# REGENERATION IN BRYOPHYLLUM CRENATUM

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In Vol. 60 (1915) of the Botanical Gazette, appeared an article by Jacques Loeb entitled "Rules and Mechanism of Inhibition and Correlation in the Regeneration of Bryophyllum Calycinum." This article was followed by several others from the same author in later numbers of the Gazette, in Science and in the Journal of General Physiology.

As there was available in the Botany Greenhouse at the University of Illinois a number of plants of Bryophyllum crenatum, the experiments given in Loeb's first article were repeated using this species. While many of the results obtained by Loeb with B. calycinum applied to B. crenatum, several differences were found sufficiently great to warrant noting. Since the plants of B. crenatum used in the experiments differed from those of Loeb in that they were mature and in flower, the differences were thought at first to be due to maturity or to the physiological state of the respective species. Subsequently it was found that very young plants of B. crenatum gave essentially the same results as the mature ones. In the cases where the results with B. crenatum differed from those obtained by Loeb with B. calycinum, the experiments were repeated using B. calycinum.

One difference between the two species should be noted here. With B calycinum, whenever growth appeared in the notches of leaves separated from the plants, roots developed before the shoots. The reverse order of development was always true with B. crenatum.

Since the study was to be a comparative one, the methods used by Loeb were followed as nearly as possible. The work was done in the greenhouse during the winter months and at a temperature of approximately 70° F. The numbers used in referring to the leaves correspond to those used by Loeb.

In the first experiment, 3 leaves of B. calycinum and of B. crenatum were prepared as follows:—leaf 1 was separated entirely from the plant, leaf 2 had a portion of

the stem, leaf 3 had a portion of the stem and also the opposite leaf attached. These leaves were suspended by means of threads from the top of an aquarium in a saturated atmosphere in such a manner that their tips were submerged in water.

After 10 days the results obtained with B. calycinum were essentially those obtained by Loeb. After 16 days, however, roots had developed at the base of the petiole in 2 of the completely isolated leaves suspended in saturated air, and by the end of 5 weeks shoots had appeared also. These results are contrary to those obtained by Loeb who says, "The advantage of this plant for the study of the problem of regeneration lies in the fact that shoots can grow out only from definitely located buds in the stem and in the notches of the leaf."[1] In another place he states, "The stalk of an isolated leaf without any piece of stem is not capable of giving rise to any regeneration. Such a leaf will form adventitious roots and shoots in its notches very rapidly."

B. crenatum gave several results different from those obtained by Loeb with B. calycinum. After 10 days, growth had occurred in leaf 1 on the aerial as well as on the submersed portion of the leaf, and in mature flowering plants the growth from the aerial was more vigorous than that from the submersed portion of the leaf. In leaf 2 from mature plants the bud grew out from the opposite axil as stated by Loeb. In many cases, however, shoots appeared in both axils. In the leaves from very young plants, only the bud from the adjacent axil had developed. With B. calycinum, Loeb reports no development of the axilliary buds in leaf 3. In B. crenatum shoots appeared from both axils in all the specimens. There was also some notch growth on most of the leaves.

Plate I shows these leaves at the end of 5 weeks. Leaf 1 with the best notch growth had developed a large shoot at the end of the petiole. The shoots and roots in both axils of leaf 3 were as large as those in leaf 2 and the growth from many notches was nearly as vigorous as that in leaf 1. Leaf 2 showed considerable notch growth. All the drawings given in this article were made from photographs of the specimens.

