual

in that they do not absciss but rather remain attached to the plant for long periods after full bloom even though no pollination has occurred. A comparison, therefore, can be made between the growth habit of a pollinated and an unpollinated gherkin ovary.

Gherkin plants were grown under controlled conditions in the Phytotron, and ten ovaries were measured to the nearest 0.5 mm, the diameters and lengths being recorded each day. The ovaries increased regularly in size until the flowers opened. A female gherkin flower stays open only one day. At that time, five of the ovaries were pollinated by hand, and the other five left unpollinated. Two days after the opening of the flowers the growth of the two groups started to be strikingly different. The pollinated ovaries continued to grow at an increasing rate, but, on the contrary, the non-pollinated ovaries stopped growing completely. Moreover, the latter soon started to turn yellow and then to shrink as if they were losing the food that they had gained previously. Finally they dried out. The growth curves of both groups are shown in fig. 7 and a picture of the fate of pollinated and unpollinated flowers is represented in fig. 8.

3) Auxin content of the ovary before pollination.

The cause of arrested growth of flower ovaries shortly after full bloom is puzzling. We know that, before full bloom, ovary enlargement proceeds largely through cell multiplication. Unfortunately little is known about the physiological factors that bring about cell

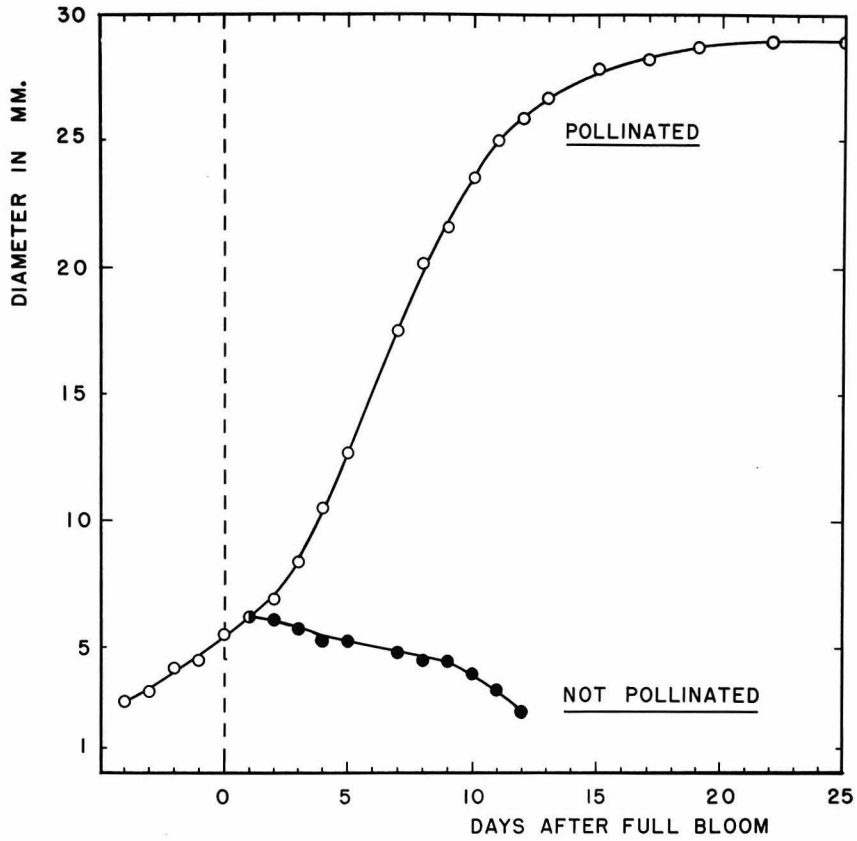


Fig. 7. Growth curves of pollinated and unpollinated gherkin ovaries.

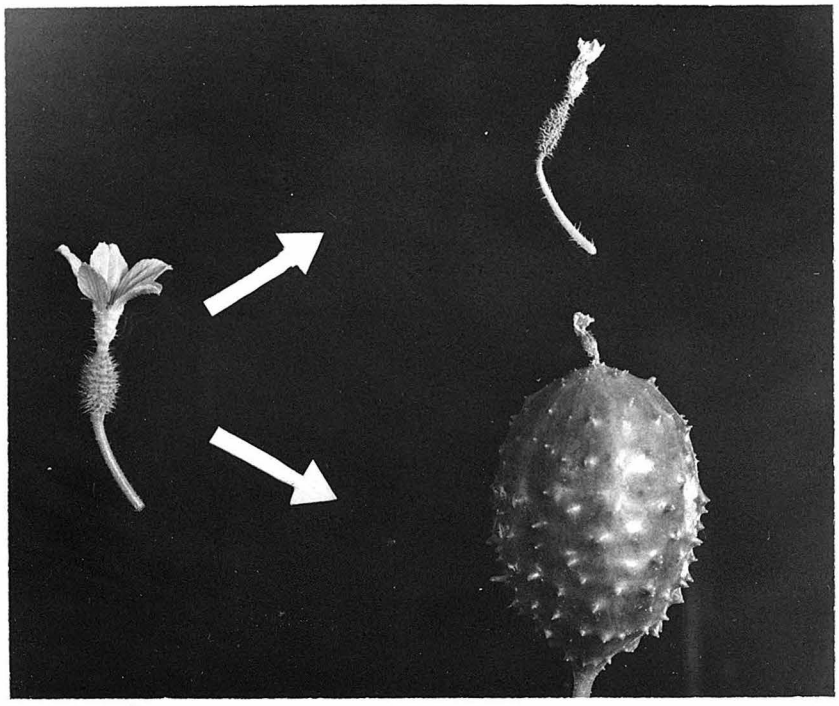


Fig. 8. The fate of a gherkin ovary after full bloom when unpollinated (above) and pollinated (below).

division. While auxins are mostly concerned with cell elongation, nevertheless there is also one clear cut instance where auxin promotes cell multiplication. This is the case for cambiums, either in vivo or in vitro. It might therefore be worth while to try to get a picture of the auxin content in the young, unpollinated ovaries, in order to find if any change in the auxin level is correlated with the cessation of ovary growth at the time of anthesis.

As a matter of fact, Söding (1936, 1938) has published experiments where he tested the activity of flower buds and open flowers of Cephalaria and Heliopsis by the Avena test. The auxin was obtained by the diffusion method. Young flower buds were found to contain appreciable quantities of diffusible auxin, in contrast to open flowers which did not release auxin. Unfortunately these experiments cannot give us any clue as to whether or not the auxin which diffused from these flower buds came from the stamens or from the ovaries. In fact, it is known that developing stamens are very rich in auxin (Wittwer, 1943).

With flowers that have an inferior ovary, it is easy to separate this ovary from the rest of the plant, even in a very young bud. Gherkin flowers present this advantage, so that flower buds were sampled 1, 2 and 3 or 4 days before full bloom, as well as at full bloom and 2 and 4 days after, the latter flowers not being pollinated. The ovaries were separated from the rest of the flowers in a cold room,

immediately frozen in a deep-freeze, then lyophilized by the procedure of Link, Eggers and Moulton (1941), and finally extracted for free auxin using redistilled wet ether at 0°C., as suggested by Wildman and Muir (1949). Since cucurbit fruits are exceedingly low in free auxin, and also since it was important to detect the presence of inhibitors, 0.25 cc of a solution of IAA in water (20 gammas/liter) was added to each sample of lyophilized tissue at the time of extraction. Fig. 9 gives the amounts of auxin obtained per 100 mg dry weight after deduction of the added IAA. Although individual variations may not be significant, the actual drop in auxin concentration at full bloom seems to be correlated with the cessation of growth in unpollinated ovaries.

4) Conclusions.

Once formed in the flower bud, the ovary will enlarge regularly, chiefly by cell multiplication. At the time of flowering, cell division ceases and the auxin content of the flower drops. The ovary will then absciss with the flower or, if it remains attached to the plant, it will shrink and dry out. From its initiation up to full bloom the ovary has been growing only on the food supplied by the mother plant. It now becomes unable in some way to use this food and to grow further. A new growth element has to enter the picture. This new element is the pollen.

CHAPTER III

EFFECT OF POLLINATION ON FRUIT GROWTH.

CONTENTS:

1) Effect of pollination on the ovary growth curve

Let us again consider the growth curve of a gherkin ovary (fig. 7). The ovary enlarges in diameter at a constant exponential rate. There is no sudden change in the shape of the curve at the time of pollination. In other words, the growth curve does not indicate in its pattern when pollination has taken place. The curve is smooth and regular as if nothing had happened. Still, if pollination does not take place, growth soon ceases. How then can we distinguish between ovary growth before and after pollination, since one growth period goes so gradually over into the other that it is impossible to say where one finishes and where the other begins?

The breakdown of a fruit growth curve into partial processes can be done, however, provided a suitable material is used. The fruits

of tropical orchids, such as Phalaenopsis or Cypripedium, are particularly slow growing, taking about 6 months from pollination to maturity. A particular point of interest is the fact that in Phalaenopsis, for example, pollen germinates only 4 days after pollination, and that fertilization takes place 60-70 days afterwards. Using such a material, Duncan and Curtis (1942) were able to demonstrate a two peak curve in the rate of ovary enlargement in Cypripedium. The first peak may be ascribed to a growth stimulus coming from the pollen, the second to a further stimulus from the developing ovules. This chapter will be concerned with the action of pollen itself. The role of the ovules will be discussed in the next chapter.

2) The action of pollen on ovary physiology.

a) Inhibition of flower abscission.

Since the flowers of most plants will drop off if the plant pollination has not taken place, it seems possible that one effect of pollination is to keep the flower on the plant. This idea gains support from the experiments of Laibach (1933) and La Rue (1936) who were able to inhibit abscission of Coleus leaves through application of a lanolin paste containing pollen of orchids and other plants. That this effect of pollen in preventing abscission may be due to the release of auxin is suggested by the fact that: first, pollen contains appreciable amounts of auxin (Laibach, 1932, Thimann, 1934); and secondly, auxin itself may prevent

abscission, at least in the pre-harvest drop of pome- and citrus-fruits (Gardner et al., 1939, Stewart and Klotz, 1947). Muir (1942) showed that, in the case of the tobacco, no auxin diffuses from the pedicel below the ovary when the flower is left unpollinated. About 40 hours after pollination, however, detectable amounts of auxin are released from the pedicel, so that Muir concluded that: "The growth hormones released in the fertilized ovary move downward through the pedicel and prevent abscission of the pistil by inhibiting the development of the abscission layer."

The prevention of abscission, however, is not sufficient by itself to cause an ovary to grow. This can be shown best in vitro, as we will see later, but also some observations made in vivo support this view. In the first place, in some plants the ovaries will not absciss when they have not been pollinated. This is true for the gherkin. Nevertheless, such ovaries will not grow, but, on the contrary, they will decrease in size, shrink and finally dry out. Secondly, even flowers that normally absciss when left unpollinated, like tomato flowers, can be induced to stay on the plant by proper control of the environment. Essex Wonder variety of tomato plants were grown in the Earhart laboratory with cool nights (day temperature: 23°C., night: 17°C.), since Went (1944) had shown that high temperatures, especially

at night, favor flower drop in tomato. The flowers were deprived of their stamens and styles as they opened, which prevented them from being pollinated. Nevertheless, these flowers remained attached to the plant for at least 1 month. The sepals enlarged but the ovaries did not develop (fig. 10).

Therefore it appears that the maintenance of the ovary on the plant is only a prerequisite for fruit set, not its cause. It is then necessary to look for a more direct stimulus in pollen.

b) Stimulation of ovary growth.

In addition to the prevention of abscission, the pollen seems to give a definite growth stimulus to the ovary. Although the details of an experiment suggesting this explanation will be given in the second part, for the purpose of discussion the results will be summarized briefly here. Pollinated ovaries of tomatoes and gherkin flowers grow on a certain basal medium when excised from the plant two or more days after pollination. On the contrary, unpollinated ovaries will not enlarge when grown on the same medium. Here abscission does not play any role because first, the ovaries are kept supplied with nutrient and, second, the ovaries have been excised above the abscission zone which cannot, therefore, function as a filter so that the uptake of certain compounds could be prevented. That this growth stimulation as well as the prevention of abscission is

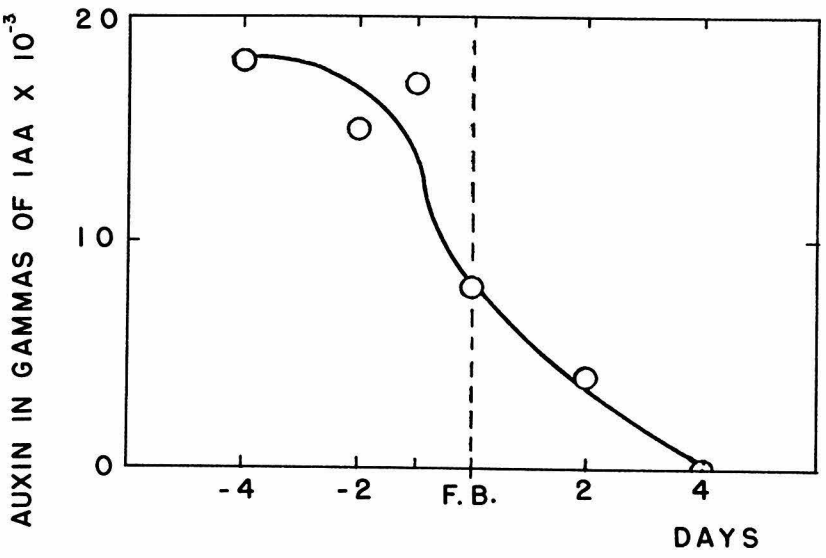


Fig. 9. Free auxin content of 100 mg lyophilized tissue of unpollinated gherkin ovaries before and after full bloom.

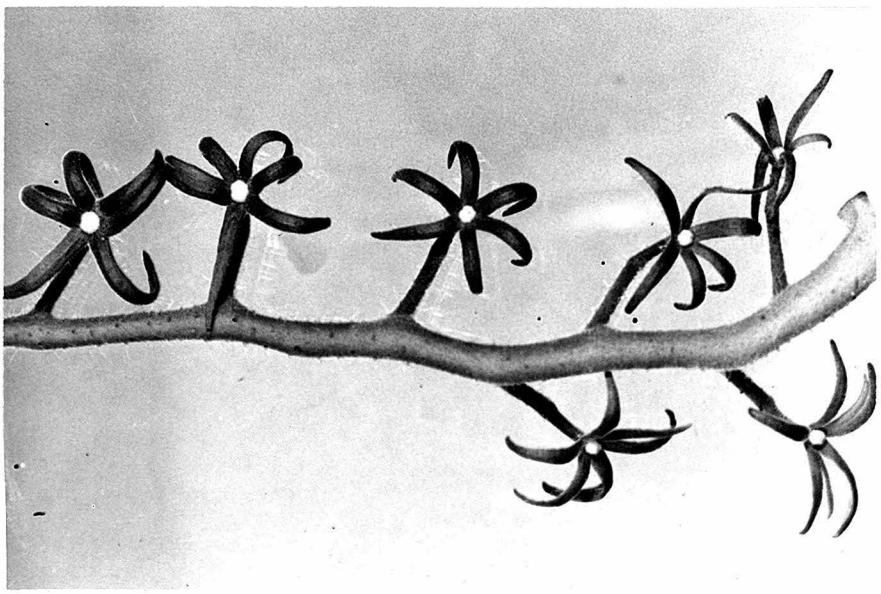


Fig. 10. Unpollinated tomato ovaries (var. Essex Wonder) which did not absciss but remained attached to the plant for 1 month after anthesis (temperature: 23°C. (day), 17°C. (night).

also caused by auxin is suggested by the fact that growth substances can stimulate the growth of such unpollinated tomato ovaries cultivated in vitro.

3) Auxin and pollination.

After pollination a marked increase of auxin has been found in the tobacco ovary by Muir (1942). From the preceding discussion we may say that this auxin is most probably responsible for the two physiological effects of pollen on ovary growth. The question now becomes: where does this auxin come from? Several possibilities come immediately to mind. Pollen may either supply the auxin itself or provide a mechanism to manufacture this auxin.

The first alternative has been extensively studied. Yasuda (1934) and Gustafson (1937) obtained the formation of parthenocarpic fruits by treating flowers with pollen extracts. Laibach (1932), Thimann (1934), and Muir (1947) showed that pollen contains large quantities of auxin. The calculations of Van Overbeek et al. (1941) however indicate that despite the fact that pollen is rich in auxin, the minute amounts of pollen sufficient to achieve fertilization do not bring enough auxin into the ovary to account for the quantity observed after pollination.

