INTUMESCENCES ON POPLAR LEAVES. II. PHYSIOLOGICAL CONSIDERATIONS[1]

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INTRODUCTION

In preceding papers (12, 13) the writer has described the occurrence and structure of intumescences on leaves of *Populus grandidentata*, *Populus tremuloides*, *Eucalyptus coccifera*, *Eucalyptus cornuta*, *Hieracium venosum*, *Mitchella repens*, and *Thurberia thespesioides*. In the present paper an account is given of an attempt to determine the factors involved in stimulating the cells to the unusual growth, which result in these abnormal protuberances.

The hypotheses which have been developed to account for the induction of intumescences have been almost as numerous as the records of their occurrence. Sorauer (18) believed that metabolic disturbance due to an excessive water supply and low assimilation accounted for the outgrowths. In a later paper (19) he added another factor to the complex, namely, the effect of a high temperature.

An excessive water supply due to low transpiration and a high rate of water absorption by the roots, was advanced by Atkinson (1, 2) as the stimulus to formation of oedemas on the tomato and the apple, though he considered that low illumination was favorable also, since it led to the development of "weak, watery tissue."

Steiner (20) discovered intumescences on leaves of *Ruellia formosa* and *Aphelandra Porteana* which he believed were caused by high humidity. By subjecting leaves of *Aphelondra* to a saturated atmosphere he was able to induce these outgrowths, but he failed to secure them by treating the leaves with copper sulfate, ammonium chlorid, formic acid and various other chemicals; by keeping them in the dark; or by submerging them in water.

Douglas (5) induced intumescences on leaves and stems of potato plants by covering them with bell jars. Since the intumescences occurred in either strong or weak light, but not in total darkness, she concluded that light as well as humidity played a part in the abnormal stimulation of the plants. She found also that the abnormalities could be produced by treating young potato plants with copper compounds, as Von Schrenk (23) had previously found in the case of cauliflower.

Küster (11) secured intumescences on leaves of *Populus* floated on water in the presence or the absence of light. In strong light, however, the

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growths were best developed on the side of the leaf in contact with the water and this, he believed, was due to the retarded transpiration on that side.

Dale (3) found that high humidity was necessary for the development of intumescences on *Hibiscus vitifolius*, but that light conditions were important also, since the developments occurred in white, red, or yellow light but not in blue, green, or weak light, or in darkness.

Intumescences on leaves of grapes grown in the greenhouse were believed by Viala and Pacottet (22) to be due to intense light and high humidity.

Haberlandt (6) painted thalli of *Conocephalus conicus* and *C. suaveolens* with a one percent solution of mercuric chlorid in alcohol, which killed the cells of the hydathodes. The development of intumescences, which he regarded as "substitute" hydathodes, resulted; due, he believed, to excess pressure developed in the absence of the normal hydathodes.

Smith (17) found that ammonia and Carnoy's fixing fluid gave intumescences on the leaves of cauliflower. He was able to induce crown-gall formation in stems of *Ricinus communis* by injecting by-products of *Bacterium tumefaciens*.

Harvey and Rose (8) and Doubt (4) found that ethylene causes abnormal growths in the tissues of various plants. Wallace (24) found that ethylene caused abnormal growths in sixteen different species of woody plants including the apple. Later (25, 26) he made an intensive study of the intumescences on *Pyrus malus* var. Transparent, induced by treatment with ethylene.

Wisniewski (27) found that intumescences in the lenticels of *Ficus australis* and *Ficus elastica* could be induced by coating the twigs with paraffin oil. He regarded the reduction of transpiration as a major factor but thought that other conditions, not yet fully understood, had an influence in the process.

Schilling (15) reported that coating stems of *Aesculus hippocastanum*, *Spiraea concinna*, *Philodendron pinnatifidum*, *Artocarpus incisa*, and *Sambucus nigra* with vaseline or paraffin oil caused outgrowths, similar to those described by Wisniewski, from the lenticels. He believed that the developments were not due to the chemical stimulus of the vaseline, or paraffin, but mainly to the reduction of transpiration, and possibly to oxygen deficit.

Hahn, Hartley, and Rhoads (7) found that intumescences were produced on the roots of several species of conifers by an excess of soil water, though they did not find that lowered transpiration was a significant factor.

Wolf (28) attributed the intumescences which he observed on leaves of cabbage to injury by wind-blown sand. On the same plant R. B. Harvey (9) induced intumescences by exposure to low temperatures.

In his first paper on this subject (12) the author has told of the production of intumescences on leaves of *Populus grandidentata* and *P. tremuloides* which had been enclosed in a damp chamber; and under any conditions, such as rolling the leaves, fastening two leaves together, etc., which simulated such an enclosure. Obviously, under such conditions reduced transpiration of the leaves and high humidity of the atmosphere would result. Such conditions are usually conducive to growth and it was not illogical to suspect that water played a major part in the growth of the intumescences.

THE ROLE OF WATER

Intumescences on poplar leaves have been found in nature by the author only on leaves rolled up by leaf-rolling insects and on those stuck closely together by web-spinning insects. These were found only after the overgrowths had been induced in damp chambers, and the similarity of conditions indicated great likelihood that there was an identity of causal factors.

