

THE PHYSIOLOGICAL BASIS OF MORPHOLOGICAL POLARITY IN REGENERATION. I.

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, November 21, 1918.)

INTRODUCTION.

In two preceding papers¹ on regeneration in *Bryophyllum calycinum* it had been shown that the mass of shoots formed in a leaf or in a piece of stem to the base of which a leaf is attached increases with the mass of the leaf. This proves that regeneration is a phenomenon of the order of nutrition and growth.

The question arises how this conclusion harmonizes with the well known fact of the polar character of regeneration. When we cut out a piece from a higher plant or a lower animal the piece regenerates, as a rule, a shoot or head at the apical end and roots or a tail at the basal end. In a preliminary paper,² published a year ago, the writer described experiments suggesting that the polar character of regeneration might be due to the existence in the circulating sap (or lymph and blood) of inhibitory substances which prevent dormant buds or resting cells from growing out even if an adequate quantity of food-stuffs is available. It was shown by experiments on *Bryophyllum calycinum* that the leaves as well as the growing shoots have an inhibitory influence upon the growth of all the dormant buds situated more basally in the stem. If we assume that these inhibitory influences are due to certain constituents in the sap sent out by growing buds and by leaves, we come to the following theory of the polar character of regeneration. When we cut out a piece of stem from *Bryophyllum* and remove all the leaves, inhibitory substances will continue to flow in a basal direction in the stem. Since the apical

¹ Loeb, J., *Bot. Gaz.*, 1918, lxv, 150; *J. Gen. Physiol.*, 1918, i, 81.

² Loeb, *Science*, 1917, xlv, 547.

region of the piece will be the first to become sufficiently free from these substances, the buds situated at this end will be the first to grow out into shoots. As soon as this happens the new shoots will in their turn send out inhibitory substances in a basal direction in the stem, thereby preventing the growth of the more basally situated buds. In this paper we shall present some of the qualitative evidence for the inhibitory effect of a leaf upon shoot formation, leaving the discussion of experiments of a more quantitative character for a future paper.



FIG. 1. Diagram showing arrangement of shoot buds in the stem of *Bryophyllum calycinum*. The line connecting the two buds in one node is always at right angles to the line connecting two buds in the next node.

We shall deal chiefly with the regeneration of shoots in the stem of *Bryophyllum calycinum*, which can proceed only from definitely located buds. In the axil of each leaf of a stem there exists one bud capable of giving rise to a shoot, which, however, never does so unless the plant is mutilated. Each node of a plant has two leaves in opposite position, and the axis connecting the two axillary buds in one node is always at right angles with the axis connecting the two buds of the next node (Fig. 1). Thus the line connecting the two buds at Node 2 (Fig. 1) is at right angles with the line connecting the two buds in Nodes 1 and 3, etc. The lower leaves in a stem fall off in time, leaving their axillary buds exposed. No other element of the stem except the two buds in each node is capable of growing into shoots.

The elements capable of giving rise to roots are not confined to the nodes but exist all over the stem in a definite layer of the cortex. It can be shown that the sap from the leaf flowing towards the base of a stem favors the growth of roots and inhibits the growth of shoots.

Experiments on Potted Plants.

When we cut off the top of a potted plant of *Bryophyllum calycinum*, leaving a stem containing only two leaves at the apical node, none

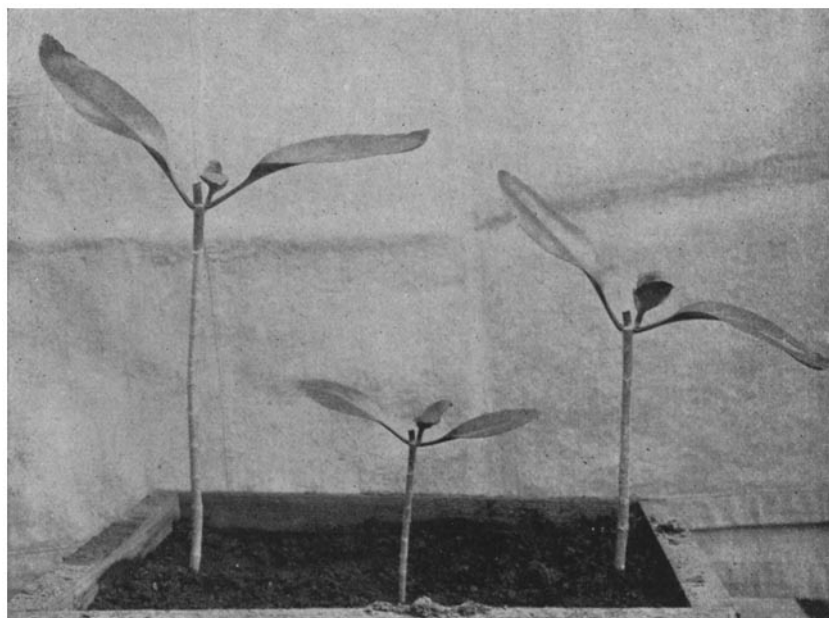


FIG. 2. Top of plant removed; two leaves at apex which prevent growth of all the buds below.

of the buds on the stem below the two leaves will grow out (Fig. 2), so long as the leaves are alive and able to send their sap to the base of the plant. The buds situated in the axil of the two leaves may after some time grow out. The two leaves inhibit therefore the growth of all the buds situated more basally (Fig. 2). Each leaf inhibits the growth of the buds situated in the same half of the stem, and in order to prove this we remove in a second set of experiments

the top of a number of potted plants leaving only one leaf at the apex (Fig. 3). We must also remove the free bud opposite this leaf, since otherwise this bud will grow out and produce the same inhibitory effect as the removed leaf would have done. In this case the experiment would only be a repetition of the preceding experiment in which two leaves were left at the apex. When, however, we remove one apical leaf with its axillary bud very often the one leaf left at the top suffices to suppress regeneration in the basal part of the stem

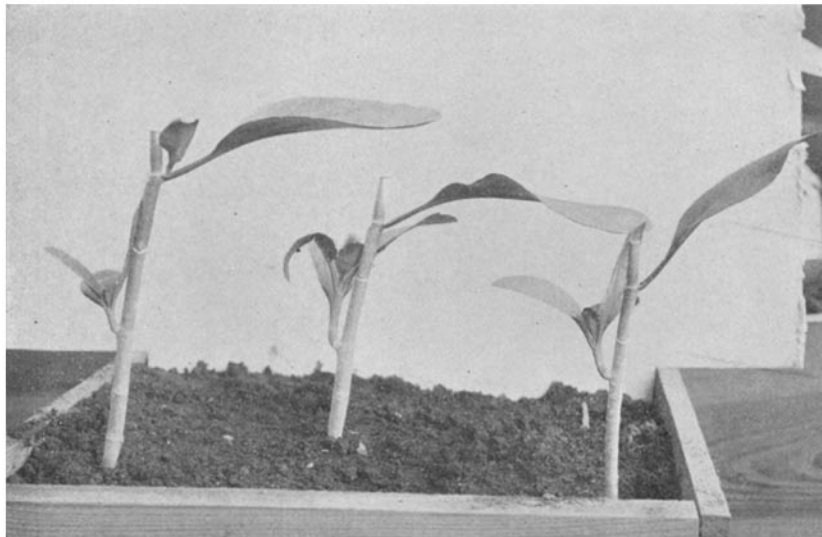


FIG. 3. One leaf left at apex. Growth of shoots in the first node below the leaf suppressed, while the shoot in the second node below the leaf, but on the opposite side of the stem can grow out.

as completely as if two leaves had been left. If regeneration occurs, it takes place in the highest node on the opposite half of the stem which is the second node below the leaf (Fig. 3). In the first node below the leaf no bud can grow out. I have never noticed an exception to this rule in a normal plant. A possible explanation of this phenomenon is furnished by Fig. 4, where that half of the stem through which the sap from the apical leaf flows to the base of the stem is shaded. Since the buds in the first node below

the apical leaf are in the path of the sap flow from the leaf, the formation of shoots is suppressed in these buds, while the bud in the second and fourth nodes below but on the opposite side from the leaf lies outside of the path of the conducting vessels from the leaf. Hence if any bud in such a stem grows out it is usually the one in the second node below but on the opposite side from the apical leaf. As soon as this bud grows out it will inhibit the growth of the lower buds in the same half of the stem.

