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Investigations on the Abnormal Outgrowths or Intumescences on Hibiscus vitifolius, Linn. A Study in Experimental Plant Pathology

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IV. *Investigations on the Abnormal Outgrowths or Intumescences on* Hibiscus vitifolius, *Linn.—A Study in Experimental Plant Pathology.*

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Communicated by Professor H. Marshall Ward, *F.R.S.*

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The peculiar pathological outgrowths termed *intumescences* are found on the leaves and young shoots of plants of various kinds. Those referred to in the following pages occur so regularly on *Hibiscus vitifolius*, Linn., in the glazed forcing pits of the Cambridge Botanical Gardens, that they might easily be, and have been, regarded as normal growths. In 1898–99 Professor Marshall Ward called attention to the probability that they are really abnormal and pathological, and I undertook their investigation to test the point. The first part of the work concerned their anatomy and development, and it was shown that they are chiefly hypertrophied outgrowths of epidermal cells, beginning at a stoma. The protrusion and division of the epidermal cells result, in the typical case, in the formation of a chimney-like outgrowth, singularly like the neck of the archegonium of a fern, and bearing the raised-up stoma at its apex. (Fig. 1, p. 164.)

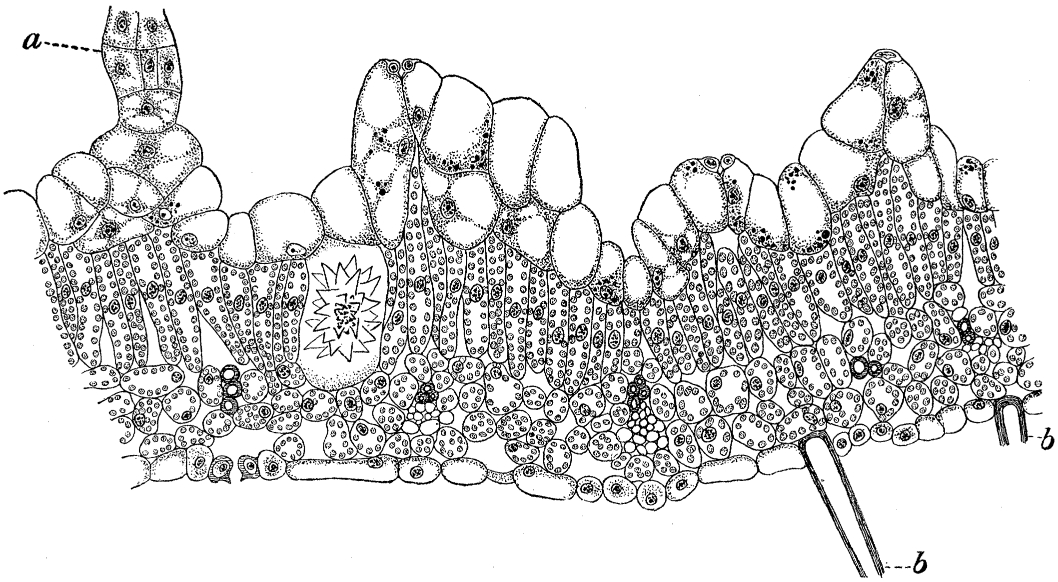
The cells of this intumescence—a term which distinguishes them from the normal hairs of the plant, and which were also examined—are highly turgid, and give a peculiarly glistening appearance to the intumescence, so that a leaf densely covered with them appears as if covered with water glands like an ice-plant. They vary much in size and in numbers, but it soon became apparent that they are very apt to predominate on plants in the moist warm atmosphere prevailing in the houses at certain seasons. No trace of minute insects, or fungi, or parasitic organisms of any kind, were found in connection with them.

As these cellular outgrowths attain maturity, a minute phellogen—cork cambium—cuts them off at the base, and the parts above this die. (Fig. 2, p. 164.) The cells shrivel, their contents dry up, the colour changes to brown, and the parts of the plant bearing them appear covered with a sort of scurf. This scurf is simply the dead intumescences.

These results, with some preliminary investigations into the causes which give rise to the intumescences, were published in detail in a paper read to the Cambridge

Philosophical Society in November, 1899,* with plates of figures illustrating the points referred to.

Fig. 1.

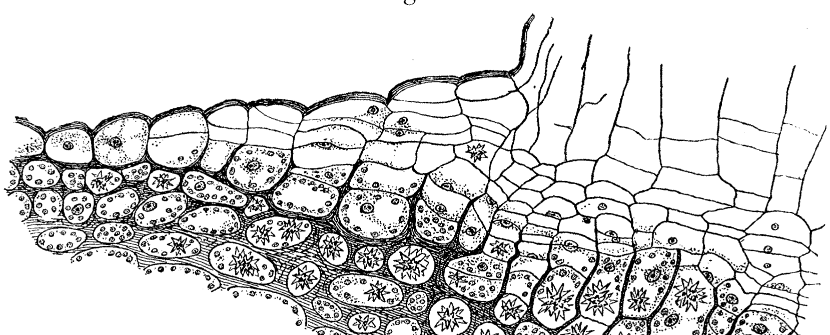


Three small outgrowths on the upper side of a leaf, each bearing a stoma at its apex. Most of the epidermal cells contain drops of oil.

a. Base of a normal glandular hair situated on hypertrophied epidermal cells.

b.b. Portion of stiff hair, also normal.

Fig. 2.



Part of an outgrowth on the stem, showing formation of cork across the base of the intumescence; the cells above this cork-layer are dead. Many calcium oxalate crystals occur in the cells of the cortex.

* DALE, "On certain Outgrowths (Intumescences) on the Green Parts of *Hibiscus vitifolius*, L.," 'Proc. Camb. Phil. Soc.,' vol. 10, Part IV., p. 192, 1900.

