Conditions influencing regeneration of hypocotyl.

By

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and

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(With 4 images in the text.)

It has long been known that the hypocotyls of many seedlings will produce, under some conditions, one or more „adventitious“ shoots.[1])

The writers cited, worked with uninjured seedlings and established the following facts.

First, that many seedlings produce „adventitious“ buds normally. Irmisch points out that about 50 % of the seedlings Anagallis arvensis do this.

Second, that many seedlings produce these buds only under exceptional conditions. Wydler found that only those seedlings which he kept in crocks produced hypocotyl buds, those in the open never doing so. Braun found the same and agreed with Irmisch and others, that an abundance of water might be the causal factor.

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[1]) Röper, Enumeratio Euphorbiarum. 1824.
Bernhardi, Linnaea VII. 1832. p. 572.
Chavannes, Monographie des Antirrhinum. 1833.
Wydler, Bot. Ztg. 1850. Nr. 22. Flora. 1856. Nr. 3.
Berner Mitteil. Nr. 485—487.
Irmisch, Über die Keimung und die Erneuerungsweise von *Convolvulus sepium* und *C. arvensis*, sowie über hypokotylische Adventivknospen bei krautartigen phanerogamen Pflanzen. (Bot. Ztg. Bd. XV. 1857. p. 433. Flora. 1853. p. 522. Flora. 1857. p. 439.)
Braun, Adventivknospenbildung am ersten (hypokotylen) Stengelglied. (Sitzungsber. der Naturf.-Ges.. Berlin. 1870; vergl. auch Bot. Ztg. Bd. XXVIII. 1870. p. 438.)
Zabel, Entwicklung der von der Achse abgetrennten Keimblätter. (Bot. Jahresber. Bd. X. 1882. p. 32.)
Beyerinck, Over regeneratieverschynselm aan gespleten vegetation punten van stengelsen over bekervornung. (Bot. Centralbl. 1883. p. 231.) Beobachtung und Betrachtungen über Wurzelknospen und Nebenwurzeln. Amsterdam 1886.

Third, that plants which as seedlings did not normally produce these buds often produce them, near the surface of the ground in the fall. These buds often live over winter and produce a new plant the following spring.

Fourth, that these adventitious shoots did not have the normal leaf arrangement. The leaves were not transversely placed but perpendicularly and that the leaves were not of the same size. The anisophylly was well marked the lower leaf being the larger.

Küster[1]) added to the above work some experiments, introducing a wound stimulus. He cut away the cotyledons at different lengths below their insertion and found that the

Fig. 1. *Linum usitatissimum.*
[Enlarged.] Cotyledons removed Jan. 25 1904 when seedling was 2 cm high. Photographed March 3. In plant to the right the first bud was removed.

tendency to the production of adventitious buds was greatly increased. While other writers found the production on a few individuals. Küster found in the case of *Anagallis coerulea* and *Linaria Cymbalaria* that every wounded individual produced one or more such hypocotyl buds. There was no fixed law as to place of origin in the hypocotyl.

Küster[2]) further confirmed the previous observations in regard to anisophylly. Experiments aimed to determine the cause of this phenomenon were not successful.

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[1]) Küster, Beobachtungen über Regenerationserscheinungen an Pflanzen. (Beih. z. Bot. Centralbl. Bd. XIV. p. 316.)

— Beobacht. über Regenerationserscheinungen an Pflanzen. II. (Beih. z. Bot. Centralb. Bd. XV. p. 421.)

Adventitious buds were also produced on the epicotyl when the main vegetation point was cut away. In this case the buds in the axil of the cotyledons develop, but their development appears to have no influence on the development op „adventitious" buds.

Material and methods. This paper deals largely with conditions influencing the production of adventitious buds on the hypocotyl.

The plants used were *Linaria bipartita splendida*, *Antirrhinum majus* and *Linum usitatissimum*.

The cotyledons were cut away just below their insertion; usually when the seedling was from two to three cm high.

After this operation the plants were placed under various external conditions which will be taken up separately.