The second alternative, that pollen provides a mechanism to produce auxin, may therefore be a better explanation of the

rise in the auxin level observed in the ovary after fertilization. This result could be achieved in several ways. First, the pollen could bring an enzyme which would manufacture auxin with a substrate coming from the ovary. Secondly, the enzyme could be in the ovary and the substrate in the pollen. Thirdly, the pollen could just contribute a co-enzyme or activate in some way the system already present with the substrate in the ovary.

The only enzyme known to date to produce an auxin is the one described by Wildman, Ferri and Bonner (1947) in spinach leaves. This enzyme transforms L-tryptophane into IAA. It was therefore of interest to find if such an enzyme could be detected in the pollen or in the ovary.

TABLE 2

ENZYMATIC CONVERSION OF L-TRYPTOPHANE INTO AUXIN BY POLLEN AND POLLEN BREIS.

Germination of the pollen was obtained on a medium containing 1% agar and 10% sucrose, forming a thin film in a Petri dish which was incubated in the dark at 25°-26°C. Redistilled ether was poured on the cultures and left at 5°C. for 10 to 12 hours at which time it was decanted and used to prepare the agar blocks for the Avena test.

Pollen breis were obtained by either grinding the pollen with glass (Muir, 1947) or by using a homogenizer. The breis were acidified to pH 3.0 before ether extraction.

Material	Total amounts of L-tryptophane	Auxin in IAA equivalents (gammas X 10^{-3})
30 mg pollen of Lilium regale, germinated 13 hours with	0 gammas	0
-id-	2 gammas	18
-id-	2 gammas	10
-id-	20 gammas	140
Culture medium incubated 13 hours, but without pollen, with	20 gammas	0
30 mg pollen of Lilium canadense, germinated 20.5 hours	0	0
-id- with	5 gammas	122
Brei of 30 mg ungerminated pollen of L. regale, incubated 7.5 hours at 25°C. with an aqueous solution containing 0.05% sucrose and	5 gammas	120

TABLE 2 (Cont.)

Material		Total amounts of L-tryptophane	Auxin in IAA equivalents (gammas X 10^{-3})
Brei of 100 mg Pinus sp. ungerminated pollen, incubated 5 hours at 25°C. in phosphate buffer (pH 7.0)	with	0 gammas	208
-id-	with	5 mg	936
Brei of 30 mg of Althæa rosea ungerminated pollen, incubated 6 hours	with	0 gammas	0
-id-	with	5 gammas	0

Nitsch and Gustafson (1948) investigated the pollen of various species (Lilium, Pinus, Althæa, etc.) for tryptophane-converting enzyme. Although the results were only preliminary it was found that the pollen of two species of lily, when germinated on sucrose-agar containing 3.3 gammas/cc of L-tryptophane would yield appreciable quantities of free auxin, while the same pollen yielded no auxin when germinated on the same medium without tryptophane. It was further apparent that such an auxin production could be obtained with ungerminated pollen merely by incubating pollen brei with tryptophane. A summary of the results is given in Table 2. As the table points out, the release of auxin by pollen incubated with tryptophane does not seem to be of a general nature, since

not every kind of pollen investigated produced auxin.

The second possibility, namely, the presence of the enzyme in the ovary, was investigated by Wildman and Muir (1949). They reported that tobacco ovaries are able to manufacture large amounts of auxin when incubated with tryptophane. However, they also found that the same enzymatic activity was present in the unpollinated ovary (private communication, 1950). If therefore, the enzyme is present in the ovary, then one should find little or no free tryptophane in that ovary, but rather tryptophane, as a substrate for the enzyme, should be brought by the pollen.

A preliminary study of the L-tryptophane content of both pollen and unpollinated ovaries was therefore undertaken. As Table 3 points out, however, there was no difference in the concentration of free tryptophane, but since the ovary was much larger it contained the larger total quantity of tryptophane. Evidently none of the hypotheses so far advanced to explain the genesis of auxin in the pollinated ovary is completely satisfactory.

TABLE 3

"FREE-TRYPTOPHANE" CONTENT OF POLLEN AND UNPOLLINATED OVARIES OF THE ACORN SQUASH (Cucurbita pepo L.)

The tryptophane assay was performed with Lactobacillus arabinosus 17-5, as described by Greene and Black (1943). The extraction of "free tryptophane" from lyophilized tissues was made by first denaturing the proteins by boiling with absolute alcohol and then extracting the tryptophane with water. Anthranilic acid and indole, which also support the growth of Lactobacillus (Snell, 1943), were removed by ether extraction after acidification to pH 3.0 - 4.0.

Material	L-tryptophane in gammas: per 100 mg (dry wt.)	per flower
Pollen	4.0	0.22
Ovaries	4.5	18.90

Note: The squash plants were raised under the following climatic conditions: temperature: 26°C. (day), 20°C. (night); light: 8 hours of sunlight.

The hypothesis of the stimulation of an enzyme is, in itself, very vague, so that the phenomenon reported by Muir (1947), who found that incubating unpollinated tobacco ovaries with pollen extracts yielded auxin yet none of the constituents alone released any auxin, still remains unexplained.

4) Conclusions.

In the second chapter we have seen that, after anthesis, an unpollinated ovary stops growing and also has a low level of auxin. On

the other hand, a pollinated ovary grows and develops into a fruit. Looking for the cause of such a difference in behavior, we investigated the action of the pollen. The conclusion is that, although pollen certainly increases the auxin level in the ovary, it does not seem to bring as much free-auxin as it was believed ten years ago.

It is, therefore, necessary to look for another source of auxin in the young fruit. Apparently, besides the pollen, there is only one difference between an unpollinated and a pollinated ovary. This difference is that in the first case the ovules remain undeveloped, while in the second case they develop into seeds. It is therefore of interest to investigate the young seeds to find out if they exert any action on the growth of the fruit, and, in the affirmative, if they perform this effect through the release of some growth hormone.

CHAPTER IV

EFFECT OF SEED DEVELOPMENT ON FRUIT GROWTH.

CONTENTS:

1) Action of the seeds on fruit growth.

a) Methods and materials.

To investigate the effect of pollen on fruit growth, we have compared the growth curves of pollinated vs. unpollinated fruits. To study the action of the seeds we may use a similar method and compare the growth of fruits with and without seeds. One series of ovaries would be subjected to pollination, fertilization and normal seed growth, while in the other series ovule

development should be prevented just after fertilization. Such an experiment is difficult to perform, mainly because seeds are located inside the ovary, so that removal of them often causes extensive injury to the ovary. Indeed Dollfuss (1936) cut open fruits of several species, including Symphoricarpus and Rosa, removed the seeds and replaced them with an IAA lanolin paste. A certain amount of growth occurred in the ovaries supplied with growth substances. Gustafson, however, was unable to repeat these experiments because of the injury inflicted to the plants (private communication, 1948).

In search of a fruit which would have the seeds located on the outside, it was found that the strawberry was a very suitable material for the planned experiments. Although the strawberry is not a single fruit -- the true fruits being the small achenes disposed around the fleshy receptacle -- nevertheless it accumulates sugars and vitamins and ripens like a true fleshy fruit, such as a cherry or a tomato. Each achene contains a single ovule and can therefore be treated as a single unit. The first experiments were performed at the University of Michigan on a local variety called "Wazete", then repeated with the variety "Marshall" at the California Institute of Technology. The plants were grown in sand in the greenhouse and were watered daily with Hoagland's nutrient solution. Each

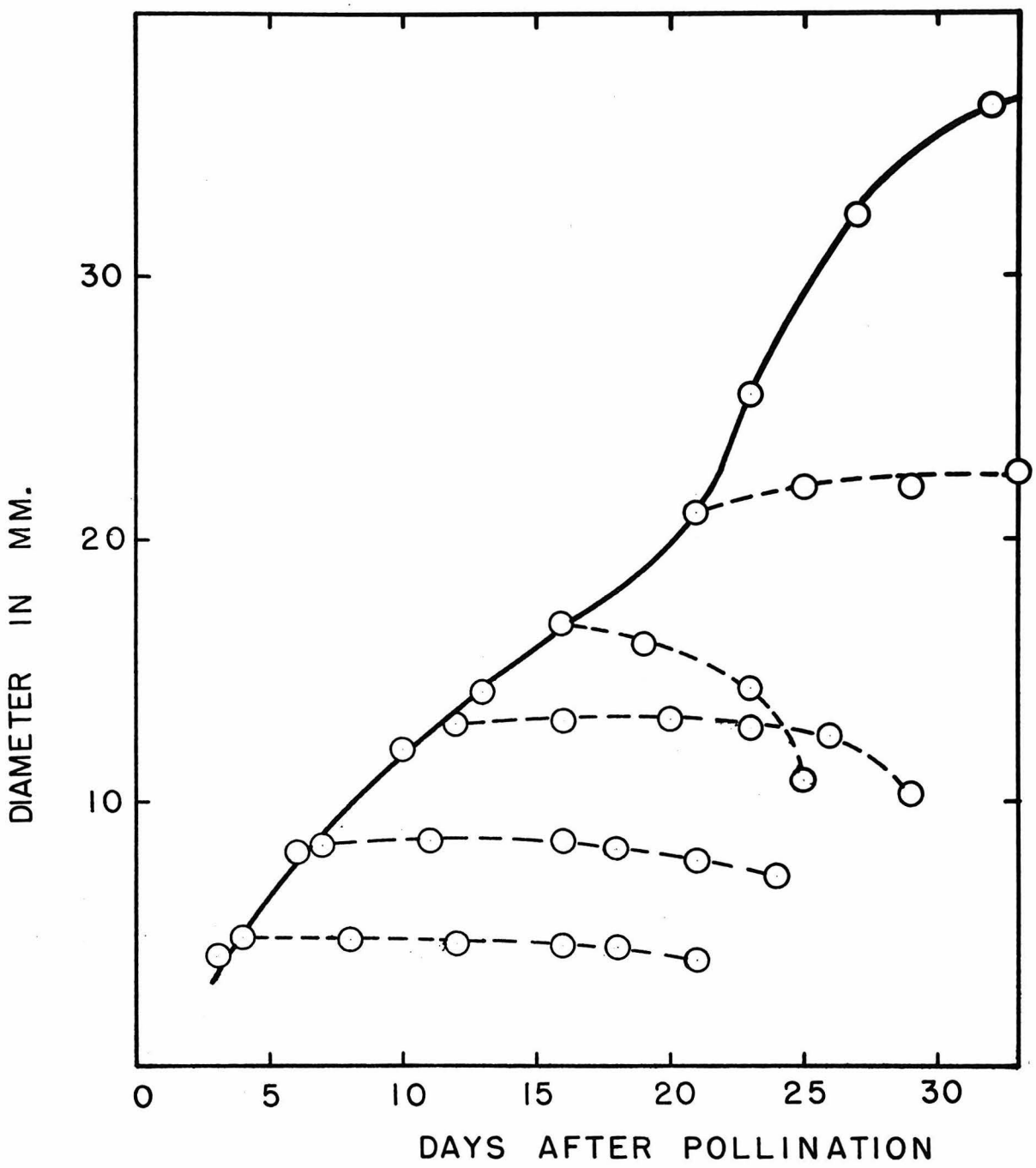


Fig. 11. Effect of removing all the achenes on the growth of a strawberry fruit. (Dotted lines: growth curves of fruits after removing the achenes. Solid line: growth curve of controls).

flower was pollinated by hand with a camels-hair brush. Diameter measurements of the strawberries were made every third day with a Vernier caliper, two perpendicular diameters being measured to the nearest 0.1 mm, and the average of the two readings recorded.

b) Experiments with strawberries

Achenes can be removed from a strawberry with the tip of a knife without apparent injury to the receptacle. When all of the achenes are removed from strawberries four days after pollination, the growth of the receptacle is completely stopped. This result also occurs when the achenes are removed 7, 12, 19, and 21 days after pollination (fig. 11). In the last instance (21 days) the "berries" turned red at the usual time (26 to 30 days after pollination) although no further growth in diameter occurred. It seems likely, therefore, that the achenes control the growth of the receptacle throughout the development.

In nature, no growth of strawberries occurs unless the ovules contained in the achenes are fertilized. However, fertilization of one ovule is sufficient to cause some growth in the area of the receptacle immediately surrounding that achene, as shown in fig. 12. Similarly, fertilization of three ovules causes three small areas of growth (fig. 13). When more ovules are fertilized, these areas of growth become more numerous

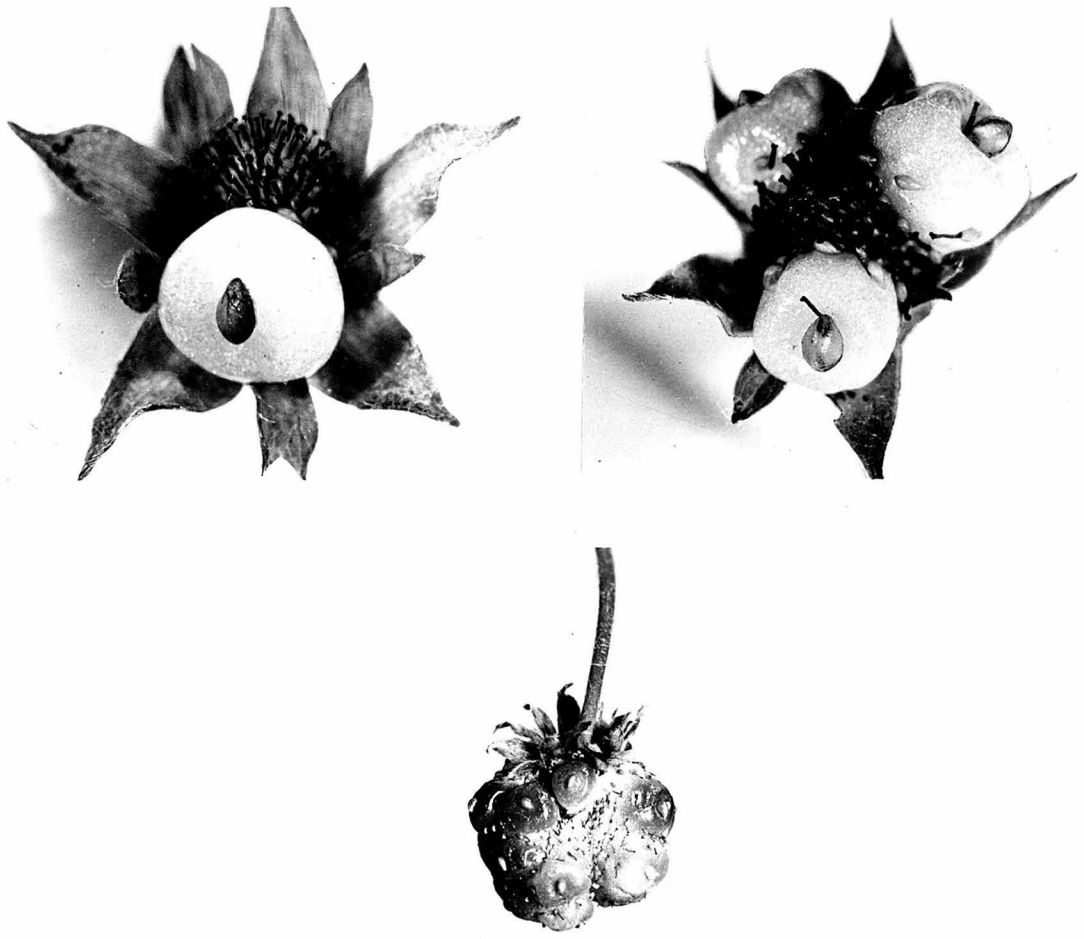


Fig. 12 (upper left). Growth of strawberry receptacle induced at one fertilized achene (magnified 3 times).

Fig. 13 (upper right). Growth of strawberry receptacle induced by 3 fertilized achenes (magnified 3 times).

Fig. 14 (below). Growth of strawberry receptacle induced by many fertilized achenes.

(fig. 14) and the berry formed assumes a more normal shape. A strawberry fruit may therefore be looked upon as constituted by the summation of the small areas of fleshy tissue which have arisen around each individual achene.

The relationship between achene and receptacle growth is shown further by the proportionality between the number of fertilized achenes and the weight of the fleshy part of the receptacle (Table 4 and fig. 15).