The preliminary experiments had already suggested that the conditions which induce the formation of intumescences are complex, but it was clear that plants in the open developed none, whereas it was sufficient to put such plants in the close atmosphere of the well-lighted glass-houses to induce their appearance. It appeared highly probable that light, as well as moisture, was one of the predominating factors of the environment concerned. The experimental results recorded in the present paper fully confirm this, and proofs are brought forward showing that certain definite conditions, easily realised, regulate their formation and abeyance; at the same time the experiments show conclusively that the intumescences are pathological structures due to disturbances in the metabolic activity of the tissues concerned.

It is believed that this is a clear case of a pathological phenomenon, due to disturbances of physiological function by variations in the non-living environment, which has been brought experimentally under control; and since it is typical of a large class of such pathological phenomena, it may lead to extensions of knowledge in a domain as yet not explored.

The plants used were chiefly those which had been raised during the winter from seeds produced by the older plants, referred to in the previous paper, and also such of the old plants as had survived.

On June 15 all these plants (together with the two cuttings which had been made in the autumn and had rooted) were planted out in an open situation in the Cambridge University Botanic Garden. In all cases the plants were sunk in their pots, because otherwise their roots spread, and when the plants are taken into the greenhouse again in the autumn they flag and die. Also they are more easily and safely moved for purposes of experiment if they are left in their pots.

The plants were left in the garden until July 19, by which time all the newly formed leaves were free from outgrowths, and some of the older ones had dropped off. In no case had any new intumescences been formed. On this date a series of experiments was begun, with a view to determining the relative importance of the supposed factors, moisture, light, and temperature, in the formation of the outgrowths. Most of the experiments were carried on out of doors, because the plants always develop a certain number of outgrowths under glass. In a few cases similar experiments were made both indoors and out.

The experiments were designed to test the effects of (1) *moisture* in air and in the soil; (2) *light*, of various colours and of different intensity; and (3) *temperature*.

I. *Moisture*.

In order to determine the effects of moisture, both in the air and in the soil, the following experiments were made :—

1. The root was kept wet and undrained, the shoot in dry air.
2. The root was kept wet but drained, the shoot in dry air.

3. The root was kept wet but drained, the shoot in damp air.
4. The root was kept dry and the shoot in damp air.
5. The root was kept dry and the shoot in dry air.
6. The root was kept very dry and the shoot in dry air.
7. A branch, still attached to a plant growing in the garden, was isolated in damp, still air.
8. A branch attached to a plant was submerged in water.

The first three experiments were made in the garden, and also repeated in the intermediate pit; all the rest were made only in the garden.

II. *Light.*

To observe the influence of light, plants were grown in the garden (Experiment 15 was repeated in the intermediate pit) under the following conditions:—

9. In a poor light with no sun.
10. Under yellow glass.
11. Under red glass.
12. Under blue glass.
13. Under green glass.
14. Under whitewashed glass.
15. In darkness.

III. *Temperature.*

It was not found necessary to carry out separate experiments to determine the influence of this factor. Its effects were noted in the experiments arranged to test the influence of light or moisture. A record of the daily maximum and minimum temperature was kept, and also the readings given by a black-bulb thermometer. These last are probably only approximate.

At the beginning of the experiments a thread was tied round the youngest leaf, and round the sixth below it (if there were as many on the branch observed), so that a record could be kept of these six leaves. At first the plants were examined almost every day, but later at less frequent intervals. At the close of the experiments the number of new leaves was counted and their condition observed. The old leaves were neglected because they are not greatly affected by changes in external conditions.

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I. Moisture.

1a and 1b. Root wet and undrained, shoot as dry as possible.

The pot was placed in a tin box and surrounded to the top with water. To prevent excessive evaporation the tin was covered with a lid made of two boards, in which grooves were made to fit round the stem of the plant.

1*a*. *In the intermediate pit.* July 19 to August 4.

On July 19 three of the four leaves examined were free from outgrowths. The oldest had a few small ones on the upper surface. By July 24, the 3rd was affected; by July 25, the 2nd; and by July 26, the 1st, *i.e.*, the youngest. By this time the two older leaves were withering, and by August 2 they had dropped off. On August 4 the plant was evidently dying, as it had lost nearly all its leaves, and the experiment was accordingly stopped. The pot was again sunk in the ground in the garden, but in a week or two the plant died.

Here we see that *with glass covering* and *wet roots, intumescences are readily formed* on the leaves.

1*b*. *In the garden.* July 19 to August 4.

The first two leaves at the beginning of the experiment were free from outgrowths, the rest had a very few small green ones which had remained since the plants had been put in the garden. By August 4 no new outgrowths had been formed, and the leaves were turning yellow and dropping off, so the experiment was stopped and the plant re-sunk in the ground. It soon recovered and began to grow. When the experiment was stopped, the base of the stem, which had been in water, was somewhat swollen, and was white and spongy.

This experiment shows that *when the plant has its leaves freely exposed to the air*, the *excessive moisture at the roots does not induce the formation of the intumescences.*

2a and 2b. July 19 to August 22. *Root kept wet, but drained; the shoot as dry as possible.*

The plant was placed in a tin box as before, but upon a brick, and only the base of the pot was under water. The box was covered with boards, as in Experiment 1. The plant was watered copiously.