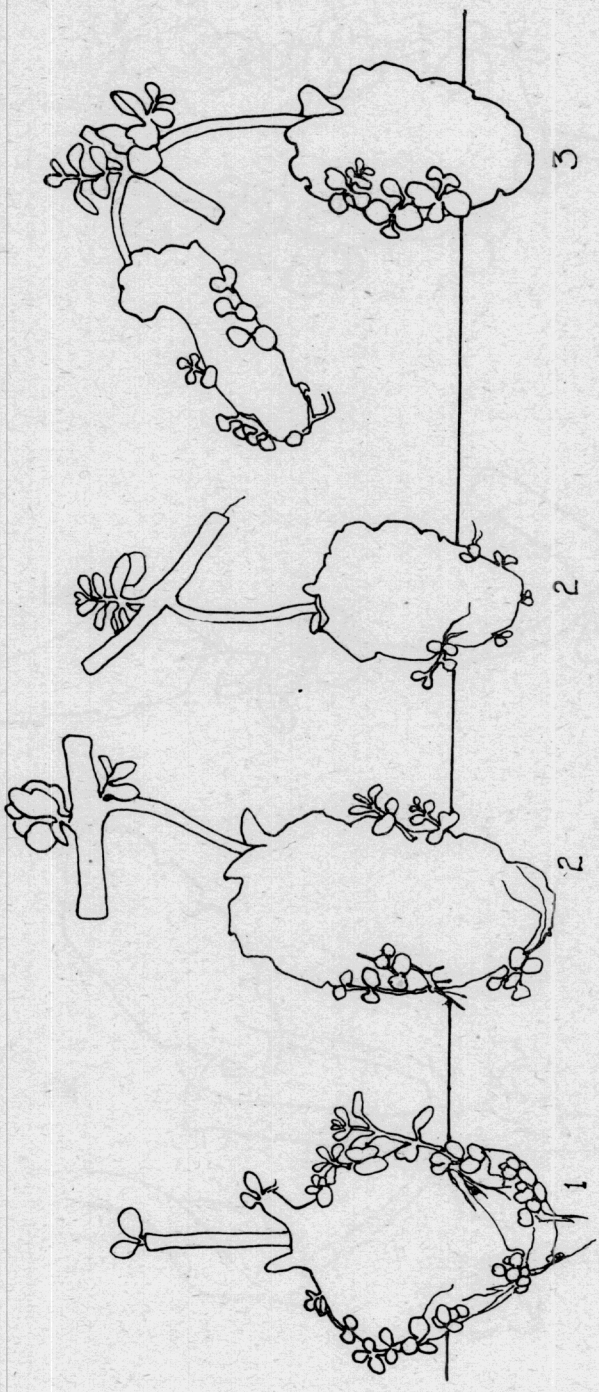



PLATE I

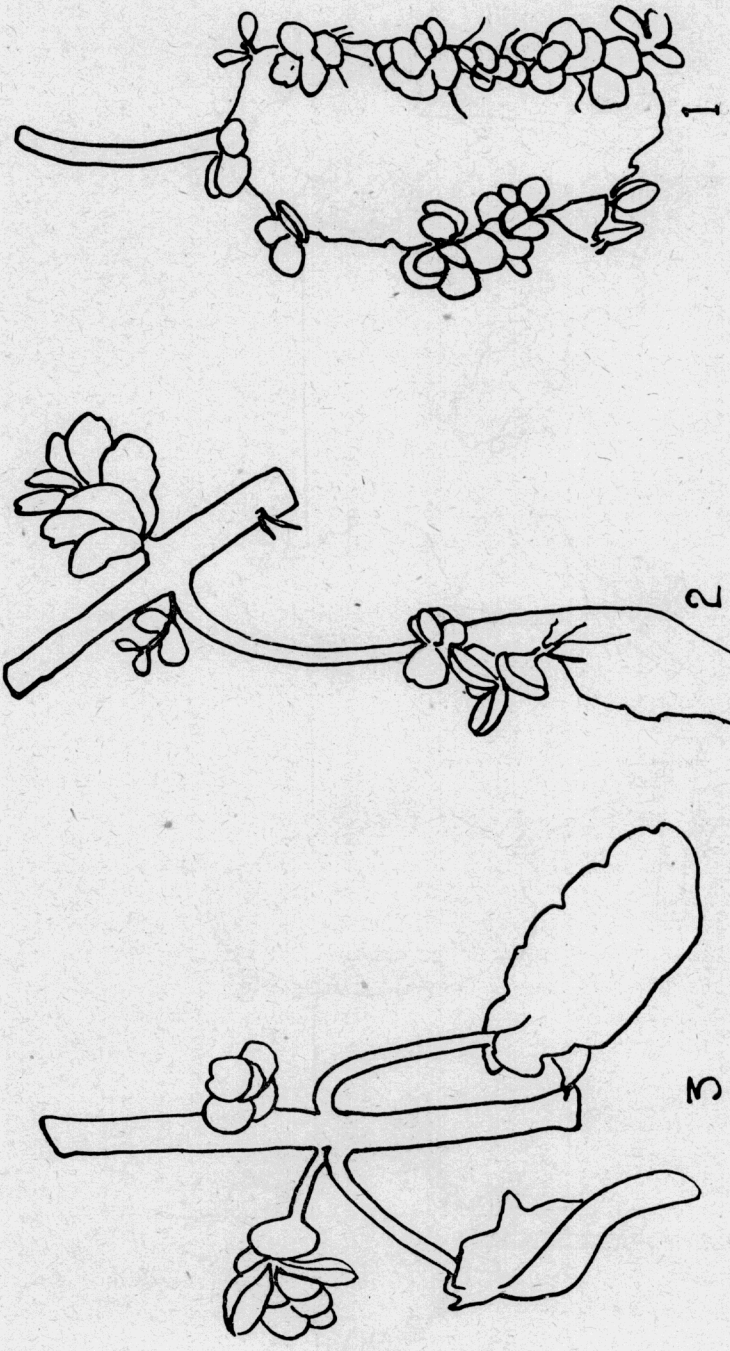



PLATE II

This same experiment was repeated suspending the leaves in a saturated atmosphere but not allowing their tips to touch the water. Plate II shows the results after 5 weeks. The growth was the same as that just described but the leaves were less turgid.