TABLE 4

RELATIONSHIP BETWEEN NUMBER OF FERTILIZED ACHENES AND WEIGHT OF THE RECEPTACLE

Total number of achenes	Number of fertilized achenes	Weight of the receptacles	Ratio: $\frac{\text{Fertilized achenes}}{\text{Weight of receptacles}}$
125	13	220 mg.	17
126	29	610	21
76	39	590	15
145	92	1,665	18
165	117	2,500	21
173	123	1,720	14
200	127	2,715	21
232	131	2,300	17

Further evidence in favor of the view that fertilized achenes directly control the growth of the receptacle lies in the following experiments. Achenes were removed in such a way as to leave only two or three narrow rows forming a ring around the whole "berry". When a vertical ring of achenes

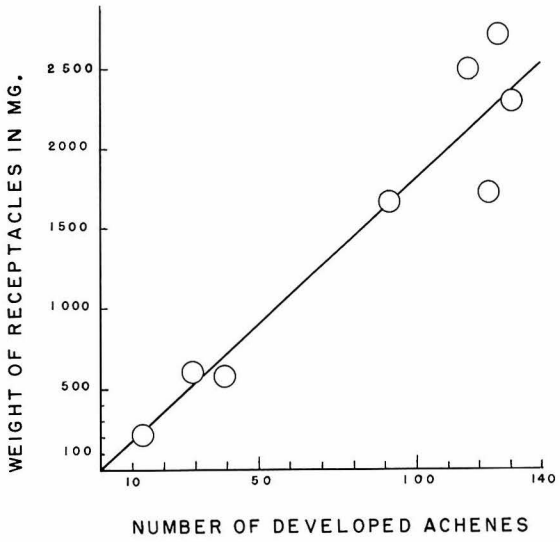


Fig. 15. Proportionality between the number of developed achenes and the weight of the receptacles of strawberries of the same age.

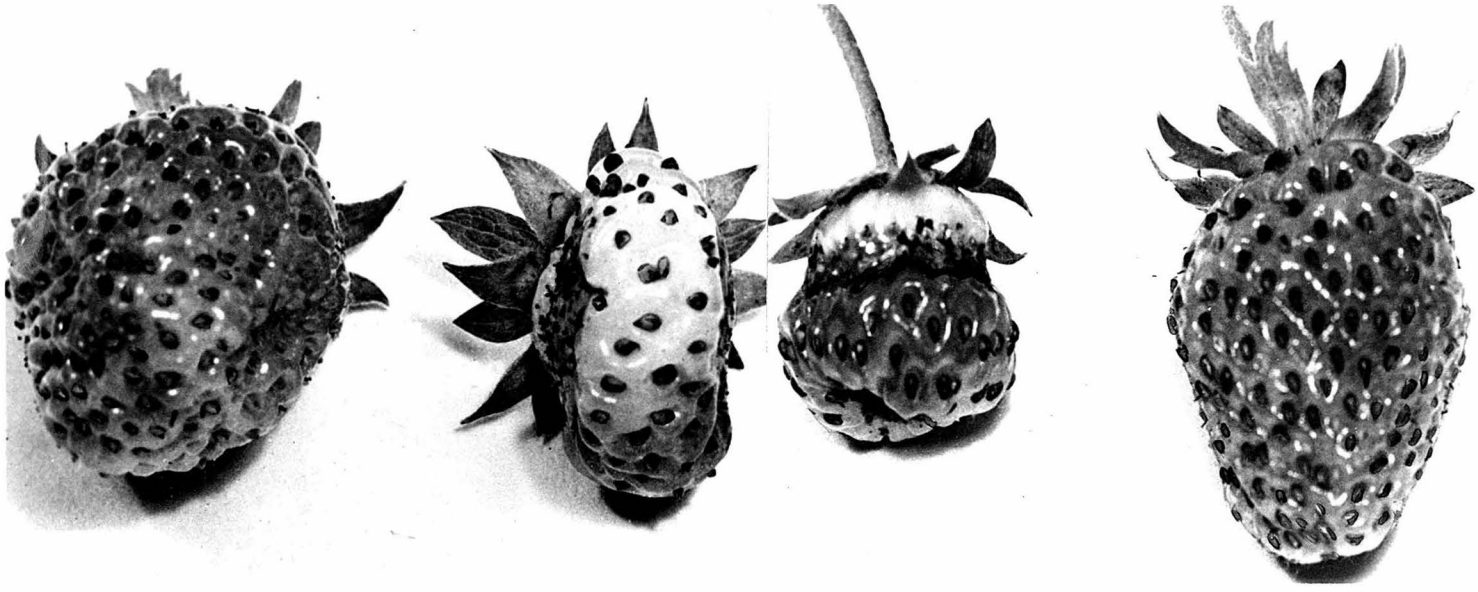


Fig. 16 (left). Growth induced by 3 rows of achenes left in a vertical position. Control strawberry at left.

Fig. 17 (right). Growth induced by 2 rows of achenes left in a horizontal position. Control strawberry at right.

was left, a flat strawberry developed (fig. 16). If the achene ring was left in a horizontal position, a short thick strawberry resulted (fig. 17). Depending upon the position of the remaining achenes, almost any shape of "berry" can be obtained (fig. 18). Control strawberries on the same plant which did not have the achenes removed developed normally.

c) Interpretation of these results.

The related experiments on the strawberry are in agreement with results obtained with other fruits. As early as 1898, Müller-Thurgau observed that in a given variety of grapes, the flesh-weight increases with increasing seed number. Kobel (1931) reports in his book that apples with many seeds grow larger than apples with only a few seeds. The shape of the apple may also be affected by the abortion of some seeds, growth being reduced around the undeveloped ovules (Roberts, 1946). In cucumber, Tiedjens (1928) observed that "uneven distribution of seed necessarily produces an irregularly shaped fruit".

The results obtained with the strawberry are therefore also representative of other fruits, so that it may be said that the growing seeds directly control the growth of the ovary. The next step now is to see how these seeds stimulate ovary growth.

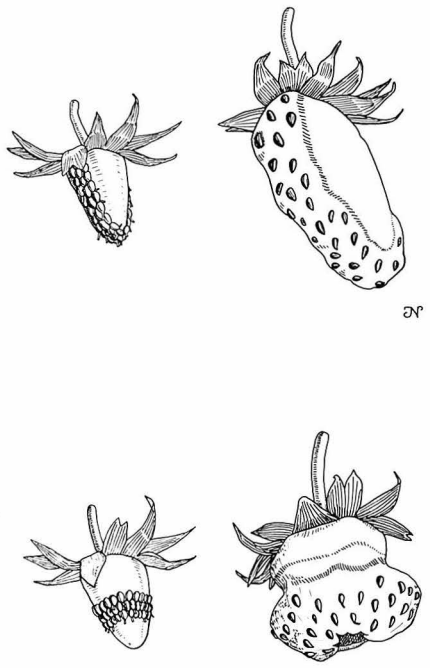


Fig. 18. According to the position of the 3 rows of achenes left on the young strawberry, a long and flat or a thick and short strawberry can be produced.

2) Auxin is the active principle released by the seeds.

a) The developing seeds produce auxin.

It has been suggested (Nitsch, 1949a) that some substances of a hormone nature diffuses from the achene to the receptacle and stimulates its growth. To investigate this hypothesis, achenes were separated from the receptacles at 3, 6, 12, 17, 20, and 30 days after pollination and 25 to 200 mg of lyophilized material were analyzed for free auxin by acid-ether extraction at 0°C. according to the method of Wildman and Muir (1949).

As shown in Table 5, no free auxin could be extracted from the receptacles at any stage of development (Nitsch, 1950a). IAA added to the receptacle tissues before ether extraction was subsequently quantitatively recovered, indicating the absence of ether extractable inhibitors of the Avena test. In contrast to the lack of free auxin in the receptacles, the achenes were rich sources of auxin. This is in agreement with the reports of other workers. Actually, developing ovules constitute such a good source of auxin that Haagen-Smit et al. (1946) used immature corn kernels as a material for extracting large quantities of a natural plant hormone which was identified as IAA. There is therefore no doubt that developing seeds produce auxin.

TABLE 5

FREE AUXIN CONTENT OF THE MARSHALL STRAWBERRY AND ITS VARIATION WITH THE STAGE OF DEVELOPMENT.

Age in days after pollination.	Auxin per 100 mg. dry weight (in gammas x 10^{-3} of IAA)		Auxin per strawberry (in gammas x 10^{-3} of IAA).
3 days	achenes	35	3.6
	receptacles	0	--
6 days	achenes	204	43.5
	receptacles	0	--
12 days	achenes	320	127.0
	receptacles	0	--
17 days	achenes	113	65.3
	receptacles	0	--
20 days	achenes	80	58.3
	receptacles	0	--
30 days	achenes	50	45.0
	receptacles	0	--

b) The effect of the seeds can be duplicated by growth regulators.

Several workers (Gardner and Marth, 1937, Hunter, 1941, and Swarbrick, 1943) have reported that spraying unpollinated

strawberry flowers with synthetic growth substances induces growth of apparently normal strawberries. Since unfertilized achenes do not induce growth around them, yet the receptacles grew in the cited experiments as a result of the hormone treatment, it seems probable that the function of the achene in causing growth of the receptacle is to supply it with auxin.

This view is supported by the following experiment. Strawberries were pollinated and allowed to develop for 9 days. At this time all the achenes were removed from some of the "berries" which were coated with a lanolin paste containing 100 p.p.m. of NOA. Others were coated with lanolin alone (containing as in the other case 1/3 of distilled water by weight). The paste was partially replenished around the berries two or three times during the duration of the experiment. Strawberries with intact achenes developed and ripened normally. Strawberries in which the achenes were removed and replaced by lanolin alone failed to grow or to ripen. On the contrary, strawberries in which the achenes were replaced by lanolin containing the growth substance developed and ripened like the controls. These results are shown photographically in fig. 19, and graphically in fig. 20 in which the growth in diameter of the "berries" is plotted against days after pollination. Other growth substances, such as IBA (0.3% in lanolin) worked as well as NOA. IAA was not used because

Fig. 19. Strawberries of the same age: (left), control, (middle), strawberry which had all its achenes removed and replaced with lanolin alone, (right), strawberry which had all its achenes removed at the same time but replaced with a lanolin paste containing 100 p.p.m. of NOA.

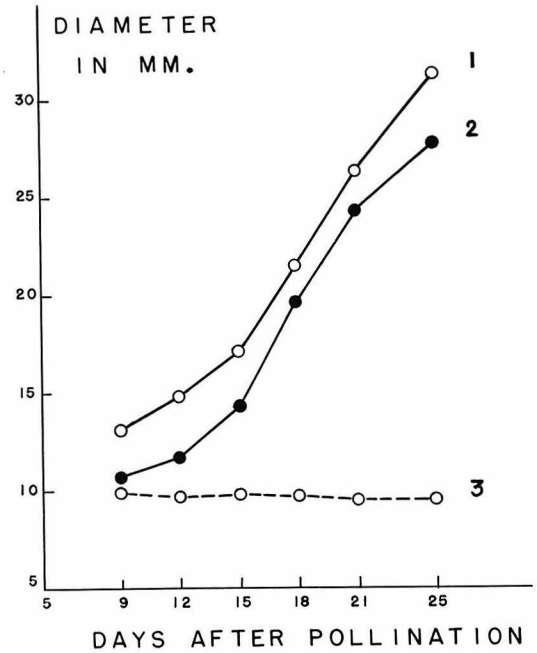


Fig. 20. Growth curves of the three strawberries shown in fig. 20. No. 1: control. No. 2: strawberry in which all achenes were removed and replaced with a lanolin paste containing 100 p.p.m. of NOA. No. 3: strawberry in which all achenes were removed and replaced with lanolin alone.

of its rapid inactivation.

In conclusion, these results show that growth substances can replace the achenes in regulating the growth of the strawberry.

3) Genesis of auxin in seeds; role of the endosperm.

The auxin level in developing seeds is not constant, as shown in fig. 21. The auxin content which gives the variations of free auxin with time increases sharply until about 12 days after pollination at which time a peak is reached. The auxin content then decreases and the curve levels off. A similar pattern of auxin content in developing seeds has been demonstrated by Avery et al. (1942) and Stehsel (1949) to exist in the corn kernel in which the free auxin production reaches a peak around the milk stage. Hatcher (1945) found a similar picture in the rye kernel, and Judkins (1945) detected an increase in auxin content in the tomato as the fruit enlarges, followed by a decrease toward ripening.

The variations in the free auxin level in strawberry and other seeds suggest that this auxin level is associated somehow with the development of ovular tissue. Randolph (1936) has investigated the anatomical changes occurring in the developing corn kernel. At the time of maximum free-auxin content the endosperm is very actively digesting the nucellus. Luckwill (1948) correlated endosperm development and auxin content of the apple seed. He further extracted

separately the embryo and the endosperm for auxin and found that the endosperm contains from 8 to 20 times more auxin than the embryo, thus confirming Hatcher's finding that 99.5% of the total auxin (extracted after alkaline hydrolysis at pH 10) is not present in the rye embryo but rather in the aleurone and endosperm tissues.

From these data it may be postulated that the actual site of auxin production in the seed is the endosperm. Such a hypothesis is even more likely when one considers that, except for the embryo, the endosperm is the only other tissue which arises from fertilization, the latter condition being generally a prerequisite for fruit growth.

4) The fate of auxin in the fleshy part of the strawberry.

As we have seen in the strawberry, the receptacle needs auxin to grow and receives it normally from the achenes. This auxin, however, which presumably diffuses out of the achenes, is no longer readily extractable with organic solvents from the receptacle tissues. It may be "used up" as a vitamin is in the growth process, or metabolized as sugars are during respiration, or it may become bound to a protein to form an enzyme which is necessary for growth, as proposed by Bonner and Wildman (1947). In this connection it is interesting to note that the maximum growth rate of a strawberry seems to occur not when the content of free-

auxin is at its peak in the achenes, but rather a few days later. Presumably, it takes some time for the auxin to diffuse out of the achenes and to participate in the growth of the receptacle. Moreover, growth of the receptacle even appears to slow down for awhile around the 17th day after pollination. Such a dip in the growth curve (fig. 22) could be explained on the basis of a competition between the embryo and the fleshy part of the fruit, both requiring a common food factor for growth. In fact, a similar break in the growth curve was found in apricot and plum by Lilleland (1930, 1933) and by Tukey et al. (1933, 1939) in peach and cherry. These authors showed that post-pollination growth of the cited fruits can be divided into three periods: a period of rapid ovary growth, followed by a period of embryo growth while the ovary stops enlarging, and a period of renewed ovary growth after the embryo has completed its development. It is therefore possible that, at a certain stage of fruit development, instead of stimulating ovary growth, the seeds actually inhibit it temporarily.

It would be of interest to discover the fate of auxin in the strawberry receptacle since no free-auxin could be extracted from receptacle tissues. Therefore lyophilized achenes and receptacle tissues have been subjected either to enzymatic digestion by trypsin or to NaOH hydrolysis. Both methods yielded appreciable amounts of auxin in all cases. Unfortunately it was found

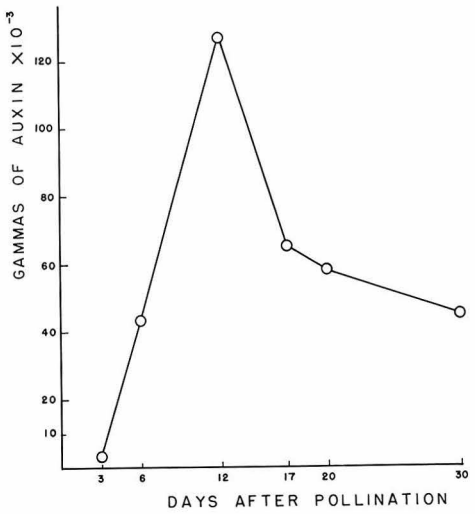


Fig. 21. Variation of the free auxin content of the achenes of the strawberry (var. Marshall) on a per fruit basis.