2*a*. *In the intermediate pit.*

On July 19 the youngest leaf was quite free, the second had a very few outgrowths at the extreme apex, third to sixth had scattered outgrowths over the upper surface. By July 25 the first leaf was still free, on the others the outgrowths had increased in number and size. By July 26 the second leaf was curling downwards on account of the numerous small outgrowths on the upper surface. On August 2 the first leaf was also curling from the same cause. On the other leaves the outgrowths on the under side had become very dense, especially on the younger leaves. The condition of the leaves at the end of the experiment (August 22) is shown in the table. The main axis had grown 8 centims.

Six new leaves were formed, of which all but the youngest, which was very immature, had formed outgrowths. The lower part of the new portion of the main axis was covered with outgrowths, and there were also a few on the leaf stalks and on the epicalyx of the buds.

Here again we see that *moisture at the roots*, if the plant is *under glass*, *rapidly induces the formation of the intumescences.*

2*b*. *In the garden.* July 19 to August 22.

At the beginning of the experiment the leaves were all practically free from outgrowths and remained so, but by August 2 they had become strongly curled downwards. The leaves did not increase in size, but five new ones were formed, all small, but free from intumescences, all more or less curled, and each with a flower bud in its axil. There were no branches formed, but the main axis grew 9 centims.

Here again, as in Experiment 1*b*, the *free exposure of the leaves* to air *prevents the development of the intumescences* even if the roots are kept wet, but drained so as to avoid water logging.

3*a and* 3*b*. July 19 to August 22. *The root wet, but drained, and the shoot in damp air.*

The pot was placed on a brick in a zinc tray partly filled with water. A bell-jar supported on bricks, was placed over the plant. The plant was freely watered.

Again the result is the *development of intumescences if the roots are kept wet, though drained*, and there is *glass overhead.*

3*a*. *In the intermediate pit.*

By July 24 the formation of outgrowths had begun on all but the youngest leaf, and by the 30th a few green intumescences had been formed on it also.

In this experiment the outgrowths were all developed on the upper surface only, where they became very thick, causing the leaves to curl strongly downwards and inwards. As in most of the experiments, the outgrowths began as small green swellings and gradually became white (see p. 176). Sections show that this is because the parenchymatous cells first hypertrophy and gradually lose their chlorophyll, and that later the epidermis grows out into the colourless parts of the intumescences. The main axis was $4\frac{1}{2}$ centims. longer, and six new leaves were formed, of which all but the youngest were affected. In these outgrowths the colourless cells were so long and narrow as to be almost hair-like. Transverse sections are one, two, or a few cells in thickness.

3*b*. *In the garden.* July 19 to August 22.

In this experiment it was found necessary to shade the plant from excessive sun to prevent burning, but as much direct sunlight as possible was given.

As in the similar experiment in the intermediate pit, by July 24 there were outgrowths on all but the youngest leaf, on which their formation began on July 30. But in this plant, although a certain number of outgrowths were formed on the upper surface, those on the under side were larger and more numerous. The first leaf only became curled, the rest remained flat. On August 22 all the leaves on the plant were pale in colour. Six new leaves had developed, but no branches; the main axis had grown 7 centims. All the new leaves except the youngest had some outgrowths on them and were curled, the older ones being thickly covered.

This experiment shows that even when in the garden, *but covered by glass so that the air is kept damp*, the intumescences are rapidly induced, although the roots were well drained.

3*c*. *In the garden*. July 19 to August 22.

This was a similar experiment somewhat differently arranged. The pot containing the plant was placed on a brick in a big flower-pot which was put in a glazed jar. Water was kept in the jar to the level of the top of the brick or a little higher, and the plant was freely watered. A bell-jar was inverted over the large flower-pot. A ring of cotton wool was put round the top of the pot, and kept wet by two more strips of cotton wool which communicated with the water in the jar.

At the beginning of the experiment the four youngest leaves were quite free, and the two oldest were almost free from outgrowths. By the 24th the third and fourth leaves, and by the 25th the second were affected, and by the 30th the first leaf also. As in 3*b*, the outgrowths were chiefly on the under side. By August 22 all the leaves but the first, which was very yellow, had dropped off. Four new leaves had developed, the two youngest being very small and free, the other two possessing outgrowths. The main axis had only grown $1\frac{1}{2}$ centims., but five new branches, some nearly as high as the main axis, had been formed. Most of the old leaves on the plant had fallen off.

Again we see that *damp air induces the rapid development of the intumescences.*

4. *The root dry, the shoot in damp air*. July 19 to August 22. In the garden. A bell-jar was placed over a plant sunk in the ground. The sides of the bell-jar were lined at the base with blotting paper kept wet by another piece of blotting paper communicating with a dish of water. A stick was placed in the ground near the plant, and a strip of cotton wool, connected with the dish of water, was hung over it. The soil was not watered, as it was kept sufficiently moist by precipitation from the damp atmosphere, and, in occasional wet weather, by obtaining moisture from the surrounding soil. The plant was shaded when necessary.

In this experiment the formation of outgrowths was very marked. By the 24th all the leaves but the youngest had responded, and on it the formation of outgrowths began on the 30th. As in experiments 3*b* and 3*c*, most of the outgrowths were on the under side, and caused all the leaves to curl upwards. At the close of the experiment the leaves were densely covered underneath, and curled upwards. There were only a few outgrowths, and some of these withering, on the upper surface. The main axis had grown $1\frac{1}{2}$ centims., and five new leaves had been formed, all with outgrowths. The three oldest were densely covered with outgrowths, two chiefly below, one above.

This experiment shows how *rapidly* an *excessively damp atmosphere* induces the formation of intumescences, *under ordinary glass.*

5. In this experiment a plant in a pot sunk in the ground was watered less freely than the others. No outgrowths were formed, but the growth of the plant was less than in the case of those which received more water.

In this case the experiment shows that if *both root and shoot are dry, no intumescences are formed*, even if the growth of the plant suffers from deficiency of water.