Experiment II was designed to show the inhibiting influence of the axillary buds on the growth in the notches of the leaves. Leaf 6, similar to leaf 1, was separated entirely from the plant; leaves 7, 8 and 9 had a portion of the stem attached. Both axillary buds were removed from leaf 7, the opposite axillary bud was removed from leaf 8, and no bud was removed from leaf 9. In this experiment B. crenatum gave results different from those obtained by Loeb with B. calycinum. Leaves 6 and 9 agreed with leaves 1 and 2 described in experiment I. After 4 weeks, 2 specimens of leaf 7 showed no growth. 3 specimens had developed a shoot from the petiole above the cut. In each of the latter, there was some notch growth although it was much smaller than in leaf 6. Leaf 8 had a shoot from each adjacent axillary bud and also some notch growth. Plate III shows these leaves after 5 weeks.

Concerning the inhibiting influence of the growth of the axillary buds on the notch growth Loeb states, "It is, therefore, obvious first, that a stem whose buds are removed has still an inhibiting influence upon the formation of roots in the notches of a leaf; and second, that if the buds of the stem are not removed, the growth of the bud opposite the leaf enhances this inhibiting effect of the stem upon the leaf considerably. Since the growth of this bud of the stem is as a rule also inhibited when the opposite leaf is not removed, as in figure 3, we understand why the non-removal of this leaf favors the growth of the adventitious roots from the notches of the other leaf." In B. crenatum the removal of the buds from the stem did inhibit the notch growth and the inhibiting effect was enhanced by the growth of the bud which was not removed, but the growth of this bud of the stem was not inhibited when the opposite leaf was not removed as was shown by leaf 3.

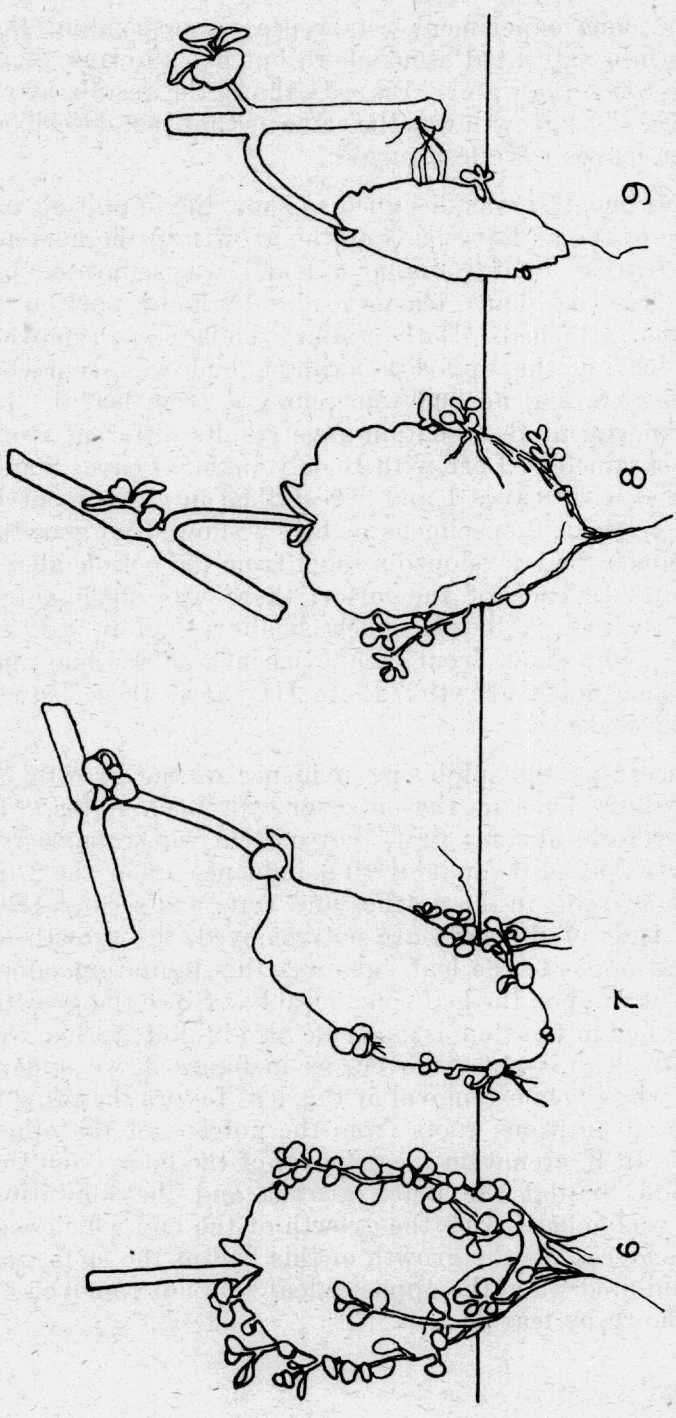
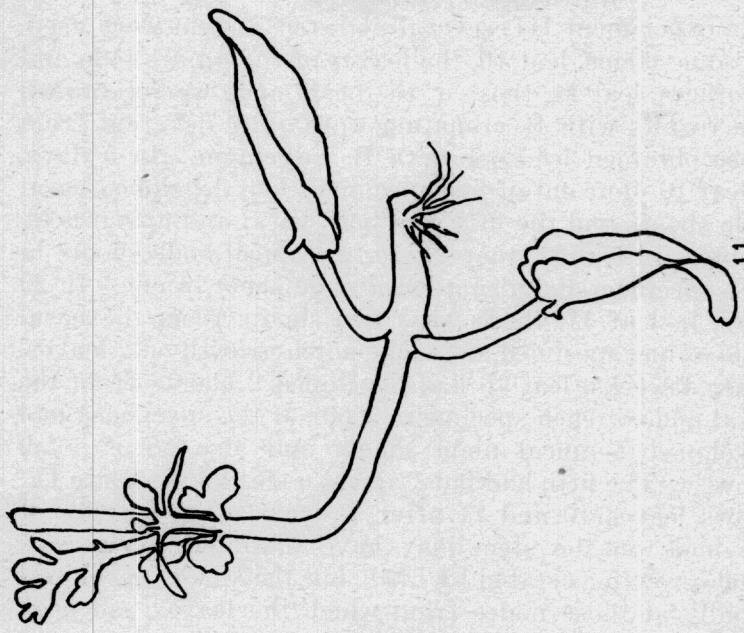