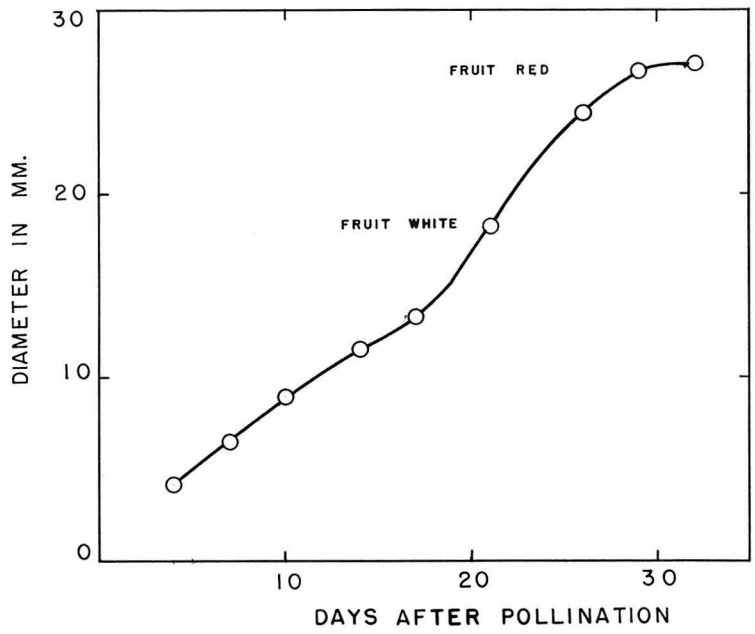


Fig. 22. Growth curve of a strawberry (var. Marshall). (The diameters given are for the receptacles only, and do not include the achenes).

that, under the same conditions, tryptophane may be partially transformed into IAA. The tryptophane content of both achenes and receptacles was also determined and found to decrease markedly through the NaOH treatment. These results confirm Schocken's view (1949) that the methods now available to measure bound auxin are equivocal. It was therefore impossible to investigate any further what happens to the auxin in the strawberry receptacle.

5) Parthenocarpy.

Having arrived at this point of our investigation, we might say that the effect of the growing seeds in controlling fruit growth is perhaps more solidly demonstrated than the effect of the pollen. One might then pertinently ask: what about seedless fruits? Since young seeds play such an important role in fruit growth, how can we find seedless oranges, grapes or pears?

A seedless fruit may be produced in a number of ways, especially:

a) through early abortion of the seeds,
b) by external stimuli (cold treatments, applications of growth substances).
c) by pollination without fertilization,
d) without any pollination.

The term "parthenocarpy" was introduced in 1902 by Noll to designate fruit formation without pollination or other external stimulation. Winkler (1908) defined parthenocarpy as the production of fruits without seeds or with empty seeds. Today,

parthenocarpy is generally understood as the formation of fruit without fertilization. This definition is less precise than Noll's and includes the possibilities b), c) and d) listed above. In practice, when investigating a seedless fruit, it is often difficult to decide if even some seed development has taken place but with an early abortion of these seeds.

In nearly all cases investigated, parthenocarpy could be traced back to some cause which increased the auxin content of the ovary. The best demonstration, of course, is the work of Gustafson (1936) who found that growth substances applied to unpollinated tomato, tobacco and other ovaries induce the formation of normal sized fruits. The original method was to cut off the style of the flower and to apply to the cut surface a lanolin paste containing the growth substance. It was later found that sprays of aqueous solutions, fumigations with volatile growth substances or even applications of chemicals to the soil could induce parthenocarpy (Hitchcock and Zimmerman, 1935, Hoffman and Smith, 1949), thus showing that growth regulators can move upward with the ascending sap.

An experiment of Nitsch (1947) also demonstrates that growth substances can move in the direction of stem--flower at physiological concentrations. An aqueous lanolin paste containing 7 p.p.m. of the sodium salt of 2,4-D and another containing only 3.3 p.p.m. of the free acid 2,4-D were smeared at the base of the flower clusters. The stamens and styles of the flowers were removed as the

flowers opened to prevent pollination, and the lanolin paste was replenished once, about two weeks after the first application. Seedless fruits developed on the treated clusters (fig. 23) whereas no fruit whatsoever was formed on the control clusters, either on the same plants or on other plants. The results of such an experiment may thus explain why parthenocarpy occurs sometimes on very vigorous plants. Maximum vigor may be associated with optimum production of auxin, which diffuses to the flower ovaries and stimulates their growth, even though no pollination or only pollination without fertilization has taken place. The case of parthenocarpy following cold treatments may also be related to some change in auxin metabolism as has been suggested in the first chapter.

Last is the case of parthenocarpy in bananas, oranges, etc., in which there is no external application of growth substances, no cold treatments, and not even incomplete fertilization (D'Angermond, 1912, Wong, 1939). Gustafson (1939) showed that the flower buds of seedless oranges, lemons and grapes contain a higher level of auxin than the flower buds of comparable seeded varieties. This higher level of auxin in some plants may be controlled by the genetic constitution. In fact, Lesley and Lesley (1941) obtained a tomato plant which had about 1/3 of a chromosome deficient. This plant and its aneuploid derivatives were very fruitful and produced parthenocarpic fruits when pollination was prevented.

6) Summary and conclusions.

The experiments of achene removal in the strawberry related in this chapter demonstrate that the young seeds directly control fruit growth. It has been shown that auxin can be extracted from the seeds in large quantities. Furthermore, growth substances applied to strawberries with removed achenes can replace the seeds in promoting the growth of the receptacle. In other plants parthenocarpic fruits may be obtained by increasing the auxin level in the ovary, either directly or indirectly by environmental or genetical changes. It seems therefore established that the seeds control the growth of the fruit by means of the auxin which they release. That auxin really plays in this case the role of a hormone can be concluded from the definition of Bayliss and Starling (1904) since auxin is a "substance produced in one organ (the ovules) and carried to another part (the ovary wall) where it brings about a specific physiological reaction" (the growth of a fruit).

CONCLUSION OF THE FIRST PART.

In Chapters I to IV, we have followed, step by step, the growth of the ovary into a fruit. Unlike a vegetative shoot, an ovary does not enlarge continuously, but goes through a succession of phases which we have called: flower initiation, ovary differentiation, ovary growth by cell multiplication (until fertilization), and ovary growth by cell enlargement (after fertilization) under the combined influence of pollen and ovules. The onset of these phases is in general determined, not by gross nutritional factors, but by factors applied in minute quantities, such as the flowering hormone, the auxin in the pollen, and the auxin from the developing seeds.

This does not mean that nutritional factors are not important. Indeed, it has been known for a long time that the nutritional level of the mother-plant affects fruitfulness. For example, Gustafson and Stoldt (1936) found that in the tomato the size of the fruits increased with the leaf area per fruit. In cucurbits we observed a very striking fact. A few days after a squash or pumpkin fruit had been pollinated and started to enlarge, the vine almost completely stopped any further growth in length. This was especially true of the pumpkins, for in this case one single fruit was able to make this inhibition almost complete. In the small gherkin, development of a fruit inhibited growth of the lateral shoot at its axil, but this shoot started to develop again when the fruit was ripe. This

phenomenon strikingly resembles bud inhibition and apical dominance in stem growth. Tiedjens (1928) made a close study of these phenomena in cucumber, especially in connection with growth of younger fruits. He found that developing fruits interfere with the growth of the fruits initiated later. He observed further that if this interference occurs at the time of pollination of the younger fruits, only the ovules located at the base of the flower grew; the malformed fruits so obtained are called "nubbins". On the other hand, if inhibition occurs only about 10 days after pollination then both ends of the fruit enlarged; the proximal end grew because it was closer to the food supply, and the distal end grew because it had received more pollen, but the middle part remained thin, and a "wasp" was produced. These experiments are interpreted in the scheme represented in fig. 24.

The related observations indicate that the study of the "food factor" in fruit growth may be of interest. Unfortunately, up to this date, only indirect experiments could be made. By changing the nutritional level of the whole plant it was attempted to modify the amount and kind of food moving into the ovary. For example, nitrogen or potassium applications to the soil affected the roots, the stem and leaves of the plant first, then, in turn, the fruits. It was, however, impossible to tell with accuracy if nitrogen or potassium fertilizers were needed by the fruits themselves or by the

Fig. 23. Induction of a parthenocarpic tomato by smearing the peduncle of the flower cluster with a lanolin paste containing 3.3 p.p.m. of 2,4-D.

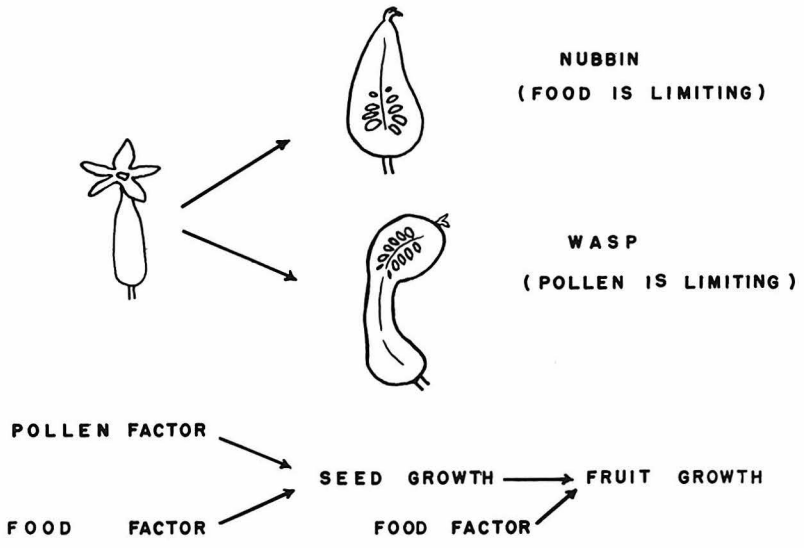


Fig. 24. Schematic interpretation of the formation of a "nubbin" (above) and a "wasp" (below) in cucumber.

rest of the plant to manufacture some more elaborated substances for the fruits. This study had come more or less to a dead-lock, and a more straightforward method was necessary to provide a fresh approach to the problem.

If we remember that it was relatively easy to study the effect of environment, pollen and ovules on the growth of the ovary because we could dissociate experimentally these factors from the ovary itself, we may think of using the same idea to study the action of the mother-plant on ovary growth. Attempts were made to separate the ovaries from the plant and to raise them in nutrient solutions. These efforts have resulted in the culture of fruits in vitro, a topic which will be discussed in the second part of this work.

PART II

IN VITRO EXPERIMENTS

CONTENTS:

I. Plant Tissue and Organ Culture.

To G. Haberlandt is generally ascribed the merit of clearly formulating the concept of tissue culture for the first time (1902). Through

consideration of the cell theory he came to the conclusion that each individual cell contains the faculty of dividing and proliferating indefinitely. His attempts to keep plant cells in the proliferating state were, however, unsuccessful. Many workers subsequently met with a similar fate. Almost all kinds of plant tissues were tried, including cells of fruit parenchyma (Börger, 1926, Pfeiffer, 1931 and 1933). Invariably the tissues did not proliferate and died after awhile. Several causes seem to have contributed to this failure. First, the techniques used were not always sterile, so that fungal and bacterial contaminations brought the cultures to a rapid end. Second, most of Haberlandt's followers used mature cells which generally do not proliferate even in vivo. Finally, it became apparent that in the gametophytes of higher plants, any one cell, when isolated, does not contain the power for indefinite multiplication.

Plant tissue culture thus remained stagnant until discoveries in another branch of plant physiology cast new light on this growth "power" in plants. F. W. Went (1926) demonstrated that apical meristems produce auxin which is indispensable for the growth of the stem tissues situated immediately below, and J. Bonner (1942) showed that leaves manufacture vitamins which are limiting factors in root growth. Thus, one part of a plant is under the control of other parts through chemical messengers called plant hormones. Any one cell is generally not capable of synthesizing all the substances it needs for growth,

but is dependent upon other cells for such substances.

These findings gave the study of plant tissue culture a new impetus. Indeed Hanning (1904), followed by La Rue (1936b) and others, already had succeeded in cultivating excised embryos, but embryos can be considered as complete plants. The culture of isolated roots, achieved by Robbins (1922), Kotte (1922), White (1934), etc., was the first true organ culture. The unlimited culture of cambiums and, through it, genuine plant tissue culture was achieved by Gautheret (1939) and Nobécourt (1939), while White (1939) attained indefinite growth with tumor tissues.

After these workers and others had established the field of plant tissue and organ culture, in vitro culture of some plant organs had yet to be accomplished. This was the case, for example, of apical meristems, leaves, and fruits. In 1945 Loo succeeded in culturing indefinitely apical meristems of Asparagus. The culture of leaves has not yet been obtained unambiguously, although cell enlargement with adenine was observed by D. Bonner and J. Bonner (1940). The last problem, the culture of excised ovaries and fruits, had not been undertaken at all despite nearly half a century of tissue culture work. Finally Nitsch (1949b, c, and d), soon confirmed by Jansen and Bonner (1949), reported growth of excised tomato ovaries in vitro. These results were extended to include other fruits such as gherkins, beans and strawberries (Nitsch, 1950). Fruits have thus to be added now to the list of plant organs that have been grown in vitro. Such a

result has been achieved partly through the use of very young ovaries that are still capable of active proliferation, and partly through the use of improved cultural techniques. It is therefore useful to describe these techniques in some detail.

II. The Technique of Fruit Culture

1) Plant material.

The following commercial varieties of plants have been used in the experiments reported below: tomato (Lycopersicon esculentum Mill.) vars. San Jose Canner and Essex Wonder; gherkin (Cucumis anguria L.); bean (Phaseolus vulgaris L.) var. Red Kidney; tobacco (Nicotiana tabacum L.) var. Cuba White; and strawberry (cross between Fragaria chiloensis and F. virginiana Duchesne) var. Marshall. These plants were grown under climatic control in the Phytotron, which gave a material of good uniformity. Since there are no insects in this laboratory, the flowers had to be pollinated by hand in the case of the gherkins and the strawberries, but occasional shaking was enough to ensure the pollination of the tomato, tobacco, and bean flowers.

2) Sterilization of the flowers.

As the flowers were cut from the plants the tip of the pedicels was dipped immediately into liquid paraffin to seal the cut end. They were then placed in sterile jars, and all subsequent operations were performed in a sterile room.

The sterilization of a living plant organ as fragile as a flower offers many difficulties. Various methods have been tried with varying degrees of success. For example, since a gas would have the advantage of penetrating easily into the many cracks that lie between the various flower parts, ethylene oxide, which is known to be a very good germicide [1], was used in some experiments at the concentration of about 1 part per thousand in air. Unfortunately, ethylene oxide proved to be also very toxic to the flowers, so that none survived even when the temperature was lowered to 10°C., the exposure time reduced to 20 minutes, or the flowers harvested at midnight to obtain a material with closed stomata. A comparison between several other disinfectants which are less injurious to plants was made. Table 6 shows

TABLE 6

STERILIZING ACTION OF DIFFERENT TREATMENTS.

The tests were made on the tomato juice-agar medium which supports the growth of many yeasts, molds and bacteria.

Disinfectant.	Concentration.	Time.	Percentage of infected cultures.
Zephiran chloride	1 part in 5,000 parts of water	30 min.	50%
Zephiran chloride, then $Ca(OCl)_2$	id. 5%	30 min. 10 min.	10%
Ethyl alcohol, then $Ca(OCl)_2$	70% 5%	15 sec. 10 min.	12%
$Ca(OCl)_2$ alone	5%	10 min.	10%

---

(1) The author is indebted to Professor A. Goetz for pointing this out and for kindly supplying the ethylene oxide.

the results of such an experiment.

The procedure which was found the most satisfactory and which has been used in most of the reported experiments is as follows. A decanted or filtered calcium hypochlorite solution (5% of $Ca(OCl)_2$, C.P. in water) is poured into the jar containing the flower material. After about 10 minutes the hypochlorite is poured off and the flowers are washed twice with sterile water and transferred to sterile Petri dishes containing two discs of filter-paper in order to absorb the excess water.

The case of the strawberries is a special one. Strawberry flowers and young fruits are so sensitive to disinfectant treatments, that it is preferable either to grow sterile strawberry flowers on the plant or to sterilize only the flower stalk, the flower itself remaining unsterilized but being prevented from dropping any spores into the medium.

3. Planting.

After disinfection of the flowers, the paraffined ends of the latter are cut off with a sterile scalpel. In the case of the tomatoes, this cut is made just above the abscission layer to prevent abscission phenomena from interfering with the growth of the ovary. The flowers are then planted on nutrient media. A variety of types of containers has been used, but test tubes have been found to be the most handy in many experiments. When liquid media were used the flowers were supported by glass-wool, or more often by discs of filter-paper provided with a central hole to allow the pedicel to plunge into the solution

below (figs. 25 and 34). Liquid media were used when any addition of auxin as an impurity in agar was to be avoided. Otherwise, the media can be conveniently solidified by 0.75 to 1.0% of agar. In the case of tomatoes care must be taken to avoid any film of liquid remaining between the sepals and the ovary, since this seems to cause the ovary to turn brown and die, perhaps by plugging up the respiratory pores situated at the base of the ovary, as shown by Clendenning (1948).

The cultures were generally incubated on shelves in a controlled temperature room (day: 23°C., night: 17°C.) where they were exposed to indirect sunlight. Others were put in the dark. In some experiments the ovaries were transferred to fresh media after about two weeks, since the plants had generally only about 25 cc. of medium at their disposal in the test tubes.