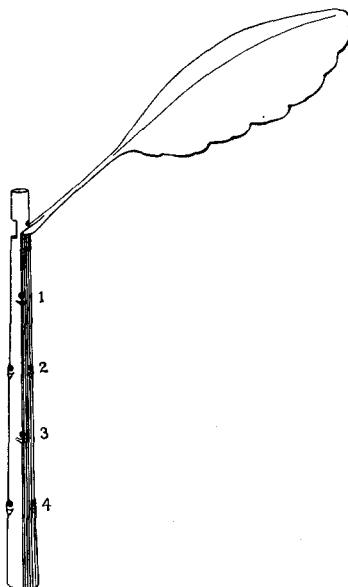


FIG. 4. Diagram explaining this inhibitory influence of the leaf on the theory that the descending sap from the leaf carries inhibitory substances. The region of the stem reached by the sap from the leaf is shaded and in the shaded part regeneration of shoots is inhibited.

The petiole of a leaf is attached with its base to one-half of the circumference of the stem. When we cut off half of the base of the petiole of a leaf, the sap sent out by that leaf can flow only through one quadrant of the next internode. This should limit the inhibitory influence of such a leaf to this quadrant of the node below, and this turns out to be the case. The top of a number of potted plants was cut off and only one leaf was left at the apex (Fig. 5). Half of the

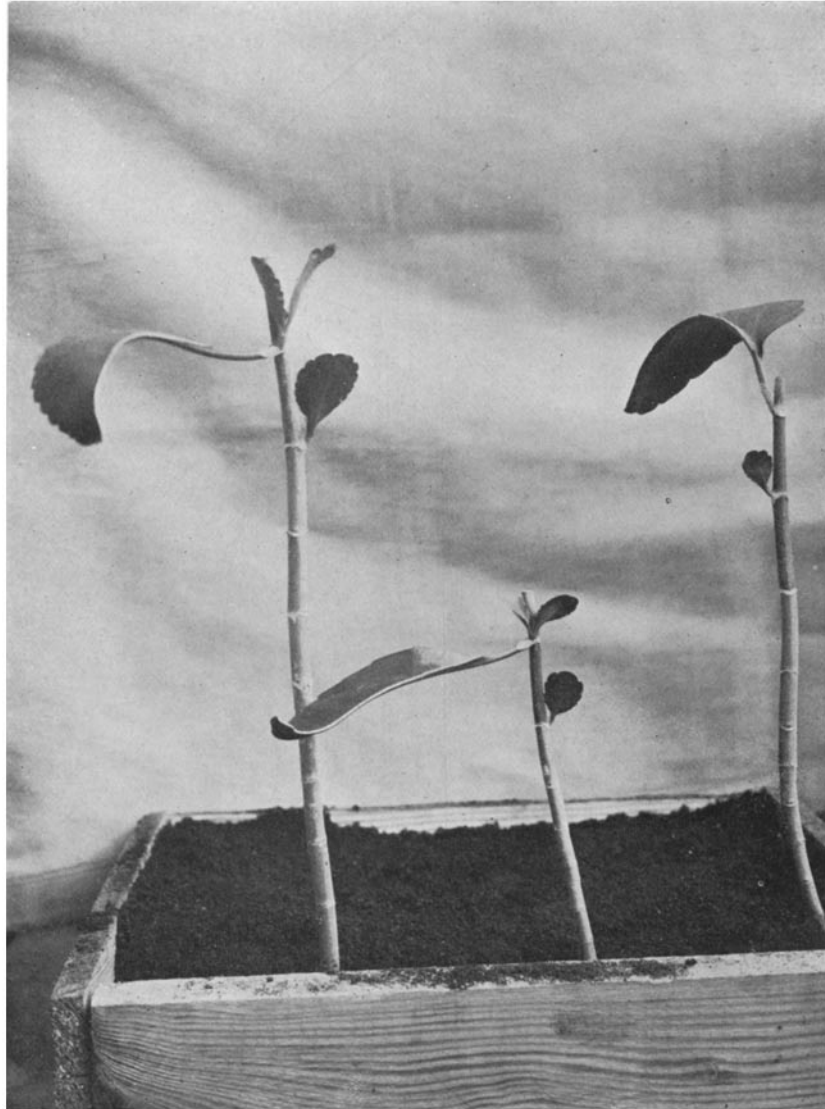


FIG. 5. One-half leaf and one-half petiole left at apex. One of the two shoots in node below leaf now grows out; namely, on the side where half the petiole is removed.

petiole of this leaf was removed at the base and also the corresponding half of the leaf itself was cut off, though this latter procedure is not essential for the result. The axillary bud of the other leaf was also cut out as in the preceding experiment. Fig. 5 gives the result of such an experiment. The reader will notice that in this case one of the two buds in the first node below the leaf will grow out; namely, that one which lies beneath the removed half of the leaf. This bud

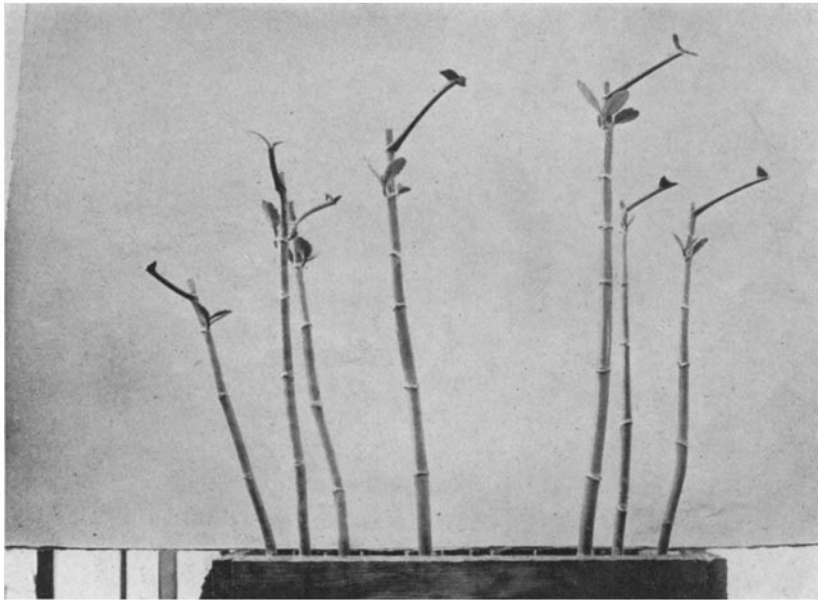


FIG. 6. Showing that the inhibitory influence of an apical leaf upon shoot formation in the node below the leaf disappears when the size of the leaf is sufficiently diminished. Duration of experiment, Oct. 25 to Nov. 14.

grows out since it no longer receives any of the sap from the leaf above. I have never seen the other bud in this node grow out. This experiment also succeeds in practically every case.