PLATE III



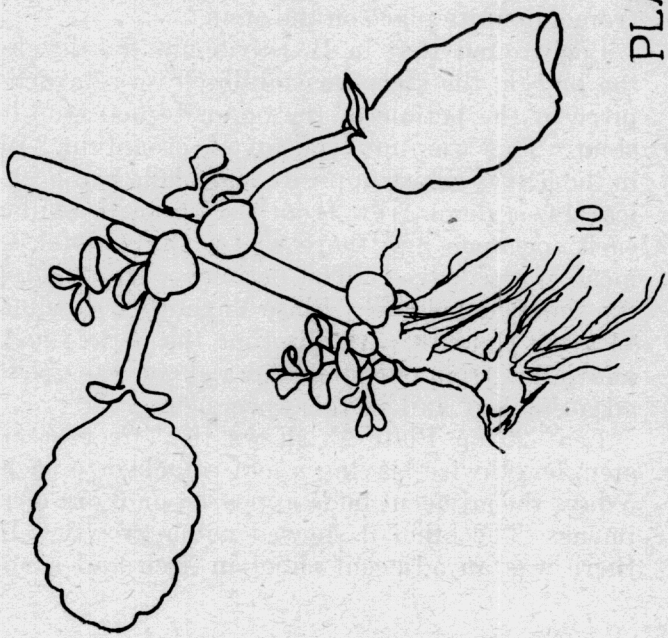




PLATE IV

In experiment III, several nodes of a stem were used. On some stems, leaf 10, the leaves of the apical node and on others, leaf 11, those of the basal node were retained. The results with B. crenatum were quite different from those obtained by Loeb with B. calycinum. In 5 days, in leaf 10, four out of five specimens had developed basal node shoots and the fifth had both basal and apical node shoots. In leaf 11, there were two apical node shoots in each specimen and also a basal node shoot in one. In 20 days, in leaf 11, there were two shoots from the basal node of one specimen and some notch growth on 2 leaves. After 4 weeks, leaf 10 had developed 2 shoots from the basal node of each specimen. Four of the specimens had developed 2 apical node shoots and also some notch growth. The fifth had but 1 apical node shoot. Plate IV. shows leaves 10 and 11 after 5 weeks. The growth of the buds on the stem may have inhibited a vigorous notch growth as stated by Loeb, but the shoots developed rapidly at those nodes from which the leaves had been removed, regardless of whether those nodes were apical or basal. From the under side of one leaf, a shoot had developed near the mid-vein. Here again we have regeneration other than that from the notches of the leaf or from a definite place on the stem.