6. A plant in its pot was dug up and the pot placed on the surface of the ground and given very little water. The new part of the main axis was $7\frac{1}{2}$ centims. long, and six new leaves were formed; but they, as well as the older leaves, remained small, although free from outgrowths. No branches were formed.

Here again *dry roots and a dry atmosphere do not induce the development of intumescences.*

7*a*. July 19 to August 4. One branch of a plant devoid of outgrowths was isolated, while still attached to the plant, in a bell-jar in which the atmosphere was kept very damp. The branch was inserted through a hole in a piece of card and covered by a bell-glass supported in a flower-pot. The sides of the bell-jar were lined at the base with wet blotting paper, and a ring of cotton wool was placed round the outside of the bell-jar where it rested on the card. A plug of cotton wool was placed in the hole where the branch passed through the card. The atmosphere was kept constantly wet by a strand of cotton-wool which connected the cotton-wool round the bell-jar with a dish of water. This experiment was never shaded, because the plant did not become burnt. Perhaps the cause of the immunity from burning may be sought in the continuous evaporation which, even though the heat of the sun was often extremely great, would prevent scorching.

The results of this experiment were the most rapid and striking of all. On July 19, when the branch was isolated in the morning, about 11 o'clock, the leaves were all quite free, flat, and dark-green in colour. By the next morning, the 20th, the formation of outgrowths had begun. On the succeeding day (the 21st) the outgrowths, all on the *under* side, were very dense, and by the 22nd the leaves were quite deformed, so strongly were they curled upwards by the mass of large intumescences. On the 24th the branch was liberated and the following observations made.

The leaves were all densely covered underneath, especially along the veins. The formation began along the veins and round the edges of the leaves. The outgrowths were of the somewhat peculiar type which arises when the formation is rapid owing to sudden change of conditions. The outgrowths are pale green and rounded, almost hemispherical in shape, but those near the veins, *i.e.*, those first formed, had large colourless outgrowths upon them. In the youngest leaves the first intumescences arise as large white swellings, one at the end of each tooth on the margin of the leaf. In older leaves they usually begin as a dense row of outgrowths along each side of the chief veins. The leaves soon begin to go yellow when shut up in the bell-jar. After the liberation of the branch a few of the older leaves dropped off, but the younger ones recovered to a greater or less extent. The leaves became flat and resumed their dark green colour, except that the position of certain of the larger outgrowths, on the under side, was indicated on the upper side by yellow spots. Ultimately these leaves also fell off. The white parts of the intumescences dried up, but some of the green ones persisted for some weeks. The new leaves formed after liberation were all devoid of outgrowths.

7*b*. On July 24 a fresh branch of the same plant was isolated as a confirmatory experiment. By July 26 one leaf was very thickly covered with big, pale-green outgrowths, especially in lines along each side of the veins. Another was similar, but less thickly covered. By July 28 the leaves on the under sides were smothered with outgrowths. On July 30 the outgrowths were going white and the leaves were curling strongly upwards. The smallest and youngest leaves were all affected, and had developed an intumescence on every leaf tooth.

On August 4 the experiment was stopped. By this time the youngest leaf had a considerable number of large white outgrowths on the upper side, as well as the green ones on the under surface. On the other leaves the intumescences were confined to the lower side, especially along the veins. After being set free the leaves partly recovered, but, as the experiment had been continued longer than the first one, they all dropped off in a week or two.

The experiment *conclusively proves that the damp air is an essential factor in inducing the development of the intumescences.*

7*c*. August 21 to August 30. A third experiment with an isolated branch was made later, when the weather was much cooler. While 7*a* and 7*b* were being performed the maximum shade temperature was at times over 90° F., and the minimum over 60° F. At the same time there was an unclouded sun, from which it was not found necessary to shade these experiments. When 7*c* was begun the maximum temperature was about 65° to 70°, the minimum about 55°, and the weather was cloudy.

The other conditions were the same, except that the branch was directed towards the north, instead of towards the south-west, so that it had less light.

The experiment was started on August 21, and, instead of being covered in two or three days with large outgrowths, by August 27 only a few small ones had been formed, some on the upper side, some on the lower, in others on both sides.

The experiment was stopped on August 30, by which date the increase in the number of outgrowths was very slight.

This experiment suggests that even in damp air a deficiency of light and low temperature in some way co-operate to bring about conditions inimical to the development of the intumescences.

8. August 14 to August 22. *A branch, attached to the plant, submerged in water.* A branch was bent down into a beaker of water and a glass dish inverted over it to prevent evaporation. No outgrowths were formed.

The experiment suggests that *something more than excessive moisture* in the leaves is needed to induce the development of the intumescences (*cf.* p. 178).

II. Light.

9. July 19 to August 22. A plant in a pot was placed under a thick cotton net, used for shading green-houses, in a north aspect, where it had no sun, but plenty of air. At first the plant showed a very slight tendency to form small outgrowths, but after a few days the formation stopped and was not begun again.

The main axis grew 5 centims. longer, and four new leaves were formed. The two youngest were free, the two older ones each had slight traces on the upper surfaces.

This experiment suggests that *a slight shading of the leaves may tend to induce the intumescences*, but whether this is owing to the diminished light or to the increased moisture—*e.g.*, in the night and dewy mornings—is not answered by the experiment itself. As we shall see, the probability is that the shading of the leaves checked the formation of the outgrowths, even in a damp atmosphere.

10*a and* 10*b*. *Yellow light.*

Two experiments were made with yellow light, but the second had to be stopped before it was complete, not, however, until the plant had responded to the changed conditions.