We have seen that if we remove one leaf and its axillary bud at the apex of a topped stem, leaving only one leaf at the apex, the buds in the node below are practically always prevented from growing out. When we diminish the mass of the leaf sufficiently (as is done in Fig. 6), this inhibitory influence ceases and every plant forms a shoot in

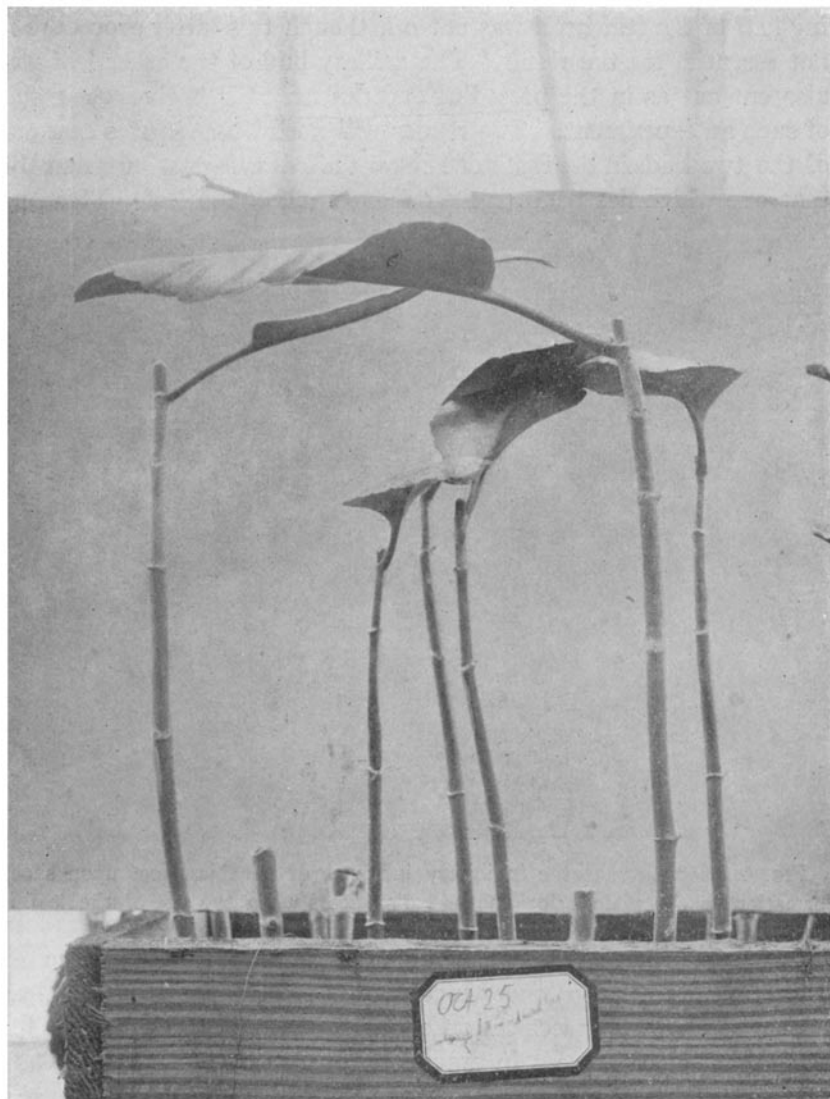


FIG. 7. Control experiment to Fig. 6. Duration of experiment also from Oct. 25 to Nov. 14.

each of the two buds in the first node. Fig. 7 shows the control experiment; namely, six stems each with one whole leaf at the apex. Not a single stem has formed a shoot in the first or any other node below the leaf. Both sets of experiments were carried out simultaneously and both sets of plants were side by side in the same flower bed.

When we reduce the mass of a leaf 10 days after the experiment is started and when the new shoots begin to form, the inhibitory effect nevertheless becomes noticeable.

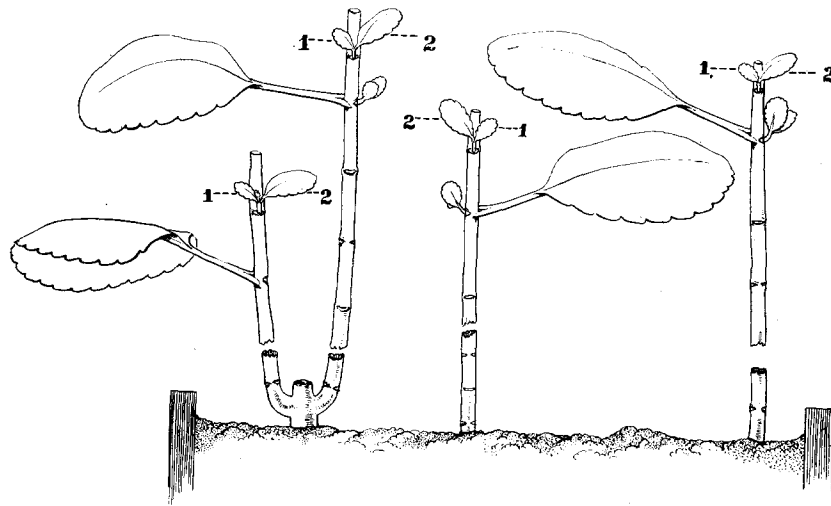


FIG. 8. Proof that traces of inhibition of a leaf upon shoot formation are also noticeable in the more apical shoots. Old leaf left at first node below the apical node. The two Leaves 1 and 2 of the new shoot in the apical node which are normally of equal size show a constant difference, Leaf 1 (on the side where the old leaf is) being smaller than Leaf 2 (on the opposite side).

Demonstration of the Inhibitory Influence of a Leaf on Shoot Formation in a More Apical Node.

We can state as a general rule that a leaf accelerates the growth of shoots at the apex and prevents or retards it in the basal parts of the stem. The leaf has, however, also a slight inhibitory effect on the more apical buds. In order to prove this it is necessary to make experiments like those represented in Fig. 8. In a number of topped

plants one leaf is left at the node below the most apical one. In this case both buds in the most apical node grow out into a shoot giving rise as usual to two small Leaves 1 and 2. While these leaves are normally of equal size, a typical and constant difference exists between the size of the two leaves when one old leaf is left in the node below. Leaflet 1, which has the same orientation as this old and large leaf in

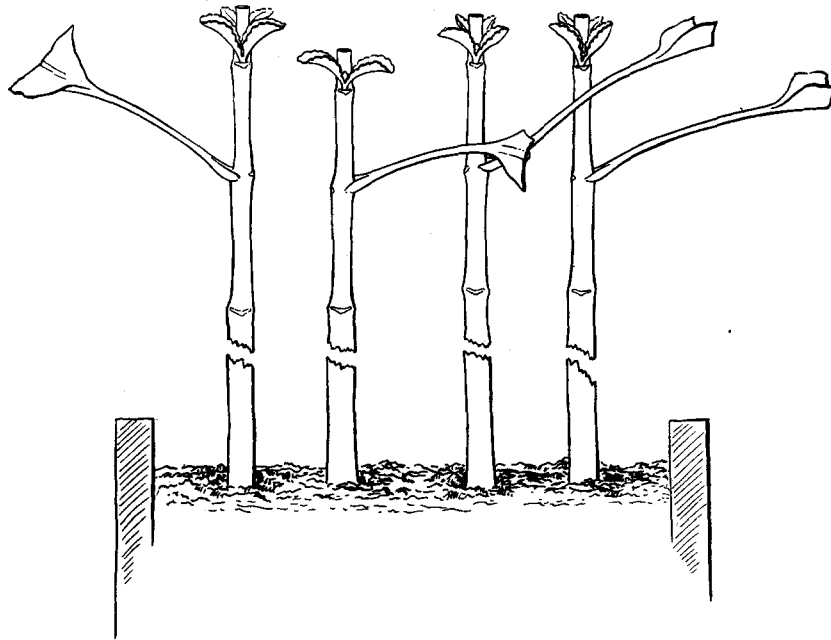


FIG. 9. The inhibitory influence of the leaf upon an apical bud disappears also when the size of the leaf is reduced. Duration of experiment, Oct. 24 to Nov. 7.

the node below, is practically always smaller than the other, Leaflet 2 (Fig. 8). This difference is intelligible on the assumption that a small quantity of the inhibitory substances from a leaf flows towards the apex of the stem; these substances will reach the young leaf facing the same side of the stem where the old leaf is, while they do not reach the other leaf. When we reduce the size of the old leaf, this inhibitory influence disappears (Fig. 9).