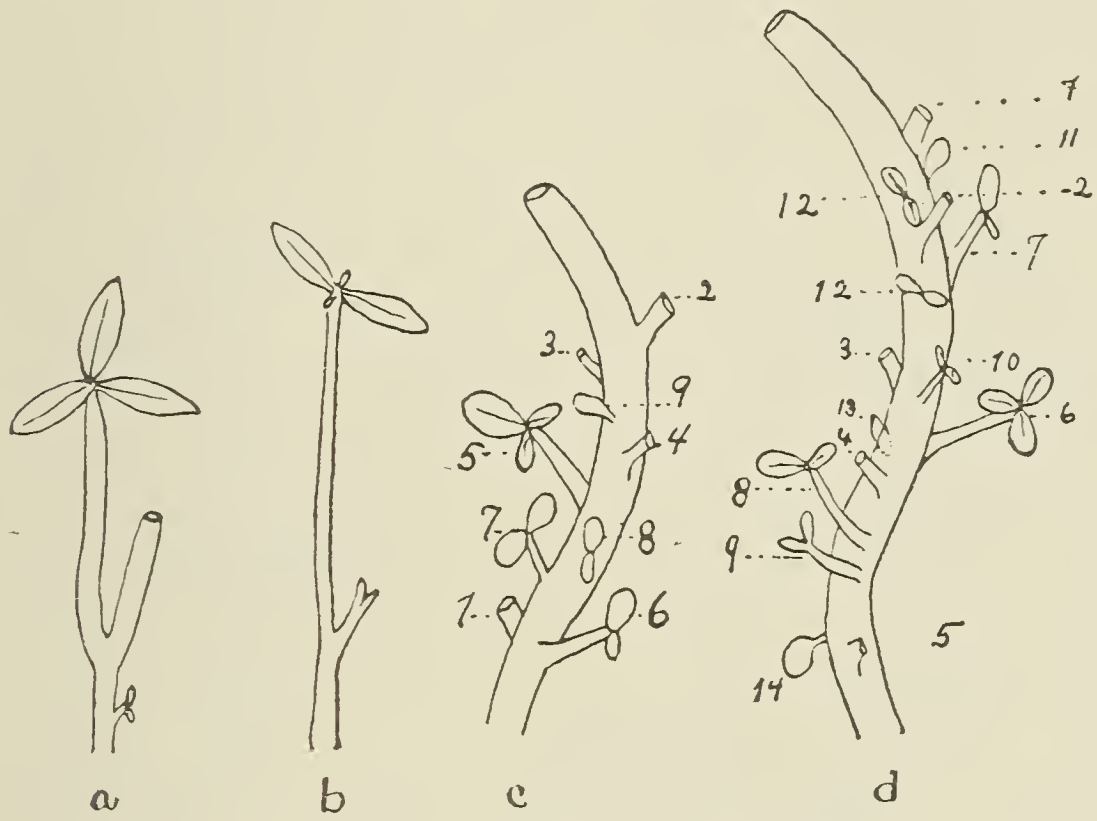

Fig. 2.
Linaria a b. The cotyledons were removed from a b was not injured. Note difference in number of leaves in regenerated short. *Antirrhinum* c and d. The order of shools is shown by the figures. Note size, number and arrangement of leaves in regenerated buds.

The experiments were started in November 1903 and carried on through the following year.

The results obtained confirm those of Küster. All individuals of the plant named whose cotyledons and part of the hypocotyl were removed produced numerous buds.

General description. In the course of from 5—9 days after the operation the hypocotyl buds began to appear as small eruption on the epidermis. A day or two before the buds appear, the hypocotyl becomes somewhat swollen.

The number of these buds varied in the three different plants *Linaria* produced from 1—6 buds, *Antirrhinum* from 1—14, and *Linum* from 1—60 or more. Every epidermal cell of *Linum*

seemed capable of forming a bud, the hypocotyl in some cases was almost completely covered with buds from base to apex.

The buds were developed near the base of the hypocotyl in *Linaria*, but in *Antirrhinum* and *Linum* they were more generally distributed.

As a rule the hypocotyl of *Antirrhinum* and *Linum* became bent sometimes as much as 90° and the buds then tended to crowd on the upper side, very few if any were produced on the under side. These buds either developed one after the other or several at a time, but no very definite arrangement was ever determined. In the majority of cases, however, the second bud developed below the first.