10*a*. July 19 to August 22. A bottomless box was placed over a plant, and propped up on bricks to give air. A sheet of yellow glass was laid over the box, but a little space was left to allow of ventilation.

After a few days all the leaves formed outgrowths, chiefly on the top. These slowly increased in number and in size. By August 22 the main axis had grown 5 centims. and five new leaves had been formed. The third of these was quite smothered in a dense mass of white outgrowths which entirely hid the leaf surface, and curled the leaf under so strongly that the under side was invisible. The two lowest new leaves were also very thickly covered.

The experiment *proves that intumescences are readily formed in the light passing through yellow glass*, when the air is damp.

10*b*. July 19 to 28. A double jar filled with potassium bichromate was inverted over a plant and supported on bricks to allow of ventilation. The plant was shaded when necessary.

On July 19 only three leaves were free, three were very slightly affected. By the 24th the second and third leaves were affected, and by the 28th the youngest also. The jar then began to leak, and the experiment had to be stopped.

The experiment confirms the last one: *in the yellow rays* the *intumescences form on leaves in moist air*, as they do in ordinary light under otherwise similar conditions.

11. *Red light*. July 19 to August 22.

A bottomless box of red glass was placed over a plant and supported by bricks. It was covered by a sheet of red glass, arranged to allow the passage of a current of air.

On July 19 four out of five leaves were free, and the fifth only had a few on the top. By the 28th all but the youngest leaf had formed outgrowths, and by the 14th of August that also had produced some.

No new leaves were formed in this plant, because the apex of the stem was injured in a storm of wind which blew off the box. Also this experiment was apparently placed too close to, and on the north side of, the blue box, which prevented sufficient light from reaching the plant.

The experiment proves that intumescences are developed in the *light passing through red glass* when the leaves are in moist air.

12*a* and 12*b*. *Blue light*.

12*a*. July 19 to August 22. A blue glass box was used as in the experiment with red light (11).

At the beginning of the experiment the first two leaves were free and flat, the others had a few outgrowths. During the experiment no new outgrowths were formed, and the younger leaves grew considerably. By August 22 the main axis had elongated 28 centims., and six new leaves, most of them very large and flat, with long petioles, had been formed. In one leaf the petiole was 10 centims. long, in another 11, which is about three times the normal length. The laminæ were also much larger than in any other plant.

This experiment shows that even in a moist atmosphere *no intumescences are developed in the light passing through blue glass*. Hence may be inferred that the

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arrest of transpiration due to the damp and close air round the leaves is *not* the sole cause.

12*b*. July 19 to July 26. This experiment was arranged as in 10*b*, but the jar contained copper sulphate and ammonia. Four leaves were examined, of which the two youngest were free, and the two oldest had a few outgrowths. Owing to the leakage of the jar the experiment had to be stopped on July 26, a week after it had been started. No new outgrowths had been formed.

The experiment *completely confirms* the conclusions arrived at in the last case, viz., that no intumescences are developed in *blue light.*

13. *Green light.* July 19 to August 22.

This experiment was arranged like 10. The five leaves observed were all free when the experiment was begun, on July 19, and at its conclusion on August 22, no outgrowths had been formed on the leaf blades, but one or two were present on the stalk of the second one. No new leaves had been formed, and the old ones had remained small, but appeared healthy.

The experiment shows that intumescences are *not* developed in light which has passed through *green* glass, although the moist atmosphere checks transpiration.

Spectroscopic analysis of the glasses referred to above showed that the red blocked all rays except a band of red extending from between the lines B and C to near the line D. The yellow glass transmitted all rays from between B and C in the red to just beyond the line F in the green, all beyond were completely absorbed. The green glass completely blocked out all red rays and part of the orange to midway between the lines C and D, as well as all beyond the blue end of the green: it transmitted the orange-yellow and green but with diminished intensity. The blue glass transmitted some red rays below the line B, some orange rays between C and D, and some green beyond D, and the greater part of the blue from between F and G onwards, but only the blue portion seemed undiminished in intensity.

14. July 19 to August 22. *Whitewashed glass.* The arrangement was like that in the preceding experiment. Light intense.

Four leaves on a lateral branch were examined, and all had traces of outgrowths at the beginning of the experiment, July 29. In the intense light, slightly shaded, new outgrowths were rapidly formed, chiefly on the under sides, but also on the top of all the leaves. At the close of the experiment the main axis was $1\frac{1}{2}$ centims. longer, and three new leaves had been formed. Of these the first two had outgrowths only round the edge, but the lowest was densely covered on the upper side.

Here we see that, *given the moist atmosphere* checking transpiration, and glass which merely reduces the intensity of white light to a slight degree, *intumescences are developed* as freely as under ordinary clear glass, or red or yellow screens.

15*a* and 15*b*. *In darkness.*

15*a*. August 4 to August 10. In the intermediate pit. On August 4 a plant, with traces of outgrowths on one leaf only, was placed in a tin, with another inverted over it. On August 10 the experiment was stopped. No outgrowths had been formed, and the leaves were becoming very yellow, so that the plant seemed to be dying. The plant was left in the pit, and by August 22, the leaves had become green again, and growth had begun. Outgrowths were forming on the upper surfaces of the leaves.

15*b*. A similar experiment in the garden gave the same results.

These experiments show that *no intumescences are formed in the absence of light*, although, as in the case of blue and green lights, which also give negative results, the check to transpiration is afforded by the enclosed atmosphere (*cf.* p. 178).

Experiments with other Plants.—For the sake of comparison with *Hibiscus*, a few experiments were made with other plants in which intumescences are known, in order to try to obtain outgrowths by altering the external conditions. The following plants were used : *Ipomea Woodi*, *Vitis vinifera*, and *potato*.