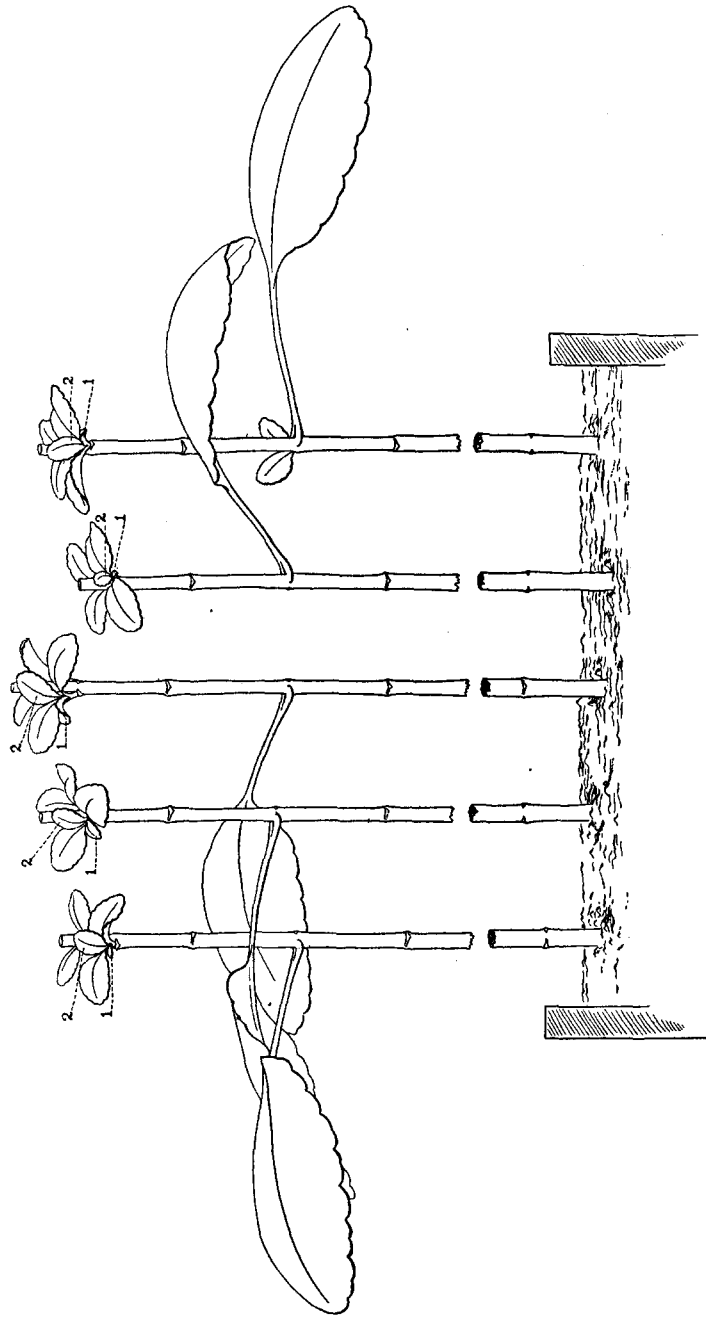


FIG. 10. In the second node above the old leaf the inhibitory effect is shown in the second pair of leaves of the new shoot which are no longer of equal size, Leaf 1 which is above the old leaf being a little smaller than the symmetrical Leaf 2. Duration of experiment, Oct. 17 to Nov. 13.

This slight inhibitory influence of a leaf upon the more apical buds shown in Fig. 8 can also be demonstrated in the growth of the second node above a leaf (Fig. 10). In this case the influence is noticeable only in the second pair of new leaves of a bud; Leaf 1 which has the same orientation as the old leaf remains smaller than Leaf 2.

While in the basal region of a leaf the inhibitory effect is complete, it is comparatively slight in the apical part.

Influence of Gravity upon the Inhibitory Action of the Leaf.

All the experiments on potted plants described in the preceding pages can be repeated with the same result in stems cut out from a plant. We may omit a description of such experiments since they would constitute only a repetition of what has already been stated. But certain of these experiments yield some additional results which are of theoretical importance.

The assumption that the inhibitory effect of the leaf upon the growth of dormant shoot buds is due to chemical substances sent out by the leaf is supported by the striking influence of gravity on regeneration in stems suspended horizontally. Long straight stems were cut out from a plant and suspended horizontally in an aquarium nearly saturated with water vapor. When two leaves are left at the most apical node of such pieces, none of the buds situated more basally will grow out. If, however, one leaf with its axillary bud is removed and the other leaf left, regeneration will occur, but the buds which will grow out will show a characteristic difference according to whether the leaf is on the upper or the lower side of the horizontally suspended stem.

We suspend such stems so that the axis of the two most apical buds (one of which is removed with its leaf) is vertical (Fig. 11). In five stems the leaf is on the upper side and in five stems on the lower side of the stem (Fig. 11). All the stems were originally horizontal but underwent the geotropic bending described in previous papers, whereby the upper side became concave.

When the leaf is below (right half of Fig. 11), shoots may be formed in the first node basally from the leaf. This occurred in three out of five stems drawn here. The other two formed shoots from the upper bud of the second node.

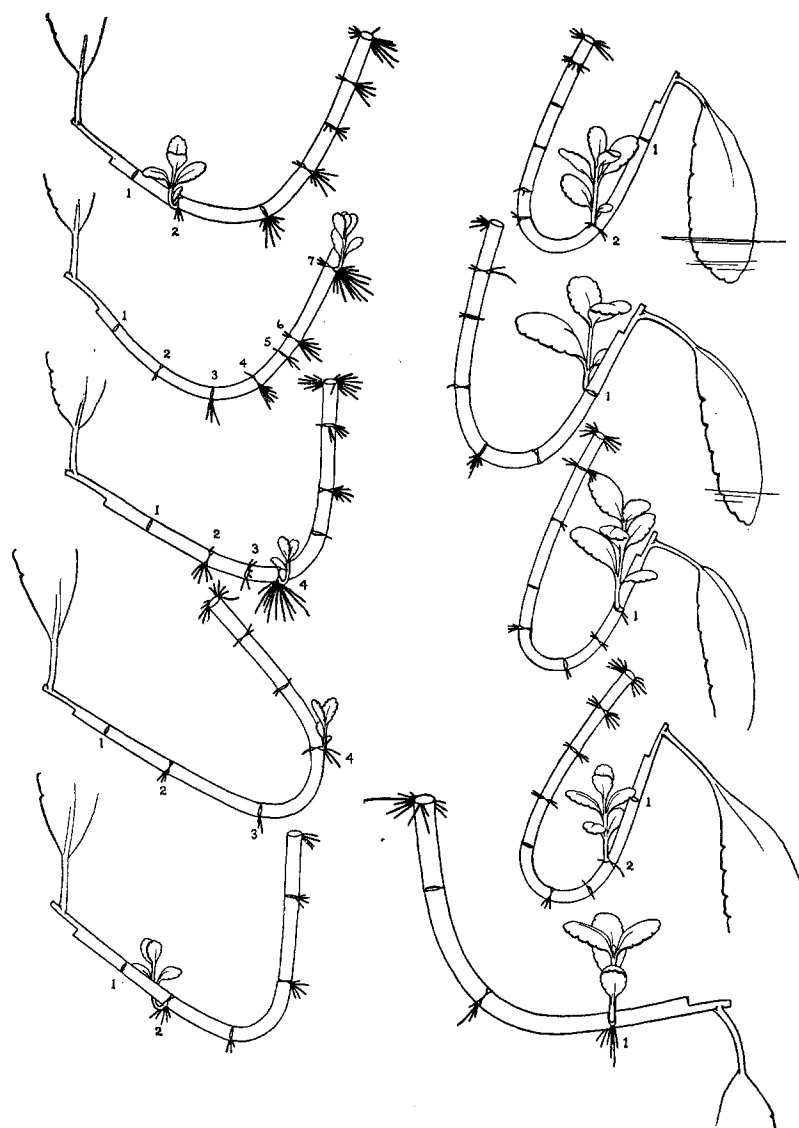


FIG. 11. Influence of gravity on shoot formation in horizontally suspended stems. The stems were originally straight but underwent geotropic bending during the experiment. In the stems to the right one leaf is left at the lower side of apex. In this case the shoots in the first node will grow out in half of the stems; in the second node only the upper bud will grow out. In the stems to the left the leaf is on the upper side. In this case the shoots in the first node are prevented from growing, and only the lower bud in the second and fourth node can grow out. Duration of experiment, Sept. 29 to Nov. 12.