4) Nutrient media.

Since nothing is known about the nutrient requirements of excised fruits, it was thought that, to start with, it was probably preferable to use complex mixtures in general, and the juice of the fruits themselves in particular. This is why tomato juice was added initially to the nutrient media. In the case of tomatoes, for example, the pulp of a given weight of green or red tomatoes was added to the same weight of the basal solution described below, supplemented with thiamin (1 mg/l) and cysteine hydrochloride (10 mg/l). The pH of such a medium was around 4.1.

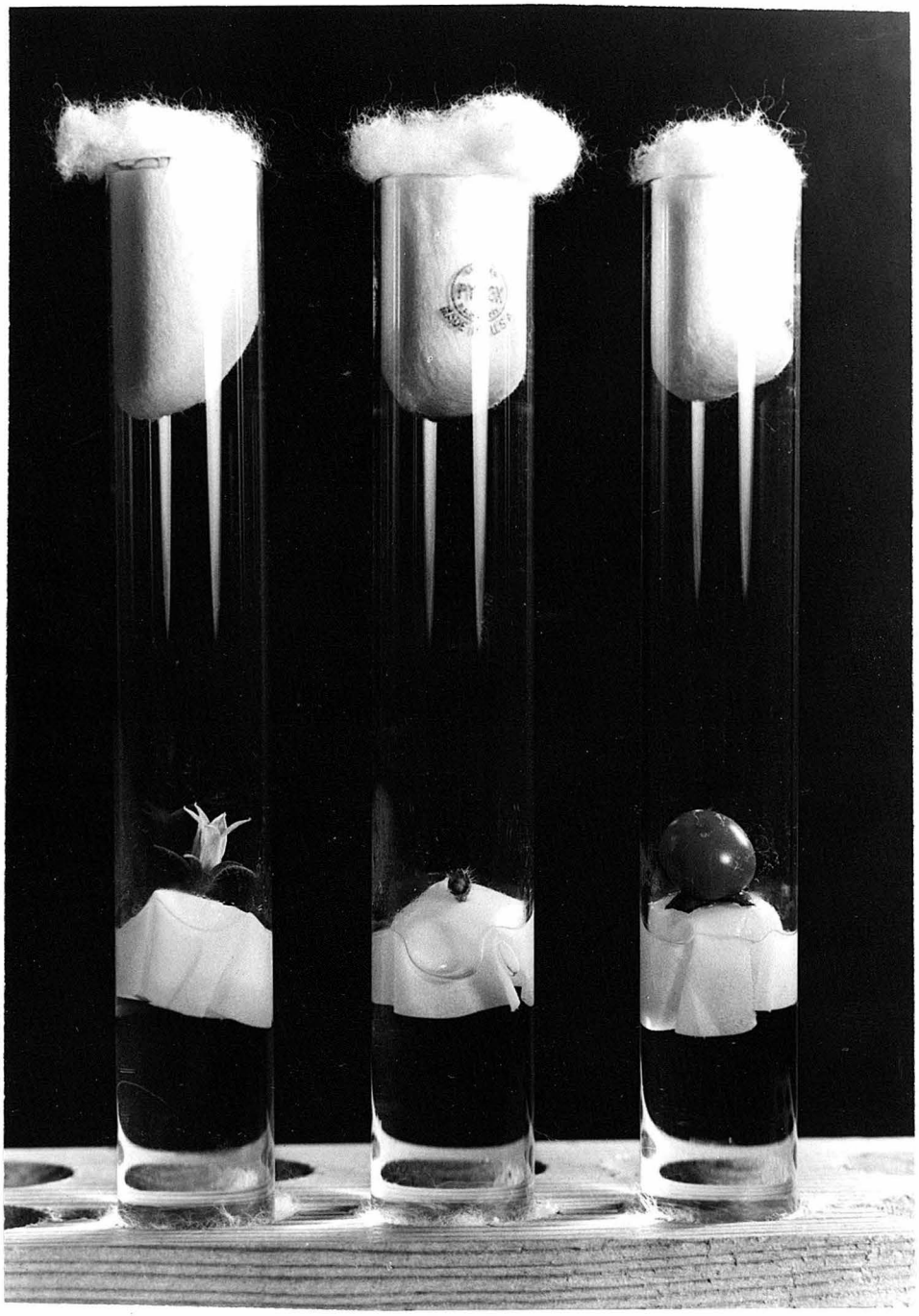


Fig. 25. A typical aspect of fruit culture. Left: a pollinated tomato flower. Middle: an ovary at the beginning of an experiment (the petals, stamens, and part of the sepals are usually cut off). Right: a pollinated ovary grown after 6 weeks of culture.

After it was found that excised ovaries could grow in vitro on such a medium, it was attempted to determine what would be the minimal medium on which such ovaries could grow. All the organic compounds were successively removed, with the exception of sucrose, and it was found that such a medium would still support the growth of excised fruits provided they had been pollinated two or more days before their separation from the mother-plant. The importance of the inorganic constituents of the medium used, on the other hand, has not yet been studied. Such a study has been done for whole plants by many workers, and their results were used to compose the following basal solution.

The basal medium consists of water, mineral salts, trace elements, and sucrose. The water which was used throughout the experiments had been redistilled in Pyrex glass to eliminate toxic salts (mainly Cu salts). Several salt-mixtures have been used, especially the following simplified Knop mixture:

$Ca(NO_3)_2 . 4 H_2O$	500 mg/l
KNO_3	125
$MgSO_4 . 7 H_2O$	125
KH_2PO_4	125

The trace elements were prepared in two solutions. The first consisted of a ferric-citrate solution containing 25 mg of $FeC_6O_5H_7 . 5 H_2O$ per cc. The second was a composite solution which includes several ions which have been shown to be essential for the growth

of higher plants. These are: Mn (Mazé, 1914), Zn (Mazé, 1914), B (Sommer and Lipman, 1926), Cu (Sommer, 1931), and Mo (Arnon and Stout, 1939). The trace element solution has the following composition (per liter):

H_2SO_4, sp. gr. 1.83	0.5	cc
$MnSO_4 \cdot 4\ H_2O$	3,000	mg
$ZnSO_4 \cdot 7\ H_2O$	500	mg
H_3BO_3	500	
$CuSO_4 \cdot 5\ H_2O$	25	
$Na_2MoO_4 \cdot 2\ H_2O$	25	

In summary, one liter of the basal medium contains:

1) The mineral salt mixture,
2) 1 cc of the ferric citrate solution,
3) 1 cc of the trace-element solution,
4) 50 gr of sucrose.

To this medium were eventually added several concentrations of various growth substances, vitamins, amino acids, purines, and plant extracts. Only a part of these experiments will be reported in this thesis, because many of the chemicals tried did not stimulate growth in an appreciable manner.

Finally, one point which received special attention was the regulation of the pH of the solution, since the effect of growth substances seems to vary with their degree of dissociation. Following the suggestion of Vacin and Went (1949), the addition of tri-calcium phosphate to the solution was tried, but this was later abandoned since Gautheret

(1947) had shown that plant tissues in culture maintain the pH around 6.0, provided that the pH is adjusted to that value at the beginning. The pH of the nutrient solutions was therefore adjusted to 6.0 with KOH or HCl before autoclaving. It was then poured in test tubes which were closed with cotton plugs. The prepared tubes were covered with paper and autoclaved 15 minutes at 15 lbs. pressure.

5) Measurements.

The diameter, or in the case of beans, the length of the fruits, was found to constitute a suitable measure of growth under the conditions of the reported experiments. Diameter measurements to the nearest 0.5 mm were made through the glass with a translucent plastic ruler. Since the tomato ovary is roughly a sphere, an increase in diameter from 4 to 24 mm (500% in diameter) as has been observed with some of the San Jose tomatoes, corresponds to an increase in volume from 33 to 7,234 mm^3, or 21,800%.

III. Experiments on the Growth of Fruits Cultivated In Vitro.

1) General pattern of the growth of excised fruits.

a) Development of the ovary.

As an example of the growth of an excised ovary in sterile culture, let us consider the case of a tomato flower of the San Jose Canner variety which was excised about two days after the flower opened and was planted on a tomato juice medium.

After 5 to 7 days the petals and the stamens are pushed upward by the growing ovary, which then enlarges regularly, and is green and healthy looking. Three weeks after, it reaches a diameter of about 20 mm, and then growth slows down. After one more week the color changes to yellow. Finally, the tomato reaches full maturity, is deep red in color, and tastes like a vine-ripened tomato (fig. 26).

The growth curve of such a tomato fruit is given in fig. 27, and it is evident that the curve is sigmoid. The beginning of the curve, however, has not been drawn on the figure because the ovary, hidden under the petals, could not be measured at that time. The growth curve of a tomato fruit attached to the plant is also sigmoid (Judkins, 1939), but in this case the slope of the curve is steeper than the slope of the growth curve of a test tube tomato. This indicates that the final size of tomatoes grown in vitro under the prevailing conditions was smaller than the size of normal tomatoes. Nevertheless, the general growth patterns are the same, for ovary enlargement starts to slow down and maturation occurs at about the same time in both cases. Thus it may be said that tomatoes cultivated in vitro retain the general growth pattern of a fruit grown on the plant.

Attempts were made to culture in vitro other fruits, such

Fig. 26. A San Jose Canner tomato grown on tomato juice medium for about 1 month. The petals, which had not been removed at planting, stayed on top of the ovary.

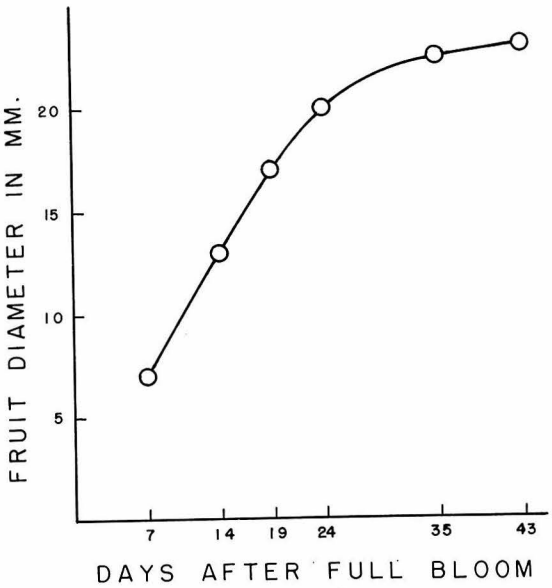


Fig. 27. Growth curve of a San Jose Canner tomato grown on tomato juice medium.

as gherkins, beans, strawberries, etc. When a gherkin ovary, excised two or more days after pollination, is planted on the basal medium, it will grow, but more slowly than a tomato ovary, and much slower than fruits left on the vine. After a month of culture on the basal medium it will reach about twice the diameter it had when it was planted. The appearance of such a gherkin fruit grown in vitro is shown in fig. 28. Bean flowers, when planted after pollination, will rapidly develop a young fruit of 10 to 15 mm length (fig. 29), but this fruit will almost stop growing afterwards. Young strawberry fruits, excised 7 to 12 days after pollination, enlarged on the basal medium and turned red like normal strawberries. However, the size of the fruits was less than those left on the plant. Finally, some growth was obtained with fertilized tobacco ovaries, also on the basal medium, but the seeds did not develop completely.

b) Development of accessory tissues: roots and calluses.

Aside from the growth of the ovary itself, development of various organs, especially sepals, roots and calluses, has been observed. Root formation on the flower pedicel has been rarely reported. It occurs readily, however, on the pedicels of the flowers of Lycopersicon esculentum, in particular when

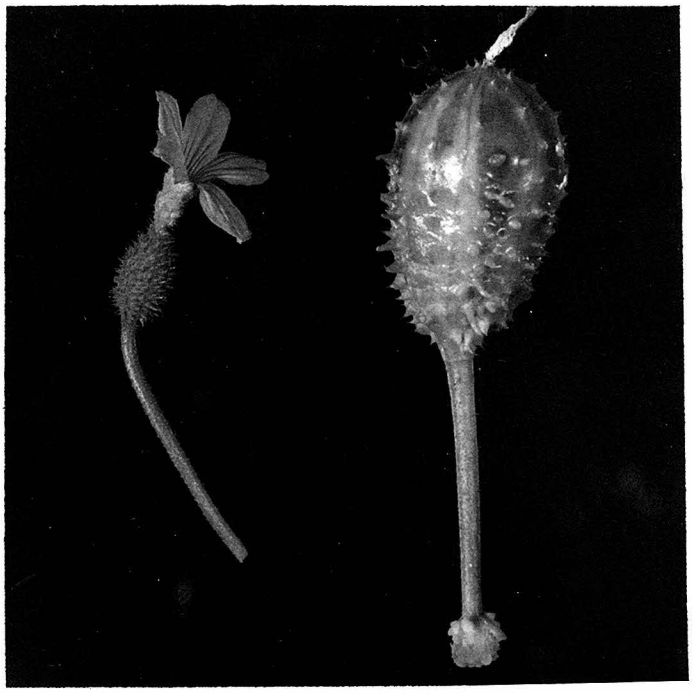


Fig. 28. Gherkin fruit grown for 1 month in vitro (note the callus at the base of the pedicel). Left: an ovary at full bloom.

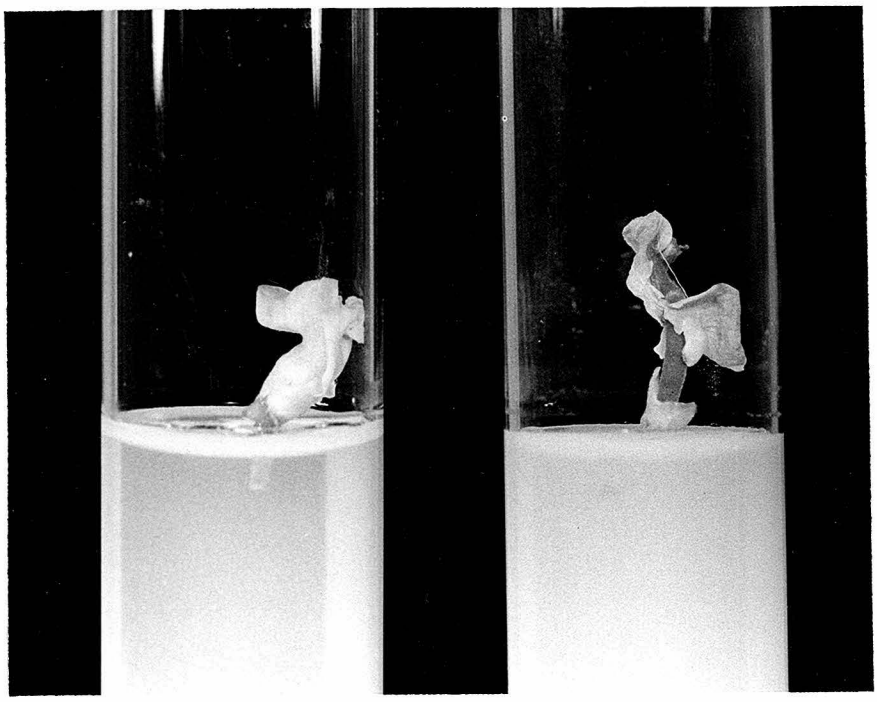


Fig. 29. Development of a Red Kidney bean in vitro. Left: flower at planting. Right: the fruit 10 days later.

the cultures are placed in the dark or subjected to reduced light. Such an effect has been reported by Galston (1948) for excised Asparagus stem tips cultivated in vitro when IAA was added to the medium. With tomato fruits, however, no growth hormone had to be added to the basal medium to cause root formation in the dark, but thiamin and other vitamins seemed to be beneficial, an effect similar to that encountered by Robbins and Bartley (1937), White (1937), and Bonner (1937) with isolated root cultures. The difference in root formation between tomato ovaries planted four days after anthesis or one day before anthesis was in the speed at which roots appeared macroscopically, but both series eventually reached 100% rooting. For example, three weeks after planting 77% of the cultures of fruits excised four days after full bloom had roots, whereas only 22% of the fruits excised one day before full bloom showed roots. Five weeks after planting, however, the latter series also had 100% of rooted cultures. A photograph of a sample of each series is shown in fig. 30. It seems, therefore, that although a tomato flower contains enough auxin to initiate roots regardless of whether it has been pollinated or not, it is possible that the auxin content of the ovary increases four days after pollination, so that root initiation occurs more readily. The effect of this root system on ovary growth is not yet very clear. While roots are forming, ovary

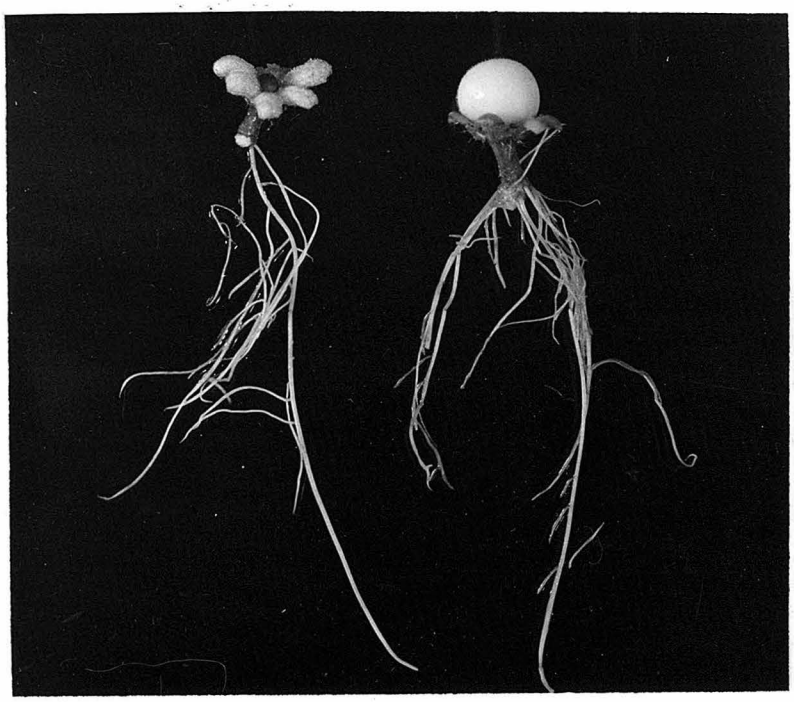


Fig. 30. Rooting of Essex Wonder tomatoes grown in the dark for about 1 month. Left: ovary excised before full bloom and which died. Right: ovary excised 4 days after anthesis.