Two plants of *Ipomea Woodi*, growing in the intermediate pit, had most of their leaves covered, *on the under side only*, with colourless outgrowths, larger and more regular in shape than those on *Hibiscus*. Microscopic examination showed that in both plants the structure is essentially the same. In both they consist of thin-walled, colourless cells, and in both stomata and hairs are present. The number and large size of the oil-drops in *Ipomea* is even more striking than in *Hibiscus*. The oil also occurs in the palisade parenchyma, to a less degree in the spongy parenchyma, and round the vascular bundles, in the neighbourhood of the outgrowth, but not in other parts of the leaf.* The drops were stained black with osmic acid† when the leaves are hardened in Flemming's solution, and they are not dissolved by subsequent treatment with absolute alcohol, xylol, and clove oil, so that in permanent preparations of microtome sections their distribution is well seen. The outgrowths in *Ipomea* are exclusively epidermal, but the spongy parenchyma below them is in some cases modified.

As in *Hibiscus*, they are extremely thin-walled, and dry up immediately if exposed to air which is not saturated with moisture.

One of the two plants of *Ipomea* was placed in the open garden, where *it at once ceased forming outgrowths.* On August 14 a branch with an old, a mature, and a very young leaf was isolated in a bell-jar containing damp air, as in Experiment 7 with *Hibiscus* (see p. 170). By August 21 numerous small outgrowths were *beginning to form* on the second leaf.

* In *Hibiscus* the oil is almost exclusively confined to the epidermal cells. In some of the very greatly elongated cells, in outgrowths produced rapidly, in a few days there is also a certain amount of oil in the parenchyma.

† The drops also stain pink with alkanna.

The structure and distribution of the outgrowths formed during the course of the above-described experiments with *Hibiscus* differ in some details according to the conditions under which they are formed.

Those formed on plants which have been for a long period under conditions moderately favourable to the formation of outgrowths are different from those which are rapidly developed after a sudden change to very favourable conditions.

In the former case, the outgrowths are generally smaller, slenderer, and more superficial. They may be developed on either side, or on both sides, of the leaf. In the latter case, the outgrowths are almost entirely confined to the under side, where they arise as large, pale green, almost hemispherical swellings of the sub-epidermal parenchyma. Later, the cells of the epidermis over these swellings divide, and swell up into very much distended thin-walled cells. Sections show that the pale colour of the young outgrowths is due to disorganisation of the chlorophyll bodies. When exposed to dry air the green outgrowths do not dry up, but those which have become white shrivel immediately.

The results of the above-described experiments may be briefly summarised as follows:—

I. *Moisture.*—Experiments 1 to 8 show that in every case in which a shoot was in *a damp atmosphere* under *ordinary glass*, outgrowths were formed, while in no case were they developed in dry air.

II. *Light.*—The experiments with light of varied intensity and of different colours show that outgrowths form under *colourless, red, and yellow glass*, and under *white-washed glass*, while they do *not* arise under *blue or green glass*, in a *poor light*, or in *darkness*, or in submerged leaves.

III. *Temperature.*—The temperature record shows that the formation of outgrowths, *ceteris paribus*, is promoted by heat.

The experiments made last year suggested the conclusion that the check to transpiration caused by a damp atmosphere was one of the causes of the formation of the outgrowths. The further experiments confirm this view, and also lead to the further inference that alterations in metabolism are also involved. The evidence relating to transpiration may be considered first, and then that bearing on metabolism. Finally, the possible meaning of the outgrowths may be discussed. That damp air and not damp soil is a cause of the formation of outgrowths is shown by the experiments. Outgrowths are formed in damp air (provided there is enough light and heat) whether the soil be wet or dry, even if it be so dry that the leaves tend to flag. On the other hand, if the shoot is in dry air, a damp soil never causes the production of intumescences, although it may hinder the healthy growth of the plant.

That transpiration is involved is shown by the distribution and structure of the outgrowths. It has been said that, both in *Hibiscus vitifolius* and in *Ipomea Woodi*, the outgrowths possess stomata, and that they are formed by the division and swelling of the epidermis, and often also of the underlying parenchymatous cells, around one or more stomata. Since the outgrowths always rise in connection with stomata, it follows that the distribution of the outgrowths must be dependent upon that of the stomata. In *Hibiscus vitifolius*, where there are stomata on both sides of the leaves, intumescences are also found on either or on both surfaces. In *Ipomea*, on the other hand, there are no stomata on the upper side, and the formation of outgrowths is confined to the lower surface. In both plants they may also occur on the petioles. These conclusions are also in agreement with observations made by TOMASCHEK on the occurrence of such structures in *Ampelopsis hederacea*, and quoted in the earlier paper.*

In *Hibiscus* normal healthy growth is greatest when transpiration is very active, and when the plants have an abundant supply of water, but well-drained roots.† Since no outgrowths are formed on the leaves of plants freely transpiring in the open air, and since they are always formed in a damp atmosphere (provided the light and heat are adequate), it is clear that diminished transpiration is one of the essential conditions.

The immediate effect of checking transpiration would be to give rise to an accumulation of water in the veins, the leaf-teeth, and the tissues adjacent to them; it is in these parts of the leaf that the formation of outgrowths first begins when the external conditions are suddenly changed.

The blocking of the tissue with water, consequent on the check to transpiration given by the damp atmosphere, would have two further results: (1) Respiration, and (2) nutrition, would both be checked. Consequently, the normal course of metabolism would be disturbed by cutting off the supply of salts.

That metabolism is involved is shown by the present experiments, which provide evidence in several directions.