When the leaf is above (left side of Fig. 11) none of the buds in the first node will grow out. If any buds grow out, they are either the second or fourth on the *lower* side of the stem. These shoots grow out with much delay compared with the growth of shoots in stems where the apical leaf is on the lower side of the stem.

The inhibitory influence is therefore greater when the apical leaf is on the upper than when it is on the lower side of a horizontally suspended stem. This influence of gravity supports the idea that it is the sap sent out by the leaf which produces the inhibition. The diagrammatic Figs. 12 and 13 make this clear. In Fig. 12 the leaf is below and the path of the conducting vessels from the leaf is marked by black lines. The two buds of the first node lie on the upper edge of the sap flow containing the hypothetical inhibitory substances. The buds of the first node may or may not receive enough of these substances to prevent their growth. When, however, the leaf is above (Fig. 13) seepage from the vessels will cause the buds in the first and third nodes to be flooded with the sap and the inhibitory substances contained in it, thus preventing their growth. The lower bud in Node 2 (or Node 4) is outside the direct path of the conducting vessels of the leaf and hence the lower bud of Node 2 as well as of Node 4 may develop. Through the influence of gravity traces of the sap may possibly reach the lower bud of the second or fourth node. This may account for the fact that growth of these buds is usually retarded.

The reader will notice that these facts give us a neat method of restricting the growth of shoots to the lower side of a horizontally suspended stem, contrary to the general rule that in such cases shoots arise on the upper side of the stem. When we remove the lower half of such a horizontally suspended stem (containing one leaf at the upper side of its apex) leaving on the lower side only the region of the second node (Fig. 14), regeneration of a shoot will occur only from the bud on the under side of this second node. The growth of the buds in the intact upper half of the stem is completely suppressed and the growth of the bud on the under side of Node 2 is slow for reasons stated. No growth will occur on the upper side, except after the leaf is wilted or conduction of its sap through the stem is interrupted.

The correctness of this idea is supported by the further fact that this inhibitory effect of a leaf on the growth of shoots, especially in the basal parts of the stem, is diminished when the mass of the leaf is reduced. Fig. 15 shows such an experiment. Of the five stems on the left each had one whole leaf on the upper side of the originally horizontal stem. In one stem only did a shoot form and this shoot

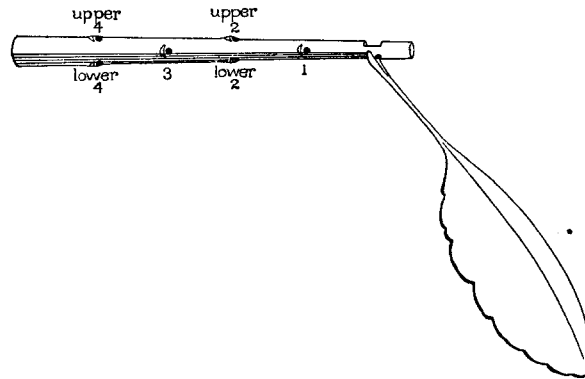


FIG. 12.

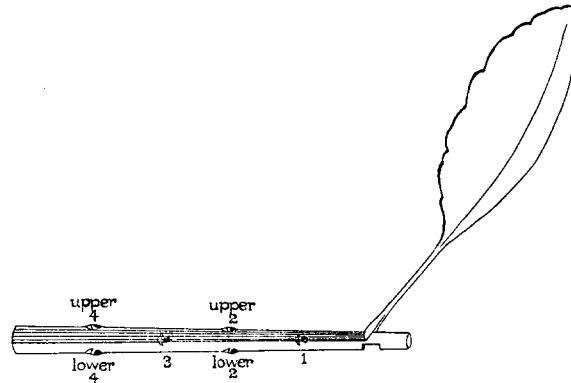


FIG. 13.

FIGS. 12 and 13. Explanation of the influence of gravity on regeneration in horizontally suspended stems on the assumption that inhibition is due to substances carried by the sap sent out by the leaf. When the leaf is below (Fig. 12), the buds in Nodes 1 and 3 are at the upper edge of the sap flow and these buds may or may not escape the inhibitory effect. In Fig. 13 the leaf is above and the sap flowing in the upper half is bound to reach the buds in Nodes 1 and 3 and hence their growth is necessarily suppressed. The lower Buds 2 and 4 are outside the sap flow and may develop.

developed on the lower side in the second node. In the five stems on the right the mass of the leaf was reduced considerably. Four of the five stems formed shoots, two even in the second node on the upper side of the stem. In a repetition of this experiment half of the stems with a reduced apical leaf on the upper side formed shoots in the first node basally from the leaf.

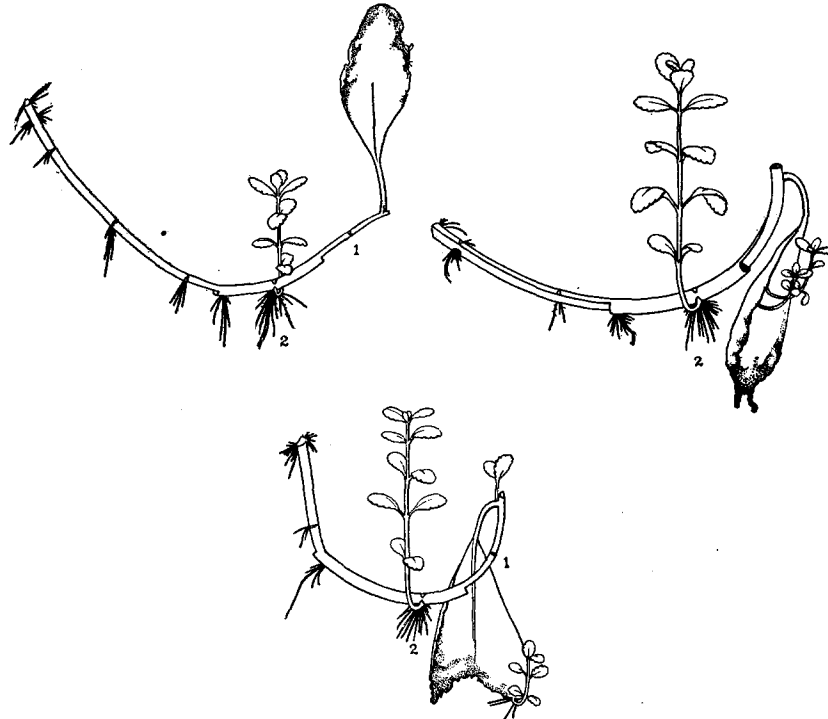


FIG. 14. Horizontally suspended stems. Leaf on upper side. The lower half of stem is removed with exception of second node. The lower bud of the second node grows out into a shoot, while the shoot formation on the upper side of the stem is inhibited by the leaf. Duration of experiment, Feb. 13 to June 24.