Loeb found that in B. calycinum the development of the bud on the stem was inhibited or retarded if only a piece of the petiole of the opposite leaf was left on the stem. This was not true for B. crenatum. In 14 days, in the leaves whose opposite leaf blade had been removed, leaf 14, a shoot grew from the opposite axillary bud in each specimen and there was a slight notch growth on most of the leaves. The petioles were still intact when the shoots developed. Plate V. shows 2 specimens of leaf 14 after 1 month. At this time the petiole had withered and fallen from one stem and a shoot was growing in the adjacent leaf axil in this specimen.

Leaf 20 on Plate V. shows the effect of cutting the stem lengthwise leaving a leaf attached to each half. In 5 days the adjacent buds appeared on 3 out of the 6 specimens. The other 3 showed notch growth. In 21 days there was an adjacent shoot in each and a slight notch

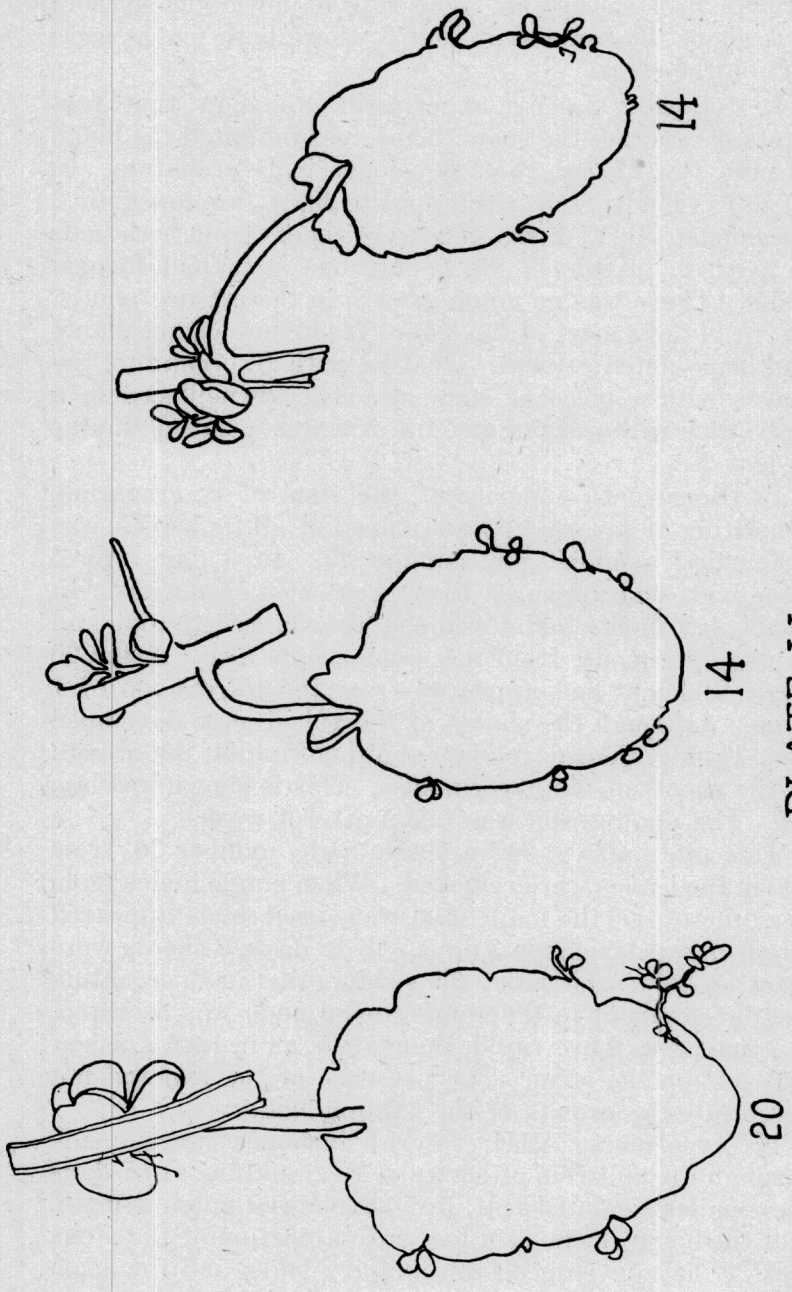



PLATE V

growth in 4 specimens. The rate of development here was about the same as in leaf 3 where both leaves were left on the stem.