In the first place, the conditions of the formation of outgrowths are those necessary for assimilation, namely, light and heat. For example, given a sufficiently moist (and, since moving air promotes transpiration, probably also still) atmosphere, the outgrowths are formed most numerously and are largest the greater the intensity of the light, and, within limits, the heat. The most abundant formation of the largest outgrowths occurred under clear glass, where as much direct sunlight was given as was possible without scorching the plants.

Outgrowths were also formed under yellow, red, and whitewashed glass, but not under green or blue glass, and never in darkness, however great the warmth and moisture, nor under water.

In this connection it is also of importance to note that the formation of intumescences is most active in leaves which are young, but mature, *i.e.*, in those

* DALE, *loc. cit.*, p. 206.

† In *Hibiscus* transpiration is, doubtless, promoted by the presence of stomata on both sides of the leaves; probably some cuticular transpiration also occurs, on account of the thin cuticle.

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which are most actively transpiring and assimilating. When the external conditions are suddenly changed from those which are unfavourable to the production of outgrowths to those which are favourable, comparatively few are generally formed on the old leaves or on those which are very young. In very young leaves the formation usually begins on the teeth of the leaf, on the upper side, and causes the edges of the leaf to curl downwards. In older leaves, the outgrowths generally arise along the veins on the under side, so that the leaf curls upwards.

Further evidence of the relation between assimilation and the formation of outgrowths is furnished by the distribution of starch. Starch was plentifully stored in the leaves of normal out-door plants, and in those grown under yellow glass; it was also fairly abundant under red glass; very little was produced under blue glass; and none was found in leaves in poor light, under green glass, and under whitewashed glass. This, of course, refers to accumulated starch, indicating an excess over that used at the time. That starch is not formed in the submerged leaves of land-plants which can be wetted, has been shown by NAGAMATZ.*

The fact that outgrowths were formed under whitewashed glass, while no starch was found in the leaves, is probably to be explained in the following way. When the outgrowths were developed, the light, even under the whitewashed glass, was of very considerable intensity, whereas, when the leaves were examined for starch at the close of the experiments, the weather had for several days been cooler, and the sky almost continually overcast, so that the amount of light was reduced.

Comparing the distribution of starch with that of outgrowths, we find that, given the necessary conditions of moisture, light, and heat, intumescences are formed when starch is present and not when it is absent, except in the case of the plant grown under whitewashed glass. But if transpiration is active, no intumescences are formed even when starch is abundant, *e.g.*, under free conditions in open air.

The tendency of the plant in Experiment 9 to form outgrowths at first, may be due to the fact that at the beginning of the experiment the plant already possessed carbohydrates in excess. The non-development of outgrowths in darkness (in Experiment 15) and in submerged leaves (Experiment 8) is doubtless due to lack of carbohydrates.

Another fact which points to the disturbance of the normal metabolism of the plant is the abnormally large quantity of oil present in the outgrowths and, especially in *Ipomea*, in the adjacent parts of the leaf, more particularly in the neighbourhood of the vascular tissue and in the palisade cells. This is striking, because oil is not observable in the cells of the normal tissue of these leaves. A similar increase in oil formation has also been noted by SORAUER† in *Acacia pendula*, in the ends of young branches which had developed outgrowths on the side turned towards the light.

* 'Arbeiten des Bot. Inst. in Würzburg.' See BLACKMAN, 'Phil. Trans.,' B, 1895, vol. 186, p. 557.

† SORAUER. In a paper subsequent to mine, "Ueber Intumescenzen," 'Berichte der Deutschen Bot. Gesell.,' January, 1900, p. 459

The importance of light and heat is clearly shown by comparing the three experiments (7*a*, 7*b*, and 7*c*) in which single branches, still attached to the plant, were isolated, out-of-doors, in a damp atmosphere. During the first two experiments the maximum temperature was about 90° F., the minimum above 60°, and the highest daily sun temperature about 130°. The third experiment was made later, when the temperature was much lower and the weather cloudy and dull.

In the two earlier experiments, after two or three days, the under sides of the leaves were densely covered with large outgrowths which caused the leaves to curl strongly upwards. In the later experiment, after about ten days, only some of the leaves had a few outgrowths, some on the upper and some on the lower side (*cf.* pp. 170–172).

But though light and heat are essential, it seems probable that, given the necessary moisture in the atmosphere, the other two factors may be interdependent, viz., that in a strong light outgrowths will form at a lower temperature, while in a poor light, great warmth is required. That a certain minimum of light is required is proved by the fact that outgrowths never form in darkness. Also, the minimum temperature at which assimilation will take place cannot be very low in a tropical plant like *Hibiscus*. That direct sunlight of full intensity is not necessary is shown by the formation of outgrowths under whitewashed glass, or in a warm shaded greenhouse; that it promotes their formation is suggested by their more rapid development under the clear glass of a bell-jar.

If the light and temperature are inadequate to promote the necessary assimilation, the plant becomes starved, and dies without producing any outgrowths, as was the case with the plant grown in the cool and dimly lighted Filmy Fern house.*

It is not easy to explain why, in *Hibiscus*, where stomata occur on both sides ot the leaves, the outgrowths should appear on only one surface or on both. It is possible that, since the stomata are more numerous on the under side, where transpiration is most active, any *sudden* check to transpiration would affect the lower surface first, and most strongly. If, on the other hand, the plant had grown up in a damp atmosphere, every part of it would be gradually adapted to its external conditions and would be able to produce outgrowths.