The reader will notice that the geotropic bending of the stems was considerably less in the five stems on the right with the reduced leaf than in the five stems on the left with a whole leaf.³

³Loeb, *Science*, 1917, xlvi, 115; *Ann. Inst. Pasteur*, 1918, xxxii, 1; *Forced movements, tropisms, and animal conduct*, Monographs on Experimental Biology, Philadelphia, 1918.

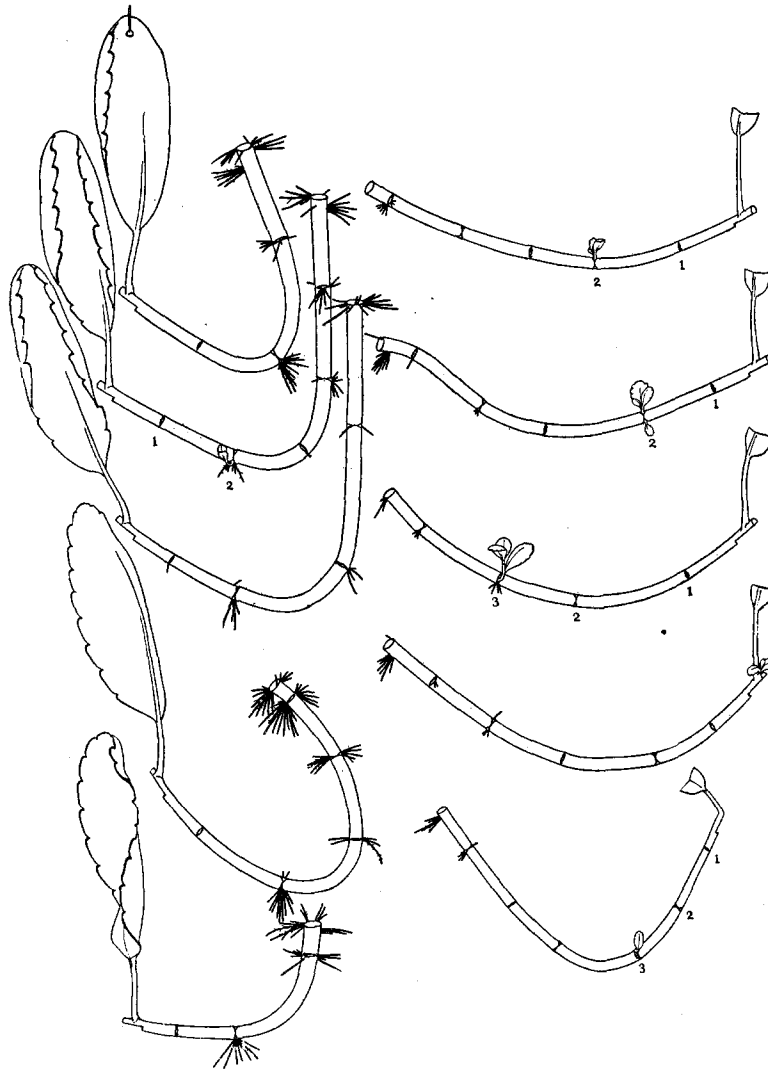


FIG. 15. Showing that reduction in size of apical leaf diminishes its inhibitory power. On the left, five stems each with large size leaf at apex, on upper side of stem. Inhibition of shoot formation complete except in one stem where a shoot is formed in the lower bud of the second node. On the right, five stems each with reduced leaf on the upper side. Four out of five stems form shoots and two of these do so in the upper bud of the second node. Root formation and geotropic curvature are considerably larger in stems with whole leaf than in stems with reduced leaf. Duration of experiment, Oct. 29 to Nov. 23.

Correlation between Inhibitory Effect of a Leaf on Shoot Production and the Opposite Effect on Root Production in a Stem.

The writer has already called attention to the correlation existing between the inhibitory effect of a leaf upon shoot formation and the opposite effect on root formation. This is expressed among others in Fig. 15. The stems on the left side, with a full size leaf at the apex, formed a considerably larger mass of roots in the same time than the stems on the right whose leaves are reduced in size. The larger the apical leaf the greater the mass of roots produced by the basal part of the stem in the same time and under equal conditions; and the greater the inhibitory effect upon the shoot production in this part of the stem.

A striking demonstration of this correlation is given in the upper row of drawings in Fig. 16. One-half of the leaf and one-half of its petiole were cut off. The leaf was at the base of the stem which contained only one node in front of the leaf. Generally only one of the two buds in the node situated apically from the leaf grew into a shoot; namely, the one on that side where half of the leaf was removed. The growth of the bud on the side where the half leaf was preserved was retarded or suppressed. At the basal end of the stems roots developed, but at first only on that side of the stem where the leaf was preserved.⁴ Hence the leaf behaves as if it sent out, in addition to the material needed for regeneration, substances retarding shoot formation and favoring root formation.

In the lower row of stems (Fig. 16) the leaf was preserved at the apical end of the stem. In this case the inhibitory effect of the half leaf on shoot formation is much greater than when it is at the base of the stem (upper row). Most of the stems in the lower row have not yet formed any shoots, but where a shoot was formed (as in IIIa) it was formed on the opposite side from that where the half leaf was preserved, while the roots were on the same side with the leaf.

In Fig. 16 the half leaf was above. In Fig. 17 the half leaf was always below. The result in Fig. 17 is the same as in Fig. 16, *i.e.*, on the side where the half leaf is preserved the shoot formation is always retarded compared with that on the other side. The inhibition is more complete when the leaf is at the apex (lower row) than when the leaf is at the base (upper row).

⁴Later on roots may form on both sides of the basal end of the stem.

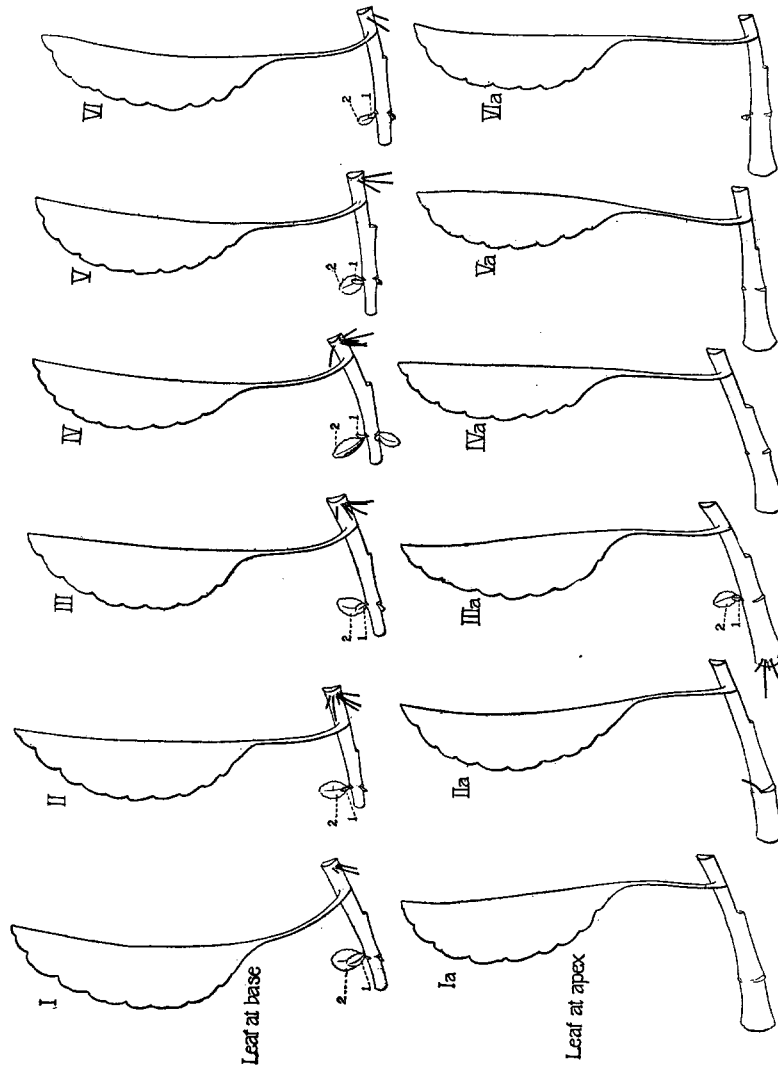


FIG. 16. Upper row: half leaf at base of stem. Shoot formation inhibited and root formation favored on that side of stem where petiole of leaf is preserved. Lower row: half leaf at apex. Inhibitory effect of leaf on shoot formation is more complete than in upper row. Duration of experiment, Oct. 24 to Nov. 9.