Fig. 31. Essex Wonder tomato flowers (top row) and ovaries (bottom row) at various stages of development. From left to right: 2 days before anthesis, anthesis, 2 days and 4 days after anthesis.

growth seems to slow down somewhat, but after the roots have reached an appreciable size they seem to contribute to the development of the whole flower, that is the sepals become much greener if the cultures are placed in the light and the ovary grows somewhat better. Root formation has also been observed in excised gherkins and beans. In the latter case, para-chlorophenoxyacetic acid stimulated root formation, as shown in Table 7.

TABLE 7

ROOT FORMATION IN RED KIDNEY BEANS CULTIVATED IN VITRO

Ovaries excised about one week after anthesis and grown on basal medium supplemented with thiamin (1 mg/l), cysteine hydrochloride (10 mg/l), and 1.5% agar. pH: 5.0.

Concentrations of P-CPA in p.p.m.	Percentage of rooted cultures after 3 weeks in the dark.
10	0%
1	13
0.1	60
0	14

The enlargement of the fruit pedicel into a callus has also been observed repeatedly. For example, pedicels of tomato flowers increased extensively in diameter when the basal medium was supplemented with NOA (10 mg/l), 2,4-D (10 or 1 mg/l), autoclaved coconut milk (20%), etc. These results agree with our

knowledge derived from the culture of cambiums in which the cited substances have proven very stimulatory.

2) Influence of pollination on the growth of excised fruits.

The experiments performed with the San Jose Canner tomato have shown that excised flowers of this variety grow on the basal medium supplemented with tomato juice. It was found later, however, that a small tomato would develop occasionally on the basal medium alone. It was thought that such instances of growth of ovaries on the basal medium might be related to pollination; therefore the following experiment was devised.

Tomato flowers were excised at various stages of development, before and after pollination. The Essex Wonder variety was used instead of San Jose Canner because the latter variety may develop parthenocarpic fruits spontaneously even at rather high temperatures. The Essex Wonder tomato does not produce parthenocarpic fruits spontaneously at the temperature at which the plants were grown--23°C. (day) and 17°C. (night)--, as shown by Went (1951). The stages at which the flowers were excised, and which are represented in fig. 31, correspond to the following steps in flower and fruit development:

a. 2 days before full bloom; petals just starting to separate at the tip.

b. full bloom; petals curved downward.

c. 2 days after anthesis; petals curved upward again.

d. 4 days after anthesis; petals closed and ready to fall off.

Flowers at such stages have been cultivated on the basal medium and the diameter recorded 20 days after planting. The results of such an experiment are shown in Table 8.

TABLE 8

EFFECT OF THE STAGE OF FLORAL DEVELOPMENT

ON THE GROWTH OF TOMATO OVARIES IN VITRO

Stage of floral development	Number of ovaries	Average diam. at planting mm.	Average diam. after 3 weeks mm.	% increase in diam.
2 days before anthesis	6	1.92 ± 0.08	2.42 ± 0.08	26
anthesis	7	2.07 ± 0.07	2.86 ± 0.17	38
2 days after anthesis	5	2.10 ± 0.10	4.90 ± 0.24	133
4 days after anthesis	7	3.50 ± 0.22	8.14 ± 0.57	133

The table shows that flowers collected before or at full bloom do not grow appreciably on the basal medium. On the other hand, only 2 days after full bloom, ovaries having the same initial diameter of 2 mm grow to almost 5 mm in diameter on the same solution. This is also true for flowers planted 4 days after pollination but it may be noted that the percentage of increase is not greater. The age of the ovaries is not the determining factor because if flowers are emasculated and left on the plant protected from pollination when they are planted in test tubes they do not grow appreciably on the basal medium.

An experiment of the same type has been performed with flowers of the small gherkin because, on account of separation of male and female flowers, they are even easier to protect from accidental pollination than the tomato flowers. Ovaries of pollinated gherkin flowers, which had been excised two days after full bloom, enlarged from an average of 5.8 mm in diameter to 11.0 mm after 24 days on the basal medium, which was renewed once (25 cc each time). This represents an 89% increase in diameter. In contrast to the growth of the pollinated ovaries, the unpollinated ones did not enlarge to the slightest degree, although they remained green and healthy looking for 3 to 4 weeks.

Both the experiments performed on the tomato and on the gherkin show that an ovary may grow in vitro on a relatively simple nutrient. The results indicate further that, even though the ovary is kept supplied with nutrient, it will not enlarge unless it has been pollinated. This conclusion is in agreement with the view expressed in Chapter III that pollen gives the ovary a definite growth stimulus in addition to preventing flower abscission.

3) Influence of seed development on the growth of excised fruits.

In view of the results presented in Chapter IV, it is of interest to find out what influence the growing seeds have on the growth of fruits in vitro. The following experiment, performed on gherkin ovaries, gives some information about this point.

For this study flowers were pollinated by hand--each one receiving the pollen of two male flowers--and were then left on the mother-plant for 1, 2, 3, 4, and 5 days thereafter. They were then excised from the vine and planted on the basal medium and transferred to fresh solution after 2 weeks. All the plants developed calluses. These calluses appeared on the pedicels of the flowers collected 3 or more days after pollination, in as few as 5 days after planting, but 8 to 9 days were required for callus development on flowers excised 1 or 2 days after pollination. The calluses were larger in the case of the flowers excised 3 or more days after pollination than in the case of the flowers harvested earlier. Fig. 32 shows the appearance of one of the fruits. These results, together with the data on the increase in diameter of the ovaries and the seed production, are summarized in Table 9. In contrast to the flowers excised shortly after pollination, the ovaries which were cut from the vine 2 or more days after pollination grew on the basal medium, which did not contain any growth substance. Undeveloped seeds, empty and with soft seed coats, were found in nearly all cases, but only the ones which had developed apparently normal seed coats are listed as "seeds" in the table. These seeds looked more or less normal (fig. 33), but when they were put on moist sand to germinate, only a few of them developed, these giving rise to apparently normal seedlings. Another result of this experiment is the observation that, when ovaries are allowed to stay on the plant longer

Fig. 32. Growth of gherkin ovaries on the basal medium (1 month of culture), when planted unpollinated (left), and 4 days after pollination (right). Note the development of a callus.

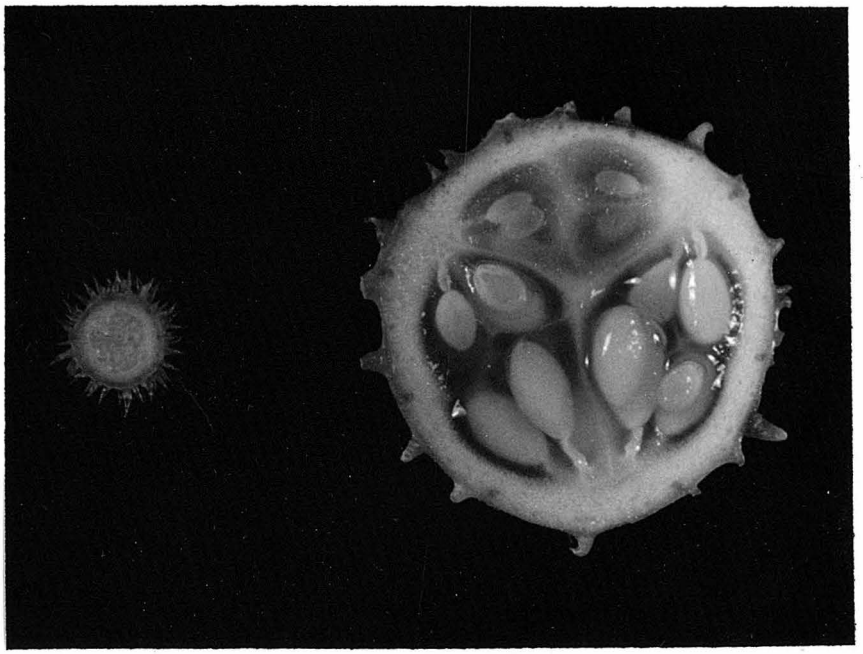


Fig. 33. Formation of seeds in gherkins cultivated on the basal medium, when planted unpollinated (left), and 4 days after pollination (right).

than 3 days, their relative increase in diameter following excision is less than the increase of ovaries collected at an earlier stage. If we consider that this smaller increase in diameter occurs in the cases in which seeds develop, then we might perhaps explain this result on the basis of a nutritional competition between seeds and ovary tissues. In fact, Tukey's experiments on peach and cherry discussed in Chapter IV, have shown that in these fruits the growth of the ovary tissues stops when the embryo is developing. Finally, it can be seen that many of the ovaries which were planted 2 days after pollination enlarged mostly at the distal end (fig. 34), thus resembling inverted "nubbins" schematized in fig. 24. It seems, therefore, that at the time the flowers were excised from the plant, the pollen tubes had not yet fertilized the most remote ovules which are located at the basal end of the flower. This fact again illustrates the effect of pollination on ovary growth.

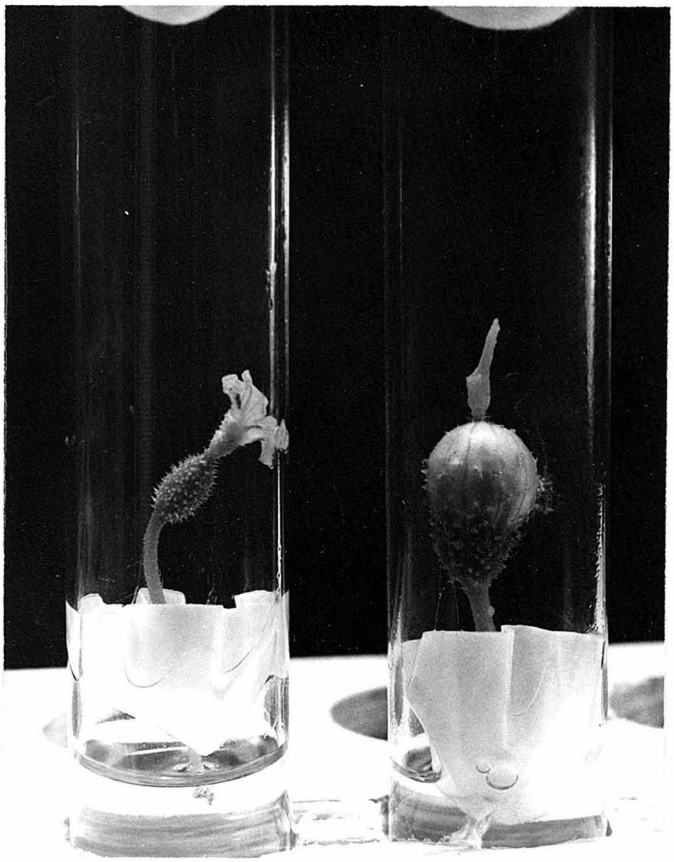


Fig. 34. Gherkin ovaries grown in vitro. Left: unpollinated female flower. Right: fruit developed after 1 month from an ovary excised 2 days after pollination. Note that the top part of the ovary (in which fertilization of the ovules had time to occur) developed more than the bottom part.

TABLE 9

EFFECT OF SEED DEVELOPMENT ON THE

GROWTH OF GHERKIN OVARIES IN VITRO

Days after poll.	Number of ovaries	Diam. at planting mm	Diam. after 3 weeks mm	% increase in diam.	No. of seeds per fruit	% germination	Calluses (after 9 days)
1	5	5.10 ± 0.24	4.80 ± 0.25	-6	-	-	+
2	3	6.66 ± 0.02	9.00 ± 0.00	35	-	-	++
3	3	7.83 ± 0.02	10.83 ± 0.002	38	4.5	0	+++
4	4	10.75 ± 0.19	14.00 ± 0.33	30	11.0	6.0	+++
5	3	11.83 ± 0.19	14.00 ± 0.33	18	21.7	7.7	+++

4) Influence of synthetic growth substances on the growth of excised fruits.

In Part I we have reviewed evidence which indicates that auxin is responsible for both the effect of the pollen and the effect of the seeds on fruit growth. It was therefore of interest to find out if growth substances could also promote the growth of unpollinated ovaries in vitro. Only the experiments performed on gherkin and tomato ovaries will be presented.

a) Experiments with gherkins

In the case of gherkins, the amount of auxin brought into the ovary by pollination was measured. The same amount of pollen that is used to pollinate flowers was allowed to germinate on sucrose-agar blocks for 2 days. The pollen tubes developed very well. These blocks were

used in the Avena test and were found to contain the equivalent of about 50 gammas/liter of IAA. This means that the amount of auxin that pollen brings into the ovary in a normal pollination could be around 4×10^{-4} gammas. If then, such agar blocks with germinated pollen are placed on the top of decapitated ovaries planted in vitro, one could expect that the auxin would diffuse into the ovary and induce the latter to grow. In fact, no swelling of the ovary was observed. An experiment with synthetic growth substances gave the same negative results. Unpollinated ovaries were planted in test tubes, and the ovary tops cut off with a sterile razor blade. Sterile agar blocks containing various concentrations of IAA (0.01, 0.1 and 1 mg/l), 2,4-D (0.01, 0.1 and 1 mg/l), and IBA (0.01, 0.1, 1, 10, 100 and 1,000 mg/l) were deposited on the cut surfaces. Other trials were made without cutting the tops but instead by smearing them with a lanolin paste containing the growth substances. As in the previous attempts, not a single ovary showed any tendency to swell or enlarge. That auxin was able to penetrate into the ovary is shown by the appearance of calluses at the base of some of the pedicels. This recalls similar observations made previously on pollinated gherkins. As fig. 33 shows, pollinated ovaries form calluses readily at the base of the pedicel, while unpollinated ones generally do not, providing that no growth substance has been added to the basal medium. When ovaries have been allowed to remain on the plant 3 or more days after pollination, these calluses show up clearly 6 days after planting. They are localized at

the very base of the pedicel, and as they grow larger they look white and rather compact. Morphologically they appear to be formed of a large number of root primordia and giant parenchymatous cells.

This side effect of pollination in the form of callus formation can be reproduced artificially with non-pollinated ovaries by use of auxins. The following example illustrates this point. Various amounts of IBA were incorporated either in the medium on which unpollinated ovaries were planted or in agar blocks placed on top of them. Figs. 35 and 36 show the general appearance of the ovaries. The results, as recorded in Table 10, show that it is possible to duplicate the callus formation observed on pollinated ovaries by application of growth substances. In order to produce a callus of the same size however, a 10,000 times greater concentration is necessary in the agar block than in the medium. This is necessary on account of the smallness of the agar block, since the results show that the effect is here more dependent on the total amount of growth substance which may eventually penetrate the tissues than on the concentration actually present outside of them, in the agar or in the nutrient medium.