From the results of the experiments we must conclude that the sudden checking of transpiration by a damp atmosphere interferes with normal metabolism by cutting off the supply of salts brought by the transpiration current. But, if the light and heat are sufficient for continued active assimilation, the plant is provided with surplus carbohydrates and originates a new and abnormal course of metabolism, which results in the formation of outgrowths and the production of oil. From what is known of the connection between respiration and oil-formation, we may infer that the saturation of the tissues with water lessens the oxidation processes involved in

* Dale, *loc. cit.*, pp. 200, 201

respiration, and that incomplete combustion results, oil being stored as in succulents. One consequence may well be the accumulation of vegetable acids and powerfully osmotic bodies in the cells, which, therefore, commence to bulge outwards under the increased pressure, and so form intumescences.

With regard to the possible meaning and function of the outgrowths, the position and structure of the outgrowths themselves, and the structure of the bundle-endings in the leaf teeth, offer a suggestion. In a normal leaf the median vein in each tooth (and frequently some of its branches) dilates at its end, owing to an increase in the number of tracheides accompanying the vessels. These tracheides, some of which resemble transfusion tissue, are in contact with the small number of chlorophyll-containing cells which here lie between the vascular tissue and the epidermis, but there is no specialised epithema. In the epidermis round the apex are stomata communicating with air spaces. The whole structure resembles a water gland, and suggests an organ which at least promotes transpiration, if it does not also secrete drops of water. It also throws light upon the fact that the formation of outgrowths often begins on the leaf teeth, since, transpiration being checked by a damp atmosphere, water would tend to collect at the point where it is normally most abundantly discharged.

In order to determine whether the plant secreted drops of water, a healthy branch was placed in water in a U-tube, into which mercury was poured, the whole being covered with a bell-jar standing in water. There were five leaves on the branch, which was left in a laboratory with a north aspect on August 22. No water was seen on the leaves till the 25th, when there was a large drop on the apex of the third leaf. On the 27th there was another drop on the second leaf. By the 28th the *formation of outgrowths had begun*, and the experiment was removed to the tropical pit to hasten their formation. In two days the youngest leaf was thickly covered on the upper side and strongly curled downwards. There were also a few outgrowths on the second and third leaves; the fourth had dropped off, the fifth was free.

The turgidity of the cells in the outgrowths and the extreme thinness of their walls, as well as the presence of large round stomata, taken in connection with the fact that the intumescences are formed when transpiration is checked, suggest the possibility that they may be a sort of abnormal organ for the direct excretion of water, either through the cell-walls or through the stomata by filtration under pressure.

Experiments on the secretion of drops of water by plants have been made by NESTLER,* who finds that all the Malvaceæ which he examined, which included some species of *Hisbiscus*, form drops of water in a damp atmosphere. NESTLER is of opinion that the hairs in Malvaceæ play a part in the secretion of water. But in

* NESTLER, "Die Secrettropfen an den Laubblättern von *Phaseolus multiflorus*, Willd., und der Malvaceen," 'Berichte der Deutsch. Bot. Ges.,' Heft 9, 1899, p. 332.

Hisbiscus vitifolius the glandular hairs secrete a viscid substance which does not evaporate, and which is chiefly formed on plants growing in a damp atmosphere. Plants grown out of doors, though possessing numerous glandular hairs, are almost devoid of the viscid secretion.

HABERLANDT* has made some interesting experiments on the formation of new organs which excrete water in *Conocephalus ovatus*. This plant normally excretes large quantities of water through special hydathodes, possessing water stomata, and lying over vascular bundles. By poisoning the normal hydathodes, through painting the leaves with a ·1 per cent. alcoholic solution of corrosive sublimate, HABERLANDT induced the formation, in three or four days, of what he calls "compensating hydathodes" (Ersatz-Hydathoden), which arise by the stretching of the parenchymatous cells which break through the epidermis. The compensating hydathodes performed the function of the normal hydathodes and excreted drops of water. After about a week the "compensating hydathodes" disappeared, apparently through drying up on account of their delicate structure. The leaf then formed (how soon is not recorded) outgrowths which appear to be exactly like those on *Hibiscus* and *Ipomea*, namely, "watery blisters arising through growth of the epidermis and of the underlying layers, so that after a time the leaf has a diseased appearance without dying." It is noteworthy that the "watery blisters" are formed on the *under* side of the leaf, a fact which is probably connected with the distribution of the stomata.

SORAUER,† who describes outgrowths on the under sides of the leaves in *Eucalyptus globulus* and *E. rostrata*, states that he has produced intumescences artificially in *Ficus elastica* and in *Impatiens Sultani*, but he does not describe the methods he used.

Résumé.—The conclusions to which the evidence furnished by the experiments lead may be summarised as follows: There is no doubt that the checking of transpiration (which is normally very active in this plant) by a damp atmosphere, is one cause of the development of outgrowths, but this by itself is not sufficient, for, as the experiments under blue and green glass show, assimilation must also be active. There is further evidence (partly furnished by the plants grown under coloured glass) that an altered course of metabolism is also involved—a conclusion to which the abnormally abundant development of oil also points. It seems clear that what occurs is—(1), a lack of salts, owing to arrested transpiration; (2), the assimilated carbohydrates are therefore being employed in metabolism with a deficiency of nitrates, &c.; and (3), the tissues blocked with water are not respiring normally.

The formation of outgrowths often begins round the teeth of the leaf. The structure of the bundle-endings in the teeth of a normal leaf is that which characterises

* HABERLANDT, "Ueber Experimentelle Hervorrufung eines neuen Organes," 'Festschrift für Schwendener.' Gebr. Bornträger, Berlin, 1899, *cit.* 'Naturwissenschaftliche Wochenschrift,' 1898, p. 287.

† SORAUER, "Ueber Intumescenzen," 'Berichte der Deutschen Bot. Gesell.,' January, 1900.

water glands, and this fact, taken in connection with the position and structure of the intumescences, suggests that they may be temporarily used as organs for the secretion of water.

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