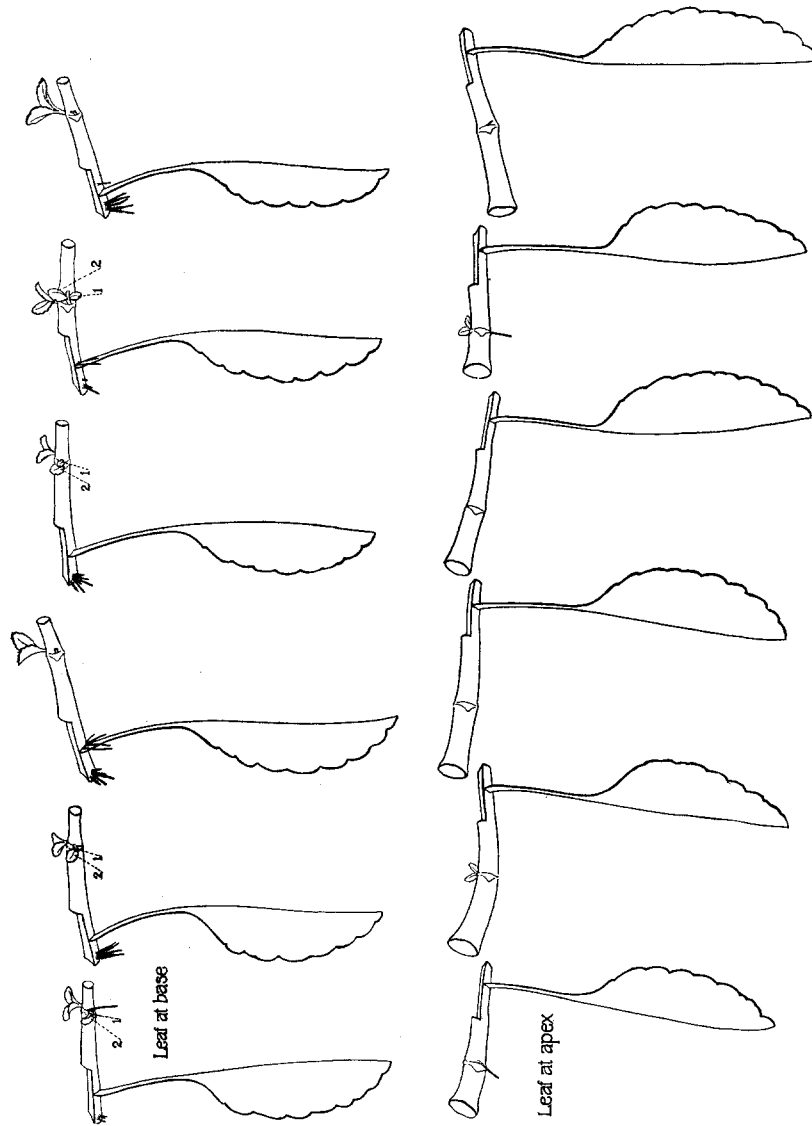


FIG. 17. Half leaf on lower side of stem. Similar result as in Fig. 16. Duration of experiment, Oct. 19 to Nov. 1.

Proof That the Leaf Sends Nutritive Material Also in the Basal Direction of the Stem.

When we suspend stems horizontally with one leaf at the apex, the inhibitory effect of the leaf upon shoot formation is much stronger when the apical leaf is on the upper side of the stem than when the leaf is on the lower side of the stem. We can use experiments of the latter type to show that the leaf sends nutritive substances to the base as well as to the apex and that the fact that the leaf inhibits shoot formation at the base is not due to the leaf failing to send nutritive material in the direction of the base of the stem. The method of proving this consists in measuring the influence of the mass of the leaf upon shoot formation in the basal part of a horizontally suspended stem.

Stems were split longitudinally and suspended horizontally; each stem having a leaf at the apex, and on the lower side of the stem.⁵ Fig. 18 gives the result of such an experiment. Pieces of stems possessing two nodes and two leaves at the apical node are split longitudinally, so that each half stem has one leaf at the apex and one bud in the basal node. One leaf is left intact while the size of the sister leaf is reduced considerably. In Fig. 18 Leaves I and Ia, II and IIa, etc., are sister leaves. Practically each stem has produced a shoot at the basal node, but the shoot is invariably greater in the stems in the upper row where a whole leaf was at the apex than in the lower row where the apical leaf was reduced in size. The drawing was made on the 34th day of the experiment. It is obvious that the growth of the basal shoots increases with the mass of the apical leaves and this is proved by the relative weight of the leaves and shoots.

Duration of Experiment, 39 Days.

	Wt. of 4 whole leaves.	Mass of 4 shoots.	Shoots per gm. of leaf.
	<i>gm.</i>	<i>gm.</i>	<i>mg.</i>
Fresh.	17.035	2.128	125
Dry.	1.196	0.193	160
	Wt. of 4 reduced sister leaves.		
Fresh.	2.182	0.384	175
Dry.	0.140	0.027	193

⁵ If the leaf is on the upper side, we get too much inhibition of shoot formation, whereby the experiment is rendered difficult.

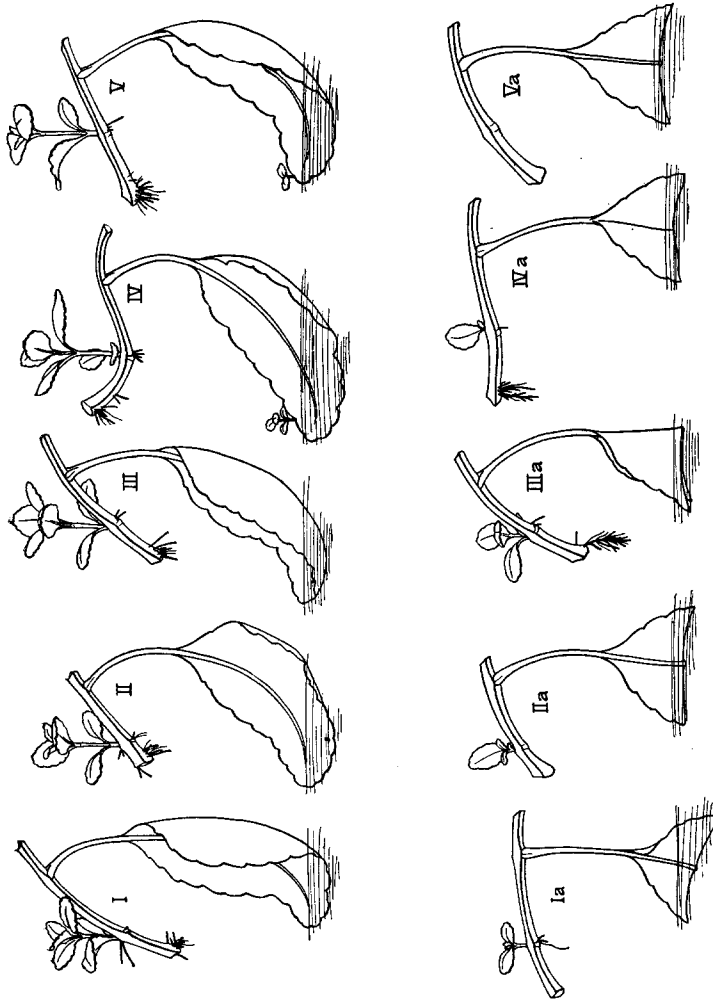


FIG. 18. Stems split longitudinally and suspended horizontally, with apical leaf on the lower side. Leaves I and Ia, II and IIa, etc., are sister leaves. The leaf in the upper row is intact, while in the lower row it is reduced in size. The mass of shoots produced is smaller in the stems of the lower row where the size of the leaf is reduced than in the upper row where the leaf is larger. This proves that inhibitory influence of leaf upon basal parts of stem is not due to lack of nutritive material.