In following Loeb's experiments to show that root pressure and not the roots themselves inhibited the notch growth, few, if any, roots developed in B. crenatum. In 10 days shoots from the opposite buds developed in 2 specimens. In 12 days, there were shoots from both buds in 4 cases. After 17 days, only one stem had formed roots. There was no notch growth in the mature plants, but in 14 days most of the leaves from very young plants had some notch growth. That is, with B. crenatum, the leaves with a piece of stem attached when placed in a Petri dish with a small amount of water behaved similar to leaf 2.

In the seventh experiment, the stem of B. crenatum, consisting of several nodes stripped of all its leaves, was suspended in moist air, number 23. In 3 days apical node buds had appeared from 2 out of 5 specimens. In 12 days, 2 shoots had developed at each apical node and in two specimens, from the second node also. After 20 days, 2 shoots had appeared from the third node of 1 stem. Although the shoots of the apical node developed most rapidly their development did not inhibit the growth of the shoots at the lower nodes. This is shown in Plate VI. The photograph was taken after 4 weeks.

This plate also shows a single node, number 28, from which the leaves were removed. When single nodes from near the top of the main stem were used, buds appeared on all the specimens in 3 days. In 12 days, 2 shoots were growing from each node. This shows that in B. crenatum the development in the single apical node was as rapid, in some cases more rapid, than when, as in leaf 2, a leaf was left on the stem. The presence of the leaf did not accelerate the growth of the axillary bud.

In experiment VIII, lateral incisions were made through the mid-vein of leaves of B. crenatum. In 7 days the smallest isolated leaf, leaf 38, showed notch growth. 3 of the 5 specimens of a leaf with a portion of the stem, leaf 39, had developed both axillary buds, and the adjacent bud of the other 2 just showed. In 9 days, there was