In several cases the hypocotyl buds were cut off as soon as they formed, and in this way an almost indefinite number could be forced to develop. They were produced either above or below the origin of the first buds; there was no fixed rule. Seldom more than two of the buds developed into shoots. generally one, but if they were cut off some of the undeveloped buds took their places, or a new shoot was formed on the stem of the old one. As soon as the hypocotyl shoots began to develop they grew very rapidly and took the place of the main axis, which became bent over to one side. After a week or two the undeveloped buds died, the shoots evidently growing at their expense. The hypocotyl buds when produced on uninjured plants of *Linaria*, as observed by Bernhardi, became the flower stalk and the main stem soon died. One other fact should be mentioned in this connection. Often the seed coats cling to the cotyledons and do not allow them to open. They are not then exposed to the light and cannot serve as assimilating organs. In such cases the hypocotyl produced buds as if it had been wounded. This was tested further by covering the cotyledons with plaster of paris. In this case regeneration took place. It is thus evident that not the wound[1]) but the absence of the normally present functioning cotyledons give the first stimulus. The length of time required for the production of such „adventitious" buds differs, widely depending as we will see to some extent upon external conditions. This is not entirely true. In November a large number of seedlings were decapitated and kept in the greenhouse. The first buds appeared only after 37 days. The same experiments conducted in January later gave results in one third of that time.

Anisophylly. The phenomenon[2]) varies with different individuals but not with changing external conditions as far as learned.

Linaria bipartita splendida develops only a few buds when the cotyledons are removed. Out of 140 individuals, 2 developed

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[1]) Goebel, Über Regeneration im Pflanzenreich. (Biolog. Centralblatt. Bd. XXII.) 1902. Weitere Studien über Regeneration. (Flora. 1903. p. 132.)

[2]) Küster, l. c.

a bud with one leaf 12 buds with two leaves, 126 buds with three leaves at the first node. Subsequent nodes produced the normal number of leaves.

The same fact is true but less marked in *Linum* and *Antirrhinum majus*. Many of the first plant produce the first adventitious buds with three leaves while the second bud on the same individual had only two at the first node.

With *Anthirrhinum*, 9 in 51 produced three leaves, one in 51 produced four while the others produced two, on the first node.

Experiments.

A. Uninjured plants. It may be best perhaps to give result of experiments and observations on uninjured seedlings. Previous reference has been made to the facts that potted plants and plants in places produce these buds in some species while in others the phenomenon is a regular occurrence on a large number of individuals.