In summarizing the auxin relationships of excised gherkin fruits, it may be said that: (1) pollinated gherkin ovaries, on a medium without growth substances, produce both a fruit and a callus; (2) unpollinated ovaries, without growth substances, give neither a fruit nor a callus; (3) unpollinated ovaries, with growth substances, give a callus but no fruit.

Fig. 35. Unpollinated gherkin ovaries grown for 11 days on the basal medium supplemented with various concentration of IBA (from left to right): 0, 10, 100, and 1,000 gammas/liter.

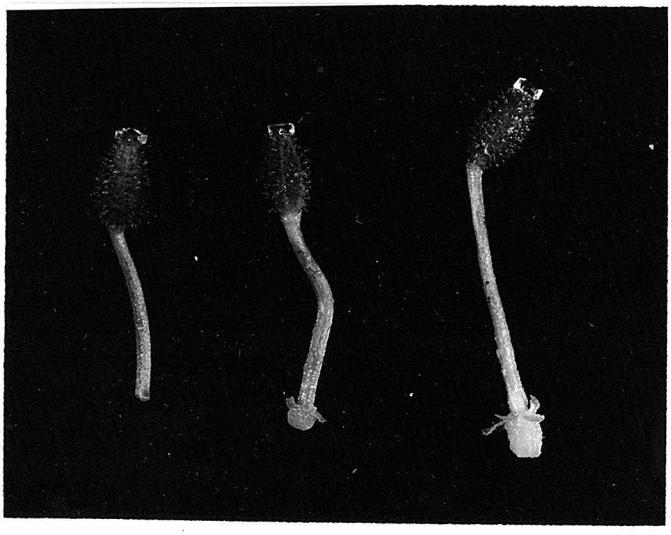


Fig. 36. Unpollinated gherkin ovaries grown on the basal medium for 14 days. Concentrations of IBA in the agar blocks (from left to right): 0, 100, and 1,000 mg/l.

TABLE 10

ACTION OF POLLINATION AND β-INDOLEBUTYRIC ACID ON THE FORMATION OF CALLUSES AT THE END OF THE PEDICELS OF GHERKINS CULTIVATED IN VITRO

Pollination of ovaries	Number of ovaries	Concentration of IBA: In the medium (25 cc)	In agar blocks (8 mm^3)	Total amount of IBA in gammas	Calluses (9 days after planting)
2 days after pollination	5	0	0	-	+
3 days after pollination	5	0	0	-	++
unpollinated	5	0	0	-	-
unpollinated	10	1 mg/l	0	25	abnormal effects(1)
id.	10	0.1	0	2.5	abnormal effects
id.	10	0.01	0	0.25	++
id.	5	0	1,000 mg/l	8	+++
id.	6	0	100	0.8	++
id.	5	0	10	0.08	-
id.	5	0	1	0.008	-
id.	6	0	0.1	0.0008	-
id.	7	0	0.01	0.00008	-

(1) Not a callus localized at the tip of the pedicel, but a swelling on the whole length of the immerged part.

The failure to induce parthenocarpy in gherkin fruits grown in vitro is surprising in view of the reports of many investigators who induced parthenocarpy in cucurbits attached to the plant. It was later found, however, that artificial parthenocarpy of gherkin fruits was unusually difficult to achieve on the vine, so that the material which has been used in the reported experiment might not have been the best. New attempts to induce parthenocarpy in vitro were therefore made with a material which responds well to application of growth substances, the tomato ovary.

b) Experiments with tomatoes.

Essex Wonder tomato flowers were excised before pollination and planted on artificial media containing various concentrations of 2,4-D, 2,4,5-T, and NOA. In each case ovaries grew in the presence of certain concentrations of growth substances, while they enlarged very little on the control medium without growth substances. As an example, the results obtained with 2,4-D and NOA are represented in figs. 37 and 38. The graphs represent the percentage of increase in diameter 3 weeks after planting. It appears that 2,4-D is about 10 times more active than NOA on a weight basis, which is in agreement with the findings on tomatoes attached to the plant that have been reported in the literature. These results

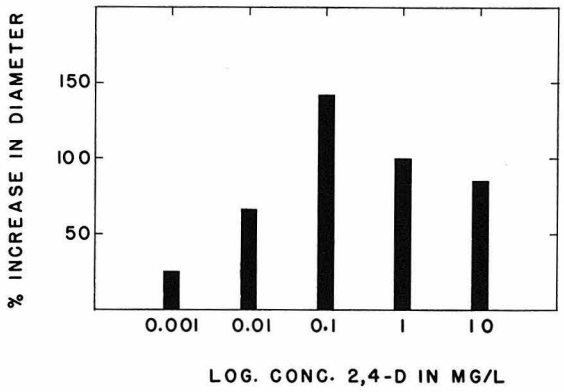


Fig. 37. Effect of various concentrations of 2.4-D added to the basal medium on the growth of Essex Wonder tomato ovaries cultivated 3 weeks in vitro.

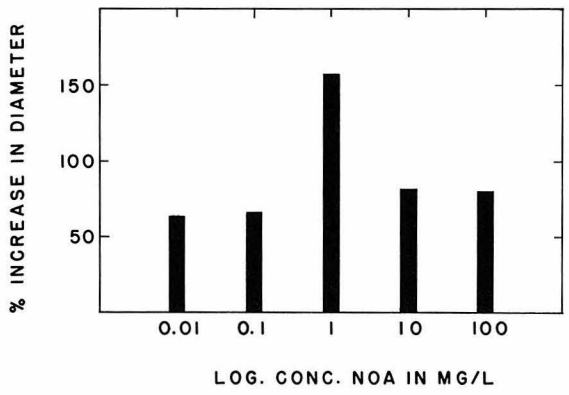


Fig. 38. Effect of various concentrations of NOA added to the basal medium on the growth of Essex Wonder tomato ovaries cultivated 3 weeks in vitro.

demonstrate that growth substances can replace the effect of pollination and seed development on ovary growth in vitro as well as in vivo.

IV. Value of the culture of fruits in vitro.

The technique of fruit culture offers a new method of approach in the study of fruit physiology. The first results of this technique, which have been presented in the second part of this thesis, show that the main characteristics of fruit growth (shape of the growth curve, ripening, action of pollination, seed development and growth substances) are not altered by in vitro culture. The size of the fruits was in general small in comparison with the size of the fruits left on the vine, but a difference in size is less important to the plant physiologist than a difference in growth pattern. Actually, no great effort has been made to grow large fruits and it is likely that, in the future, full size fruits will be obtained.

The culture of fruits in vitro, however, will probably prove useful, as it was in the case of the present investigation, as a tool to investigate such physiological problems as early embryo development, fruit maturation, fruit respiration, and even fruit diseases.

GENERAL CONCLUSIONS AND SUMMARY

In the course of this investigation, which was aimed to build a coordinate picture of the hormonal relationships in fruit growth, it has been found that auxin plays a role in nearly all the phases of the development of an ovary into a fruit: flower induction, ovary differentiation and growth before anthesis, pollination, and ovary enlargement after full bloom.

Many of the results which have been presented, however, have been obtained using synthetic substances, such as IBA, NOA, 2,4-D 2,4,5-T, and P-CPA, which are not known to exist in the plant. It is important to know if conclusions drawn from such experiments may be integrated into the same scheme legitimately. In fact, all the substances which have been used are active on several of the tests which measure auxin activity, for example the pea-test or the Avena section test. In addition, providing the correct concentrations are used, these substances can often be used interchangeably to promote growth responses characteristic of auxin action: cell elongation of stems, root initiation, cambial activity, inhibition of bud growth or of root elongation, etc. While it remains true that each growth substance has its own characteristics (transport inside of the plant tissues, activity at a given concentration, etc.), it nevertheless can be said that the bulk of their effects is similar. We feel, therefore, that

the results presented in this thesis and repeated in many instances with two or more different substances, are representative of the action of auxin in fruits. Actually, the interchangeability of synthetic growth regulators in causing certain physiological responses has puzzled many workers. The explanations given are of two types. The first is to explain the similar effects of 2,4-D, NOA, IAA, etc., by similarities in the stereochemical configuration of the molecules. Thus, various chemicals having the right "key" at one end of their molecule would play the same role by opening the same "lock" in the growth process. Another explanation of the comparable action of different growth substances is that these chemicals act upon the chain of reactions which lead to the formation of a single growth hormone in the tissues by either stimulating its production or inhibiting its destruction. Since the mechanism of action of neither the "native auxin" nor the synthetic growth regulators is known, it is difficult to give an actual proof to either one of the possibilities presented.

The results of the present investigation of the role of plant hormones in fruit development may be summarized as follows:

1) The shift of a vegetative plant to a flowering one is brought about by a mechanism in which the level of auxin in the plant appears to play a role; high levels of auxin lead to vegetative growth. The differentiation of an ovary primordium is under control of environmental factors, as it has been demonstrated in cucurbits. Low

temperatures and reduced light favor ovary formation, presumably by increasing the amount or the effectivity of available auxin.

2) Once initiated, the ovary primordium enlarges mainly by cell multiplication. When cell multiplication ceases, approximately at the time of full bloom, ovary enlargement also stops. This arrest in growth seems to be correlated with a decrease in the auxin level of the ovary.

3) In most cases an ovary will enlarge after anthesis only if pollination occurs. The immediate action of pollination on fruit growth seems to be twofold: a) prevention of flower abscission, and b) positive stimulation of growth. The relative importance of these two simultaneous effects can be demonstrated best by the technique of fruit culture in vitro, where it appears that auxin is responsible for the effect of the pollen on fruit growth.

4) Auxin is responsible also for the action of the developing seeds on fruit growth, which proceeds mostly by cell enlargement after fertilization. The site of auxin production in the seeds is most likely the endosperm.

5) A new approach to the study of fruit physiology has been devised by the realization of the culture of several kinds of excised ovaries in vitro.

REFERENCES

1. D'ANGERMOND, A. 1912. Parthenokarpie und Samenbildung bei Bananen. Ber. deut. Bot. Ges. 30:686-691.

2. ARNON, D. I., and P. R. STOUT. 1939. Molybdenum as an essential element for higher plants. Plant Phys. 14:599-602.

3. AVERY, G. S., Jr., J. BERGER, and BARBARA SCHALUCHA. 1942. Auxin content of maize kernels during ontogeny, from plants of varying heterotic vigor. Amer. Jour. Bot. 29:765-772.

4. BAYLISS, W.M., and E. STARLING. 1904. The chemical regulation of the secretory process. Proc. Roy. Soc. B 73:310-322.

5. BLAAUW, A. H., IDA LUYTEN, and ANNIE M. HARTSEMA. 1932. Grundzahl der Tulpenblüte in ihrer Abhängigikeit von der Temperatur. Kon. Akad. van Wet., Amsterdam, 35:485-497.

6. BONNER, D. M., and JAMES BONNER. 1940. On the influence of various growth factors on the growth of green plants. Amer. Jour. Bot. 27:38-42.

7. BONNER, JAMES. 1937. Vitamin B_1 a growth factor for higher plants. Science 85:183-184.

8. BONNER, JAMES, and HARRIET BONNER. 1948. The B-vitamins as plant hormones. Vitamins and Hormones 6:225-275, Academic Press Inc., New York.

9. BONNER, J., and JOHN THURLOW. 1949. Inhibition of photoperiodic induction in Xanthium by applied auxin. Bot. Gaz. 110:613-624.

10. BONNER, JAMES, and SAM G. WILDMAN. 1947. Contributions to the study of auxin physiology. Sixth Growth Symposium, 51-68.

11. BÖRGER, H. 1926. Über die Kultur von isolierten Zellen und Gewebefragmenten. Arch. für exp. Zellf. 2:123-190.

12. CLENDENNING, K. A. 1948. Growth studies of normal and parthenocarpic tomato fruits. Canadian Jour. Res. C 26:507-513.

13. CURRENCE, T. M. 1932. Nodal sequence of flower type in cucumber. Proc. Amer. Soc. Hort. Sci. 29:477-479.

14. DANIELSON, L. L. 1944. Effect of daylength on growth and reproduction of the cucumber. Plant Phys. 19:638-648.

15. DOLLFUSS, H. 1936. Wuchsstoffstudien. Planta 25:1-21.

16. DUNCAN, ROBERT E., and JOHN T. CURTIS. 1942. Intermittent growth of fruits of Cypripedium and Paphiopedilum. A correlation of the growth of orchid fruits with their internal development. Torrey Bot. Club Bull. 69:353-359.

17. EDMOND, J. B. 1930. Seasonal variation in sex expression of certain cucumber varieties. Proc. Amer. Soc. Hort. Sci. 27:329-332.

18. GALSTON, ARTHUR W. 1948. On the physiology of root initiation in excised Asparagus stem tips. Amer. Jour. Bot. 35:281-287.

19. GARDNER, F. E., and P. C. MARTH. 1937. Parthenocarpic fruits induced by spraying with growth promoting compounds. Bot. Gaz. 99:184-195.

20. GARDNER, F. E., P. C. MARTH, and L. P. BATJER. 1939. Spraying with plant growth substances for control of the pre-harvest drop of apples. Proc. Amer. Soc. Hort. Sci. 37:415-428.

21. GAUTHERET, R. J. 1947. pH et cultures de tissus végétaux. Rev. Gen. Bot. 54:5-34.

22. GREENE, R. D., and BLACK. 1944. The microbiological assay of tryptophane in proteins and foods. Jour. Biol. Chem. 155:1-8.

23. GUSTAFSON, F. G., and ELNORE STOLDT. 1936. Some relations between leaf area and fruit size in tomatoes. Plant Phys. 11:445-451.

24. GUSTAFSON, F. G. 1936. Inducement of fruit development by growth promoting chemicals. Proc. Nat. Acad. Sci. 22:628-636.

25. GUSTAFSON, FELIX G. 1939. The cause of natural parthenocarpy. Amer. Jour. Bot. 26:135-138.

26. HAAGEN-SMIT, A. J., W. B. DANDLIKER, S. H. WITTWER, and A. E. MURNEEK. 1946. Isolation of 3-indole acetic acid from immature corn kernels. Amer. Jour. Bot. 33:118-120.

27. HABERLANDT, G. 1902. Kulturversuche mit isolierten Pflanzenzellen. Sitzungsb. Akad. Wiss. Wien, Math.-Natur. Kl. III:69-92.

28. HALL, WAYNE C. 1949. Effects of photoperiod and nitrogen supply on growth and reproduction in the gherkin. Plant Phys. 24:753-769.

29. HATCHER, E. S. J. 1945. Studies in the vernalization of cereals. IX. Auxin production during development and ripening of the anther and carpel of spring and winter rye. Ann. Bot. N.S. 9:235-266.

30. HANNING, E. 1904. Über die Kultur von Cruciferen-Embryonen ausserhalb des Embryosacks. Bot. Zeit. 62:45-80.

31. HITCHCOCK, A. E., and P. W. ZIMMERMAN. 1935. Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. Contr. Boyce Thompson Inst. 7:447-476.

32. HOFFMANN, OTTO L., and ALLEN E. SMITH. 1949. A new group of plant growth regulators. Science 109:588.

33. HOUGHTALING, HELEN B. 1935. A developmental analysis of size and shape in tomato fruits. Torrey Bot. Club Bull. 62:243-252.

34. HOWLETT, FREEMAN S. 1936. The effect of carbohydrate and nitrogen deficiency upon microsporogenesis and the development of the male gametophyte in the tomato, Lycopersicum esculentum Mill. Ann. Bot. 50:767-803.

35. HOWLETT, FREEMAN S. 1939. The modification of flower structure by environment in varieties of Lycopersicum esculentum. Jour. Agr. Res. 58:79-117.

36. HUNTER, A. W. S. 1941. The experimental induction of parthenocarpic strawberries. Canadian Jour. Res. C 19:413-419.

37. JANSEN, L. L., and JAMES BONNER. 1949. Development of fruits from excised flowers in sterile culture. Amer. Jour. Bot. 36:826 (abstract).

38. JUDKINS, W. P. 1939. Time involved in pollen tube extension through style and rate of fruit growth in tomato (Lycopersicum esculentum Mill.) Proc. Amer. Soc. Hort Sci. 37:891-894.

39. JUDKINS, WESLEY P. 1945. The extraction of auxin from tomato fruit. Amer. Jour. Bot. 32:242-249.

40. KOBEL, F. 1931. Lehrbuch des Obstbaus. Berlin.

41. KOTTE, W. 1922. Kulturversuche mit isolierten Wurzelspitzen. Beitr. zur allgem. Bot. 2:413-434.

42. LAIBACH, F. 1932. Pollenhormon und Wuchsstoff. Ber. bot. Ges. 50:383-390.

43. LAIBACH, F. 1933. Versuche mit Wuchsstoffpaste. Ber. bot. Ges. 51:386-392.

44. LAIBACH, F., and F. J. KRIBBEN. 1950. Der Einfluss von Wuchsstoff auf die Blütenbildung der Gurke. Die Naturwissenschaften 37:114-115.