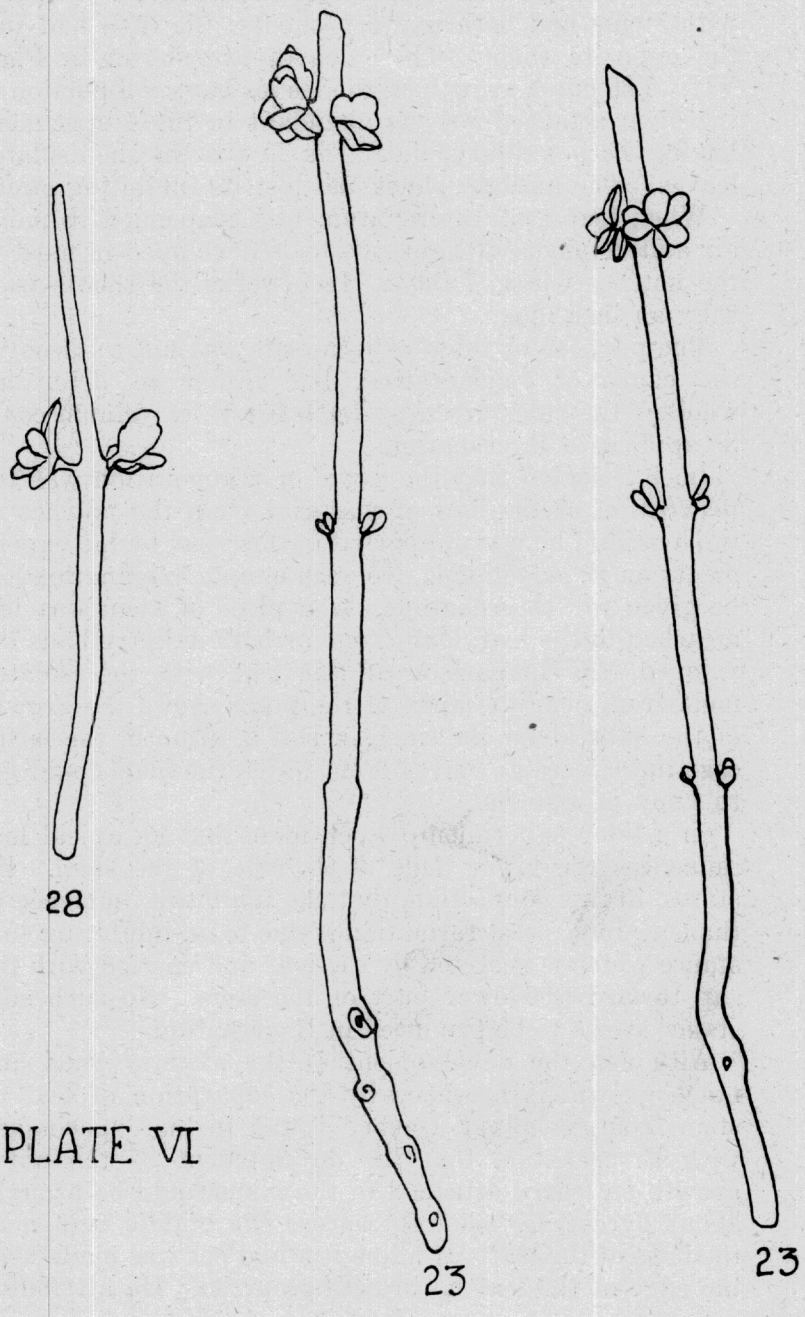



PLATE VI

notch growth in each of the 5 isolated leaves. 4 leaves with stems had both axillary shoots; the fifth had just the opposite shoot. These leaves are shown in Plate VII. The notch growth on the leaves having a portion of the stem attached was as abundant in the 4 specimens having both axillary shoots as it was in the isolated leaves. The axillary shoot had lost its inhibiting effect.

When leaf 1 of B. crenatum was suspended in moist air and deprived of light, the buds developed in most of the notches within 7 days. In 5 weeks the shoots were fully an inch long.

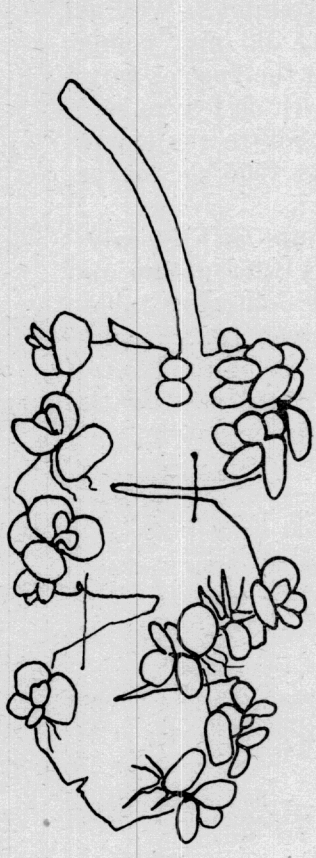
The purpose of these experiments was not to discover the cause of regeneration, but rather to determine whether the rules given by Loeb for B. calycinum could be applied to B. crenatum.

Loeb assumed that the cause of regeneration was the prevention of the flow of material from the notches of the leaf, and he was supported in this view by his experiments on B. calycinum. No such simple explanation can be given for B. crenatum. If a piece of stem was left attached to the leaf, leaf 2, one or both axillary buds developed. A comparison of this leaf with the isolated node from near the apex of the plant shows the growth of the buds about the same in leaf 2, altho in the latter case there were no leaves from which the buds could get this flow of material.

In a later experiment,[2] Loeb finds that an apical leaf influences the lower buds of its side of the stem. He states, in this connection, that the inhibiting influence of the leaf upon shoot formation is due to an inhibiting substance which is secreted by the leaf and carried with the sap toward the lower part of the stem. No such substance seems to be produced in B. crenatum.

Although the development of the axillary buds and notch growth is hastened by the separation of leaf or stem from the plant, Goebel[3] found in his experiments with B. crenatum that the development of the notch growth on leaves attached to the plant could be brought about by cutting squarely across the middle vein near the base of the leaf. If a longitudinal cut was made near the edge of the leaf no growth occurred. He attributed

this lack of growth to the fact that by the longitudinal cut the vascular bundles were not sufficiently injured, Wakker[3] said that the growth of adventitious buds on B. calycinum could be brought about by injuring or disturbing the water passages of the leaf.



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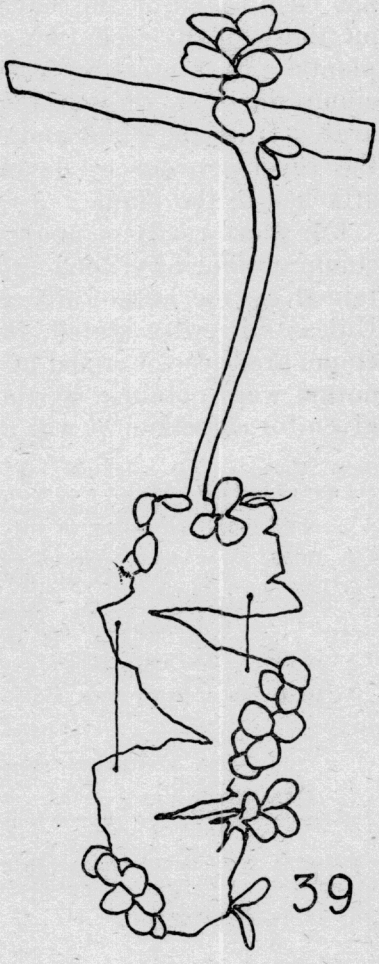

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# PLATE VII

Child and Bellamy[4] found that cooling a portion of the petiole of a B. calycinum leaf to a temperature from 2.5 to 4° C. for a few days was a very effective means of inducing notch growth.