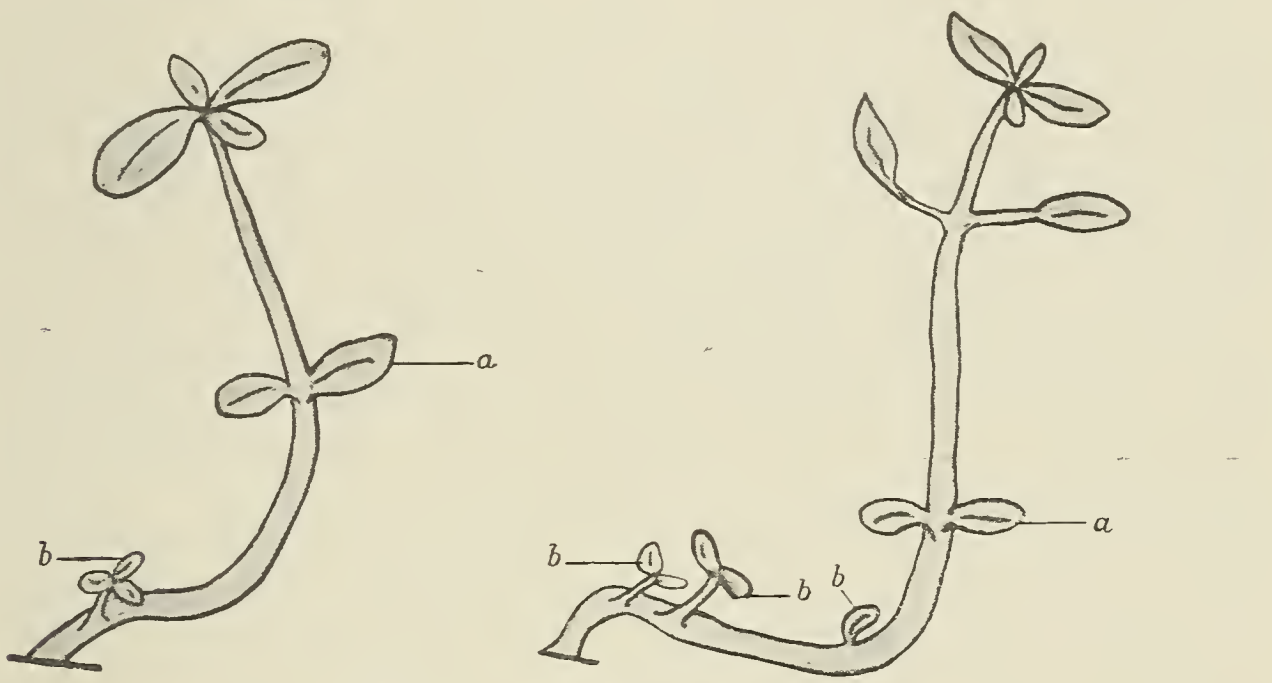

Fig. 3. *Antirrhinum majus.*
Two seedlings grown in a damp chamber without support. In boch cases „adventitious" buds were produced a. cotyledons, b. „adventitious" buds.

The number of shoots is always few and their position varied, but the larger per cent is always found near the base. The three plants named produced these buds when kept in a crock in a damp chamber in the following per cent. *Linum* 7 %, *Linaria* 19 %, and *Antirrhinum* 30 %. Usually only one bud was developed and the near the base.

Plants of *Antirrhinum* do not stand erect and often bend over on the ground. In this case buds develop on the upper side of the horizontal part. Eight plants were placed in a damp chamber and tied to wires so that they grew erect, and eight plants placed in the same conditions but without support. The latter plants developed in every case buds while none of those erect did so.

B. Wounded seedlings. Moisture. The effect of moisture on production of these buds on the hypocotyl is shown in the following:

Antirrhinum seedlings were prepared as above described and one set placed in a damp chamber, the other in the laboratory where the air was much drier. Other factors were of course held as nearly constant as possible, *Antirrhinum* showed a difference of about one day in favor of the damp atmosphere. The same held true for *Linum*. In the case of *Linaria*, this was not the case although there was a slight advantage in favor of the plants kept in the damp. In 140 seedlings, plants developed buds in three days regardless of the moisture content of the air, while of 35 plants kept in a damp chamber 5 produced buds in four days and in another crock of the same number in dry atmosphere one produced a bud in five days.

The effect of moisture is more pronounced in the number of buds formed. In all cases the plants in the damp chamber produced many more buds.

Temperature. The temperature experiments were conducted mostly with *Linum*. The experiments were set up like the preceding, except that the varying factor was temperature. The results obtained were as follows; plants at 10—15° in damp chamber produced buds in 10 days, those at 25° in 8 days, while four seedlings out of eleven at 30—35° produced buds in four days. Higher temperatures killed the plants.

Not only did the plants in higher temperatures produce buds sooner but the number of buds produced was always greater.

No Seedlings	Temp.	No days to regeneralt	Size	Moisture
12	10—15	10	25 cm	wet
8	25	8	"	"
4	25	9	"	"
11	30—35	4	"	"

Linum. Relation of temperature to regeneration. The table show the results of one set of experiments.

Age. Experiments were conducted with *Linum* to determine if possible at what age the cells of the hypocotyl lose the ability to produce these buds. It was first determined what age the hypocotyl ceaaes to grow. To determine this the hypocotyl at different ages was marked every 2 mm with India ink and observed later. These experiments showed that the last part to stop elongating is the part immediately beneath the cotyledon. The hypocotyl asually ceases elongating entirely when it is 2,5 to 3 cm long, under ordinary conditions.

A comparison of the figures already given show that on young seedlings there is no tendency toward developing shoots

on the parts still elongating. After a few days all elongation ceases and the epidermal cells of the hypocotyl are all practically alike. Plants were allowed to grow longer periods with the result shown in the table:

Age in days	No. used	Temp.	Jime to regenerate	No. Plants in giventime	to buds
5	10	30 max	4 d	5	several
15	80	30 „	7 d	80	„
29	60	30 „	7 d	80	„
48	60	30 „	8 d	60	„
55	8	30 „	19 d	3	few
69	16	30 „	11 d	4	esch. 2

Linum. Relation of age to regeneration.