Other experiments gave similar results. While the experiment does not reveal any strict proportionality between mass of leaf and mass of basal shoot produced, the increase of the mass of shoots with the mass of the leaves is unmistakable.

We therefore come to the following conclusion. The leaf sends material for growth in both directions of the stem, to the apex as well as to the base. It also behaves as if it sent out inhibitory substances in both directions, but if this be the case there must exist a considerable difference in regard to the mass of the latter. While much or almost all of the inhibitory substance is sent in basal direction, only traces of it are sent in an apical direction, so that special experiments are required to demonstrate the inhibitory effect in the apical parts of the stem.

A glance at Fig. 18 confirms also the statement that the mass of roots formed in the basal part of a stem increases with the mass of the apical leaf. The stems in the upper row with a whole leaf at the apex have a greater mass of shoots and roots than the stems in the lower row with a reduced leaf.

DISCUSSION.

We have shown in this paper that a leaf inhibits the regeneration of shoots in the basal parts of the stem and that this inhibition is diminished or ceases when the mass of the leaf is reduced below a certain limit. If the inhibitory influence of the leaf is due to inhibitory substances sent out by the leaf to the basal parts of the stem we must conclude that traces of these inhibitory substances flow also to the apex of the leaf since it is possible to demonstrate slight inhibitory influences of the leaf in the buds situated apically.

The influence of the leaf upon the regeneration of roots is exactly the reverse from that on the regeneration of shoots. The leaf favors the formation of roots in the basal parts of the stem and this favorable influence upon regeneration of roots in the basal part of a stem increases with the mass of the apical leaf.

This gives us an indication of the rôle which a leaf plays in the establishment of the polar character of regeneration in the stem of *Bryophyllum calycinum*. When a piece of stem is cut out with a leaf in the middle, the leaf sends out nutritive material in both directions of

the stem, since it can be shown that if once a shoot is caused to grow, it increases with the mass of the leaf, no matter whether the shoot is situated at the base or the apex. The leaf has a powerful inhibitory effect upon the development of basal shoots. If we assume this inhibitory influence to be due to inhibitory substances we must further assume that not more than mere traces of these inhibitory substances reach the apex which are not sufficient to interfere with the growth of shoots. At the moment we cut out the piece of stem from a plant the stem contains throughout a sufficient quantity of these inhibitory substances to prevent shoots from growing, and these inhibitory substances will continue to flow in the descending sap towards the base of the stem. The most apical buds in the stem will hence be the first ones to become sufficiently free from inhibitory substances to be able to grow and the regeneration of shoots will start at the apex of the piece of stem. As soon as the shoots are beginning to grow at the apex they in turn act like a leaf so that now the further growth of shoots at the base is permanently inhibited. On the other hand, the influences which inhibit shoot formation at the base are associated or identical with influences favoring root production. Hence the leaf will favor root formation at the base of the stem and shoot formation at the apex. This gives an idea how the leaf may contribute by its "internal secretion" to the establishment of the polar character of regeneration.

If it could be shown that plants possess a closed circulatory system comparable to that of animals, all these facts might become easily intelligible if we assume that inhibitory substances for shoot formation (and favorable substances for root formation) are carried in the descending sap from the leaf to the root, where they are retained or altered, so that the ascending sap becomes practically (but not absolutely) free from these substances and contains only the nutritive material for the formation of shoots.

The assumption that the inhibitory influence of a leaf upon shoot formation in the basal part of a stem is due to inhibitory substances, is not without analogy in biology. It is known that when twins in cattle have different sex the female is in the majority of cases sterile, and Lillie⁶ has shown that there exists an exchange of blood between

⁶ Lillie, F. R., *J. Exp. Zool.*, 1917, xxiii, 371.

such embryos. This indicates that there exists in the blood of the male cattle embryo an inhibitory substance which prevents the normal development of the sex glands of the female embryo.

A second case is that of the prevention of the development of the male plumage in the female fowl. Boring and Pearl⁷ have shown that the ovary of such females contains specific cells, the luteal cells, which are absent in the male. Boring and Morgan⁸ have found that in the Sebright, where the male shows hen-feathering, luteal cells exist in the testes of the male bird. Since extirpation of the ovary in fowl and duck leads to the assumption of the full male plumage by the female (Goodale⁹), it seems as if some specific substance in the ovary inhibited the development of male plumage in the female. This inhibitory substance may be contained in the luteal cells, which, however, cannot well influence the development of feathers in any other way than by the secretion of some substance into the blood. The assumption that the inhibition of shoot formation in the basal part of a stem by a leaf is due to an inhibitory substance secreted by the leaf is therefore not without a precedent.

It is, however, necessary to call attention to the fact that even if the inhibitory influence of the leaf upon shoot formation should turn out to be based on the chemical character of the sap sent out by the leaf, it does not follow that all phenomena of inhibition and correlation in regeneration will find their explanation on the same basis. Quantitative experiments published in a former paper suggest that the inhibitory influence of a piece of stem on shoot formation in the leaf of *Bryophyllum calycinum* is due to the fact that the leaf sends its sap normally to the stem, and that as long as this happens the buds in the notches of the leaf cannot grow out.¹⁰

We shall show in the next communication that growing buds have inhibitory influences upon the formation of shoots comparable to the same influences caused by a leaf.

⁷ Boring, A. M., and Pearl, R., *Anat. Rec.*, 1917, xiii, 253. Pearl and Boring, *Am. J. Anat.*, 1918, xxiii, 1.

⁸ Boring, A. M., and Morgan, T. H., *J. Gen. Physiol.*, 1918, i, 127.

⁹ Goodale, H. D., *Biol. Bull.*, 1910-11, xx, 35; *Am. Nat.*, 1913, xlvii, 159; *J. Exp. Zool.*, 1916, xx, 421.

¹⁰ Loeb, *Ann. Inst. Pasteur*, 1918, xxxii, 1.

SUMMARY.

1. In *Bryophyllum calycinum* two apical leaves suppress the shoot formation in all the dormant buds situated basally from the leaf; one apical leaf suppresses the shoot formation in the basal buds situated in the same half of the stem where the leaf is, and, if one-half of the petiole of such a leaf is removed, the growth of basal buds in one quadrant of the stem is suppressed.
2. This inhibitory influence of a leaf upon shoot formation in the basal part of a stem is diminished or disappears when the mass of the leaf is reduced below a certain limit.
3. The inhibitory influence of an apical leaf upon the growth of shoots in horizontally suspended stems is greater when the leaf is on the upper than when it is on the lower side of the stem.
4. All these facts suggest the possibility that the inhibitory influence of the leaf upon shoot formation is due to inhibitory substances secreted by the leaf and carried by the sap from the leaf towards the base of the stem.
5. An apical leaf accelerates root formation in the basal part of a stem and this accelerating effect increases with the mass of the leaf.
6. This inhibitory influence of a leaf upon shoot formation and the favoring influence upon root formation in the more basally situated parts of the stem is one of the factors determining the polar character of regeneration.