45. LA RUE, C. D. 1936a. The effect of auxin on the abscission of petioles. Proc. Nat. Acad. Sci. 22:254-259.

46. LA RUE, C. D. 1936b. The growth of plant embryos in culture. Bull. Torrey Bot. Club 63:365-382.

47. LESLEY, MARGARET M., and J. W. LESLEY. 1941. Parthenocarpy in a tomato deficient for a part of a chromosome in its aneuploid prophase. Genetics 26:374-386.

48. LILLELAND, O. 1930. Growth study of the apricot fruit. Proc. Amer. Soc. Hort. Sci. 27:237-245.

49. LILLELAND, O. 1933. Growth study of the plum fruit. I. The growth and changes in chemical composition of the climax plum. Proc. Amer. Soc. Hort. Sci. 30:203-208.

50. LINK, G. K., V. EGGERS, and J. E. MOULTON. 1941. Use of frozen vacuum-dried material in auxin and other chemical analyses of plant organs: its extraction with dry ether. Bot. Gaz. 102:590-601.

51. LOO, S. W. 1945. Cultivation of excised stem tips of Asparagus in vitro. Amer. Jour. Bot. 32:13-17.

52. MAC ARTHUR, MARY, and R. H. WETMORE. 1941. Developmental studies of the apple fruit in the varieties McIntosh Red and Wagener. II. An analysis of development. Canadian Jour. Res. C 19:371-382.

53. MANN, LOUIS K. 1942. Effects of photoperiod on sex expression in Ambrosia trifida. Bot. Gaz. 103:780-787.

54. MAZÉ, P. 1914. Influences respectives des éléments de la solution minérale sur le developpement du mais. Ann. Inst. Pasteur 28:21-46.

55. MUIR, ROBERT M. 1942. Growth hormones as related to the setting and development of fruit of Nicotiana tabacum. Amer. Jour. Bot. 29:716-720.

56. MUIR, ROBERT M. 1947. The relationship of growth hormones and fruit development. Proc. Nat. Acad. Sci. 33:303-312.

57. MÜLLER-THURGAU, H. 1898. Abhängigkeit der Ausbildung der Traubenbeeren und einiger anderer Früchte von der Entwickelung der Samen. Landwirt. Jahrb. Schweiz 12:135-205.

58. NITSCH, J. 1947. Action du 2,4-D sur la parthénocarpie de la tomate. (Unpublished).

59. NITSCH, J. P., and F. G. GUSTAFSON. 1948. Enzymatic conversion of L-tryptophane to auxin by pollen and pollen breis. (Unpublished).

60. NITSCH, J. 1949a. Influence des akènes sur la croissance du réceptacle du fraisier. Compt. Rend. Acad. Sci., Paris 228:120-122.

61. NITSCH J. P. 1949b. Obtention de fruits charnus en culture in vitro. Compt. Rend. Acad. Sci. Paris 229:445-446.

62. NITSCH, J. P. 1949c. Culture of fruits in vitro. Science 110:499.

63. NITSCH, J. P. 1949d. Culture of fruits in vitro. Amer. Jour. Bot. 36:827 (abstract).

64. NITSCH, J. P. 1950. Growth and morphogenesis of the strawberry as related to auxin. Amer. Jour. Bot. 37:211-215.

65. NOBÉCOURT, P. 1939. Sur la pérennité de l'augmentation de volume des cultures de tissus végétaux. Compt. Rend. Soc. Biol. Paris 130:1270.

66. NOLL, F. 1902. Fruchtbildung ohne vorausgegangene Bestäubung (Parthenokarpie) bei der Gurke. Sitzungsb. Niederrhein. Ges. Nat. Heilk. Bonn 149-162.

67. PFEIFFER, HANS. 1931. Beobachtungen an Kulturen nackter Zellen aus pflanzlichen Beeren Perikarpien. Arch. für exp. Zellf. 11:424-434.

68. PFEIFFER, HANS. 1933. Über das Migrationsvermögen pflanzlicher Zellen in situ und in vitro, Arch. für exp. Zellf. 14:152-170.

69. RANDOLPH, L. F. 1936. Developmental morphology of the caryopsis in maize. Jour. Agr. Res. 53:881-916.

70. ROBBINS, W.-J. 1922. Cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. 73:376-390.

71. ROBBINS, W. J., and M. A. BARTLEY. 1937. Thiazole and the growth of excised tomato roots. Proc. Nat. Acad. Sci. 23:385-388.

72. ROBERTS, R. H. 1946. Notes on apple set and growth. 1945. Proc. Amer. Soc. Hort. Sci. 48:59-62.

73. SCHAFFNER, J. H. 1930. Sex reversal and the experimental production of neutral tassels in Zea Mays. Bot. Gaz. 90:279-298.

74. SCHOCKEN, VICTOR. 1949. The genesis of auxin during the decomposition of proteins. Arch. Biochem. 23:198-204.

75. SINNOTT, EDMUND W. 1939. A developmental analysis of the relation between cell size and fruit size in cucurbits. Amer. Jour. Bot. 26:179-189.

76. SINNOTT, EDMUND W. 1945a. The relation of cell division to growth rate in cucurbit fruits. Growth 9:189-194.

77. SINNOTT, EDMUND W. 1945b. The relation of growth to size in cucurbit fruits. Amer. Jour. Bot. 32:439-446.

78. SMITH, W. HUGH. 1950. Cell multiplication and cell-enlargement in the development of the flesh of the apple fruit. Ann. Bot., N.s. 14:23-38.

79. SNELL, E. E. 1943. Growth promotion on tryptophane-deficient media by o-amino-benzoic acid and its attempted reversal with orthanilamide. Arch. Biochem. 2:289-394.

80. SÖDING, HANS. 1936. Wirkt der Wuchsstoff artspezifisch? Jahr. für wiss. Bot. 82:534-554.

81. SÖDING, HANS. 1930. Wuchsstoffbildung und Wuchsstoffverteilung in der Kompositenstaude Heliopsis laevis im Laufe einer Vegetationsperiode. Flora, N.s. 32:425-446.

82. SOMMER, A. L., and C. B. LIPMAN. 1926. Evidence on the indispensable nature of zinc and boron for higher plants. Plant Phys. 1:231-249.

83. SOMMER, A.L. 1931. Copper as an essential for plant growth. Plant Phys. 6:339-345.

84. STEHSEL, MELVIN. 1949. Thesis. University of California. Berkeley, California.

85. STEWART, W. S., and L. J. KLOTZ. 1947. Some effects of 2,4-dichlorophenoxyacetic acid on fruit drop and morphology of oranges. Bot. Gaz. 109:150-162.

86. SWARBRICK, T. 1943. Progress report on the use of naphthoxyacetic acid to increase the fruit set of the strawberry variety Tardive de Leopold. Ann. Rept. Long Ashton Res. Sta.

87. TIEDJENS, V. A. 1928. Sex ratios in cucumber flowers as affected by different conditions of soil and light. Jour. Agr. Res. 36:720-746.

88. THIMANN, K. V. 1934. Studies on the growth hormones of plants. VI. The distribution of the growth substances in plant tissues. Jour. Gen. Physiol. 18:23-34.

89. TUKEY, H. B. 1933. Growth of the peach embryo in relation to growth of fruit and season ripening. Proc. Amer. Soc. Hort. Sci. 30:209-218.

90. TUKEY, H. B., and J. ORAN YOUNG. 1939. Histological study of the developing fruit of the sour cherry. Bot. Gaz. 100:723-749.

91. TUKEY, H. B., and J. ORAN YOUNG. 1942. Gross morphology and histology of developing fruit of the apple. Bot. Gaz. 104:3-25.

92. VACIN, EMIL F., and F. W. WENT. 1949. Some pH changes in nutrient solutions. Bot. Gaz. 110:605-613.

93. VAN OVERBEEK, J., M. E. CONKLIN, and A. F. BLAKESLEE. 1941. Chemical stimulation of ovule development and its possible relation to parthenogenesis. Amer. Jour. Bot. 28:647-656.

94. VAN OVERBEEK, J. 1946. Control of flower formation and fruit size in the pineapple. Bot. Gax. 108:64-73.

95. VAN OVERBEEK, J., and HECTOR J. CRUSADO. 1948. Note on the flower formation in the pineapple induced by low night temperatures. Plant Phys. 23:282-285.

96. WENT, F. W. 1926. On growth accelerating substances in the coleoptile of Avena sativa. Proc. Kon. Akad. van Wet., Amsterdam, 30:10.

97. WENT, F. W. 1944. Plants grown under controlled conditions. II. Thermoperiodicity in growth and fruiting of the tomato. Amer. Jour. Bot. 31:135-150.

98. WENT, F. W. 1951. Tomato growth and fruit production under controlled temperature and light. Communication No. 1 from the Earhart Plant Research Laboratory. Amer. Jour. Bot., in press.

99. WHITE, P. R. 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Phys. 9:585-600.

100. WHITE, P. R. 1937. Vitamin B_1 in the nutritient of excised tomato roots. Plant Phys. 12:803-811.

101. WHITE, P. R. 1939. Potentially unlimited growth of excised plant callus in an artificial nutrient. Amer. Jour. Bot. 26:59-64.

102. WILDMAN, S. G., MARIO G. FERRI, and JAMES BONNER. 1947. The conversion of L-tryptophane by spinach leaves. Arch. Biochem. 13:131-144.

103. WILDMAN, S. G., and R. M. MUIR. 1949. Observations on the mechanism of auxin formation in plant tissues. Plant Phys. 24:84-92.

104. WINKLER, H. 1907-8. Über Parthenogenesis and Apogamie im Pflanzenreiche. Prog. Rei. Bot. 2:293-454.

105. WITTWER, S. H. 1943. Growth hormone production during sexual reproduction of higher plants. Missouri Exp. Sta. Res. Bull. No. 371.

106. WONG, CHEONG-YIN. 1939. The influence of pollination on seed development in certain varieties of citrus. Proc. Amer. Soc. Hort. Sci. 37:161-164.

107. YASUDA, S. 1934. Parthenocarpy caused by the stimulus of pollination in some plants of Solanaceae. Agr. and Hort. 9:647-656.

APPENDIX

I. Total auxin in strawberry tissues (see pages 42 to 44).

The total auxin content of the strawberry was determined both by boiling the lyophilized tissues 8 to 12 hours with 0.1 N NaOH as described by Wildman and Bonner (1947), and by digesting the tissues with pepsin (12 hours at pH 2.0 at 37°C.) and trypsin (3 days at pH 8.4 at 40°C.). Auxin was then extracted with peroxide-free ether, after removing possible inhibitors by shaking the ether extract with sodium bicarbonate. The tryptophan content was determined by the Lactobacillus assay, as described in Table 3.

TABLE 11

AUXIN AND L-TRYPTOPHAN CONTENT OF 100 mg LYOPHILIZED STRAWBERRY TISSUES EXTRACTED AFTER VARIOUS TREATMENTS

Tissue.	Treatment.	L-tryptophan (gammas).	Auxin in IAA equivalents (gammas).
Sample 1:			
1) Achenes	a) "free auxin"	-	132×10^{-3}
	b) NaOH treatment (10 hours)	9.5	375×10^{-3}
	c) digestion with pepsin and trypsin	26.0	153×10^{-3}
2) Receptacles	a) "free auxin"	-	0
	b) NaOH treatment (10 hours)	3.75	197×10^{-3}
	c) digestion with pepsin and trypsin	14.0	137×10^{-3}
Sample 2:			
Receptacles (12 days after pollination)	a) free tryptophane and free auxin	14.4	0
	b) digestion with pepsin	18.0	0
	c) digestion with trypsin	18.0	395×10^{-3}
	d) digestion with pepsin, then trypsin	18.0	260×10^{-3}

TABLE 11 (Cont'd)

Tissue.	Treatment	L-tryptophan (gammas).	Auxin in IAA equivalents (gammas).
	e) Digestion with pepsin, then trypsin, after addition of 10 gammas of L-tryptophan	28.0	545×10^{-3}
	f) 25 mg tissue boiled 3 minutes with 20 cc distilled water, then digested w th trypsin	-	340×10^{-3}
	g) - id. -, but 10 gammas L-tryptophan added with the trypsin, after cooling	-	600×10^{-3}
	h) 20 gammas L-tryptophan	-	0
	i) 20 gammas L-tryptophan digested with trypsin	-	0

The data of Table 11 indicate that the NaOH treatment increases the yields of auxin and decreases the yields of L-tryptophane, and that, in the presence of the tissue, the tryptic digestion greatly increases the auxin yields when tryptophan is present. The release of auxin from tissues treated with trypsin in contrast to tissues treated with pepsin may be due to the alkaline pH used in the first case, since it is known that some alkaline treatments transform L-tryptophan into IAA (Gordon and Wildman, 1942). While these results suggest that tryptophan may be transformed into IAA by the treatments, such an assumption cannot be proven by the above data because (1) the method used to determine tryptophane could not distinguish differences of a magnitude of 1/10 of a gamma so that small decreases in tryptophane content could not be demonstrated in the case of sample No. 2,and (2) the treatments with

NaOH may partially transform L-tryptophan into D-tryptophan, the latter being inactive on the Lactobacillus test, which could explain the marked drop in L-tryptophane content of sample No. 1.

II. Action of various amino-acids on the growth of unpollinated tomato ovaries (var. Essen Wonder) cultivated 3 weeks in vitro.

TABLE 12

Substances added to the basal medium (pH 6.0)	Number of ovaries.	Initial diameter.	Final diameter.	% increase in diameter.
Thiamin (1 mg/l), agar (1.2%) and:				
1) no amino-acids	9	1.77 mm	2.22 mm	25.2%
id., (no thiamin	10	2.05	2.50	22.0%
2) L-tryptophane (100 mg/l (no thiamin)	3	1.83	2.50	36.7%
L-tryptophane (25 mg/l) (no thiamin)	7	2.0	2.57	28.6%
L-tryptophane (250 mg/l)	9	1.90	2.50	31.6%
3) L-Cysteine HCl L-tryptophane (100 mg/l)	5	1.90	2.60	36.8%
L-tryptophane (10 mg/l), L-cysteine HCl (10 mg/l, and "vitamin'free" casein hydrolysate (10 mg/l), liquid medium	4	1.77	3.17	79%
Same compounds but at the 100 mg/l dose	8	2.0	4.81	140%
Thiamin (1 mg/l), agar (1%), L-tryptophane (100 mg/l) and:				
1) glycine (1/10 Mol.), Cysteine HCl (1/10 Mol.), L-glutamic acid (1/10 Mol.)	8	2.0	4.19	109%
2) glycine (1 Mol.), cysteine HCl (1 Mol.), and L-glutamic acid (1 Mol.)	7	2.0	4.0	100%
3) glutathione (1/10 Mol.)	7	2.0	3.80	90%
4) glutathione (1/10 Mol.)	6	2.0	3.83	91.5%

III. Action of various substances on the growth of tomato ovaries (var. Essen Wonder) excised four days after pollination and cultivated 3 weeks in vitro.

TABLE 13

Substances added to the basal medium.	Number of ovaries.	Initial diameter.	Final diameter.	% increase in diam.
Agar (1%), and:				
1) nothing else	5	4.40	5.90	34%
2) L-tryptophane (25 mg/l)	10	3.35	4.90	46.4%
3) L-tryptophane (250 mg/l)	10	3.70	5.10	38%
Unautoclaved coconut milk (20%) (pH around 5.0)	7	4.90	10.50	114%

IV. Action of the roots on the growth of unpollinated tomato ovaries (Essex Wonder) cultivated in vitro.

TABLE 14

Basal medium solidified with agar.

	Number of ovaries	Initial diameter	Diameter after 3 wks.	Diam. after 5 wks.
Thiamin (1 mg/) and darkness: roots appear after 3 weeks.	8	1.81	2.22	2.87
No thiamin and light: no visible roots.	10	2.0	2.50	2.30*

* Most of the ovaries are dead, although the sepals remain green and healthy looking.

V. Additional references.

GORDON, SOLON A., and SAM G. WILDMAN. 1942. The conversion of tryptophane to a plant growth substance by conditions of mild alkalinity. Jour. Biol. Chem. 147:389-398.

WILDMAN, S. G., and JAMES BONNER. 1947. The proteins of green leaves. I. Isolation, enzymatic properties and auxin content of spinach cytoplasmic proteins. Arch. Biochem. 14:281-413.