This shows that the time required for the first buds and the number of these buds decreases in a general way with the age of the seedling.

The buds on old seedlings appear near the base of the hypocotyl. It would thus appear that this part of the hypocotyl can produce buds easier than other parts. It will be recalled in this connection that some plants produce such buds at the base, after the movement of food material to the flowers and fruits ceases. This part is especially disposed to produce buds.[1])

Gravity. Experiments on this line failed to show that the position and number of the buds, and the anisophylly were in any way dependent upon gravity.

Light. A large number of seedlings were revolved on the klinostat in a horizontal position in the plant house for 14 days in a diffuse light. No regeneration took place, and it seemed at first to show that gravity was an important factor. However, a second set were turned the same way in another place, in bright light and every plant regenerated in 9 days. Decapitated seedlings were then turned to give equal illumination on all sides and buds developed equally on all sides and with no preference for either end.

A third set of seedlings were then turned horizontally in the laboratory with the wounded end toward the south window and in 8 days buds. began to appear. They were however, crowded toward the wounded end.

A fourth set of decapitated seedlings were placed in a horizontal position in a south window and a remarkable arrangement of buds was found in some cases. At first the buds appeared on the upper side near the base. The hypocotyl however, gradually turned up at the end and buds appeared on

[1]) Goebel, Über Regeneration im Pflanzenreich. (Biol. Centralblatt. 1902. p. 385.)

what had been the underside but was now the side turned toward the window. (Fig. 4, b.)

Another set (Fig. 4a) of decapitated seedlings were placed in a dark chamber with the light admitted from one side only. After seven days numerous buds appeared but all were on the side turned toward the window, with no tendency toward either end of the hypocotyl.

The crowding of buds on one side is well shown in fig. 1 and fig. 4. These experiments show that light, and a rather strong light is the direction power in the distribution of buds, at least in the case of *Linum*. Observations on the other plants make it probable that it has the same influence with other plants. Finally it was thought that perhaps the light was a factor only in so far as it produced food material and that this was the

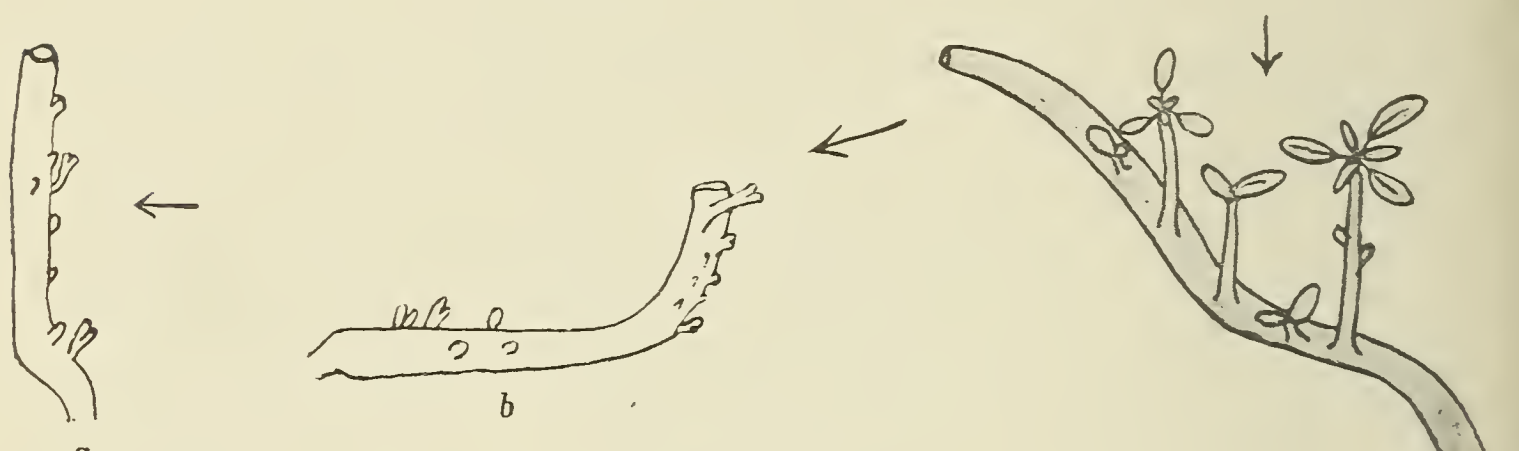

Fig. 4. *Linum usitatissimum.*
a b. Seedlings showing the effect of light on the position of shoots. Direction of light indicated by arrows. *Antirrhinum c.* Same as a and b.

direction force. According seedlings whose cotyledons were enclosed in Plaster of Paris cast, were placed in the dark. These seedlings in no case produced buds although there was still present food material in the cotyledons.