That even these injuries and disturbances are not necessary in order that shoots may develop was discovered in the plants at hand. In the Botany Greenhouse, notch growth appeared on the leaves of several plants which apparently were in normal condition. This development has been found on the leaves of B. calycinum as well as on those of B. crenatum and on both old and young plants. An interesting feature noted in the species calycinum was that, while in the notch growth on leaves separated from the plant roots appeared before the shoot, the reverse order of development was true on leaves attached to the plant.

For more ready comparison, the results with B. calycinum obtained by Loeb and those with B. crenatum obtained by the author are given in the following table. Unless otherwise stated, the results given for B. calycinum are those obtained by Loeb; those given for B. crenatum were obtained by the author. Where no time is given for calycinum it was the same as for crenatum.

| Leaf | Time | B. crenatum | Time | B. calycinum |
|---|---|---|---|---|
| 1 | 10 da. | Much notch growth, aerial and submersed. | | Roots and then shoots on submersed portion. |
| | 5 wk. | Shoot on petiole in one. | | Shoot on petiole of 2. (Author's results) |
| 2 | 10 da. | Shoot from opposite or both axils in all mature leaves. In very young, shoot from adjacent axil in each. | | Opposite axillary shoots. |
| | 5 wk. | A few notch shoots and roots. | | No notch growth. |
| 3 | 10 da. | Shoot from each axil. Notch growth on most leaves. | | Roots and shoots on submersed portion. Not quite as quickly as in leaf 1. |
| | 5 wk. | Shoots and roots as large as in leaf 2. Notch growth as vigorous as in leaf 1. | | 4 shoots from submersed portion. Adjacent axillary bud. |
| 6 | 10 da. | Abundant notch growth the same as in leaf 1. | | Roots and shoots under water, similar to leaf 1. |
| 7 | 10 da. | No growth. | | Roots on many submersed portions. Shoot on one. |
| | 4 wk. | 2—no growth. 3—shoot on petiole and some notch growth. | | |
| 8 | 10 da. | 4 out of 6 no growth. 1—adjacent shoot. 1—adjacent shoot and notch growth. | | A few had some roots and shoots on submersed portion. |
| | 4 wk. | Adjacent shoots in each. Some notch growth. | | |
| 9 | | Same as leaf 3. | | Same as leaf 3. |
| 10 | 5 da. | 4—basal shoots; 1—basal and apical node shoots. | | Notch roots and shoots, no axillary shoots. |

| Leaf | Time | B. crenatum | Time | B. calycinum |
|---|---|---|---|---|
| | 20 da. | 2 basal shoots in each, 2 apical in 4, 1 apical in 1. | 17 da. | Notch roots under water. |
| | 4 wk. | Some notch growth. | | |
| 11 | 5 da. | 2 apical node shoots in each, 1 basal in one. | A few days. | 2 apical shoots in each. |
| | 20 da. | 2 basal in one and some notch growth in 2 leaves. | 17 da. | No notch growth. |
| 14 | 14 da. | Opposite axillary shoot, slight notch growth in most leaves, petiole intact. | | A few notch roots and shoots. |
| | 4 wk. | Adjacent shoot in one. | 10 wk. | Petiole fallen off, axillary shoot growing. |
| 20 | 5 da. | Adjacent buds in 3 out of 6. Notch growth in others. | 6 wk. | Adjacent axillary shoots |
| | 21 da. | Adjacent shoots in all, slight notch growth in 4. | 14 da. | In very young plants—a few roots on base of stem, and in 6 wks. much notch growth under water. No adjacent buds. (Author's results) |
| 23 | 12 da. | 2 shoots from each apical node. 2 from second node in two cases. | | Apical node shoots only |
| | 20 da. | 2 shoots from 3rd node in 1. | | |
| 28 | 3 da. | Buds in all 5 cases. | | No growth. |
| | 12 da. | 2 shoots from each node. | | |
| 38 | 7 da. | 3 out of 5 had both axillary buds, 2 had adjacent bud growing. | | Opposite shoot, no notch growth. |
| | 9 da. | 4—both axillary shoots, 1—opposite shoot. No notch growth in 4 with both buds. | | |
| 1 | 7 da. | Most notches had growth. | | No growth. |
| (In dark) | 5 wk. | Shoots about 1 inch long but not sturdy. | | Many small roots. Shoot from base of petiole. (Author's result) |
| | | In all notch growth, shoots developed first, then roots. | | Roots develop first, then shoots, when leaves are separated from plant. |

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