Discussion.

When we compare the results obtained from the above experiments and observations with those recorded by writers we find many points of contact.

1. The wound is not the cause of the development of „adventitious" stems. This is shown in at least three cases:

a) development of buds on injured plants in damp chambers, in small pots etc.,
b) development of one or more buds at the base of the hypocotyl near the end of the growing season and
c) the production of buds on the hypocotyl when the cotyledons are enclosed in Plaster of Paris [1]) or remain the seed coats.

[1]) Winkler, Ber. d. Deut. Bot. Gesell. XX. 1902. p. 31.

In all these cases there is a hindrance to the normal movement of materials in the plant — organs which normally function — have ceased either entirely or partially to do so, in one case from internal, in the others from external causes. That is, the production is due to correlation and not to the wound.

2. The experiments confirm the observation of Goebel[1]) that embryonic tissue reacts easier than old tissue. The young hypocotyl is apparently able to produce a bud from every part of the epidermis. This ability gradually is lost as the plant gets older. In old plants however, this ability is retained by the part nearest the base, that is the oldest part of the hypocotyl. This part is especially predisposed to produce buds. Even in the young plants the first bud usually appears nearer the base than apex of the hypocotyl of decapitated seedlings.

It was not determined whether or not any special cells in the epidermis differed from others in complexity of structure or amount of protoplasm contained as is the case in certain leaves.[2])

3. Polarity. Decapitated seedlings do not show polarity. The origin of the first buds in many cases is toward the base of the hypocotyl. The origin of the bud at base of the old plant confirms this also.

Unter the paragraph on light however, we saw that „polarity“ became very marked in *Linum* when the upper part of the decapitated seedling was strongly illuminated. Without light and a relatively strong light, regeneration did not take place.

The fact has been noted in other cases that buds were produced only on the light side. On the other hand Hornschuck thinks that weakened illumination may be a factor in the development of buds on leaf of Malaxis paludosa, a suggestion with which Goebel does not agree.[3])

In many cases Goebel[4]) has pointed out the relation of the place of origin of „adventitious“ buds to the fibrovascular bundles. This is seen in case of *Begonia Rex, Utricularia, Cardamine pratensis.* (See illustration.)

The facts observed on seedlings with the light experiments seem to run parallel with these. The bundles are the places through which material is moving. In our young seedlings the bundles are not well developed. When however, the cotyledons are removed or cease to function, their work is taken up by the epidermis. The cells of this develop a vast amount of chlorophyll and all movement is to and from them. Very soon after they originate one or more are connected with the conducting system, the others perish. Only those cells exposed to light, function as the cotyledons and hence all flow of material is to and from the lightest side. Light is then an indirect cause of

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[1]) Goebel, Über „Regeneration im Pflanzenreich“. (Biol. Centralbl. 1902.) Willkürliche Entwicklungsänderungen bei Pflanzen. Jena 1903.
[2]) Klebs, Regenerationen bei Utricularia. Bd. 93. p. 118.
[3]) — Flora. 93. p. 118.
[4]) Biol. Centralbl. Flora. 93. etc.

location of buds, while the principal factor is determining the location in relation to movement of food materials in plants.

Conclusions.

1. The young hypocotyls are capable of developing more buds and in a shorter time than old ones.

2. Moisture is principal factor in formation of buds on uninjured seedlings of plants studied.

3. In injured seedlings, the time and number of buds is dependent upon light, moisture and heat.

4. The position of buds is due indirectly to light, directly to the movement of materials in plants.

5. The base of old plants is predisposed to but production,

6. Good vegetative conditions are best for production of buds on hypocotyl.

University of Michigan, February 1905.