An extensive series of experiments showed that any condition which led to the leaves being surrounded by an unchanging atmosphere and an ample water supply would induce the formation of intumescences. Under the following conditions these growths were almost invariably formed: (1) enclosure in damp chambers and under bell jars, of leafy twigs with cut ends in water; (2) rolling of leaves by insects, or experimentally; (3) sticking leaves closely together by insects, or experimentally; (4) floating leaves on water in enclosed chambers, with either the upper or the lower surface in contact with the water; (5) floating leaves as in the preceding, but on culture solutions such as Knop's, Pfeffer's, Robbins's, or Benecke's; (6) placing segments of leaves on $1\frac{1}{2}$ and 2 percent agar media in Petri dishes, the agar media being made with water only, or with Pfeffer's, Robbins's, or Benecke's nutrient solution; (7) enclosure of leafy twigs on trees, in flasks or bottles. Enclosure of single leaves on trees in test tubes. Covering of surfaces of leaves with vaselined or varnished paper so as to form a chamber between leaf and paper; (8) covering a portion of leaf surface with a chamber constructed of cork.

The effect of excessive water supply on leaves in the open air

All the foregoing tests pointed to the need of water in the development of the abnormalities. Whether or not water alone could induce their development was first tested by subjecting leaves to a continuous spray. As no convenient nozzle for producing a fine spray was at hand, one was made by wedging a short segment of grape-vine stem in a small water tap. As the water was forced through the vessels of the stem it was broken into a fine mist which served the purpose. Leafy twigs were cut, set in jars of water, and placed under the spray, but otherwise exposed to the open air. Here they were kept dripping with moisture four or sometimes five days. Although large numbers of leaves were used in the experiments, and special care was taken in the examination of those which had been constantly wet for the whole period, not a single intumescence was found. Control twigs from the same branches which had been put into water and enclosed in damp chambers showed innumerable intumescences on their leaves.

To determine whether an adequate internal supply of water was essential for the production of overgrowths—a condition which may not have been satisfied in the preceding experiment—the cut ends of leafy twigs were pushed through rubber stoppers which were secured in water taps, after which they were subjected to the full pressure of the water system. Water was injected into the twigs with such force as to cause it to drip from cut petioles. In two hours the leaves became suffused with water and dark in color, but water did not exude from them except where they bore recent cuts or tears. Old cuts and insect injuries had become so well cicatrized as to hold back the water as well as the normal epidermis did.

The leaves on these twigs were left in the open air, and on dry days they would show by their changed appearance that they had become dried to a normal water content. But on humid days they became dark and injected with water again, while on rainy days, drops of water, apparently condensed from the atmosphere, appeared on their surfaces. Though the conditions may appear to have been abnormal, the leaves showed no ill effect, but remained normal, save for the intermittent appearance of being suffused with water, for a remarkable length of time. At the end of two weeks no leaves had died, yellowed, or been dropped, though petioles from which leaves had been cut had abscissed in the meantime.

No accurate records were made of the relative lengths of time during which the leaves appeared injected or normal, but there is no doubt that the injected condition predominated. During this period leaves on twigs merely cut and placed in water in the open air dried out and withered in a few hours. None of the leaves of the experiment withered or showed any wilting at any time, and there can be no question that an adequate if not an excessive water supply was present at all times, but no intumescences ever developed. Control leaves in damp chambers showed the usual crop of abnormal growths.

A set of twigs was arranged in water taps as in the preceding experiment, but the leaves instead of being allowed to become dry externally were placed under a continuous fine spray of water. Here the maximum supply of water possible to leaves in an unenclosed atmosphere was given. That it was greater than that ordinarily available to leaves in damp chambers, either where cut twigs were used or twigs still attached to the tree, is shown by the fact that under the latter conditions the leaves never became injected with water as these did. But even with this abundant water supply no intumescences developed.

Behavior of submerged leaves

Experiments with submerged leaves, and observations on many other experiments in which leaves became submerged accidentally, have shown that these leaves never develop intumescences. Leaves partially immersed usually show a very fine development of outgrowths on the unsubmerged parts.

Although the foregoing experiments show conclusively that the presence of an excess of water, either in the atmosphere or in the tissues of the plant, is of itself insufficient to induce the formation of intumescences, further experiments were made to determine what part water does have in the process.

Behavior of leaves in damp chambers without external water supply

An experiment was made with leaves on twigs inclosed in a damp chamber, but without any water being supplied to the cut ends of the twigs. Here the leaves were surrounded with an atmosphere of high humidity, but only the water already in them and in the twigs when they were cut was available. Since transpiration was greatly reduced, the leaves did not wilt, but only a few small intumescences were developed. Control leaves on twigs set in water and enclosed in a damp chamber developed large and very numerous intumescences.

The results of surrounding leaves with dry air in closed chambers

Two experiments were run with leafy twigs, the ends of which were placed in a jar of water which was sealed to prevent evaporation from the water surface. The base of the jar was set in a dish of fused $CaCl_2$ and the whole assembly was then covered by a bell jar. In these tests the outer leaves bore no intumescences though they were not abnormally dry, but the inner leaves, which were somewhat massed together, produced very numerous ones. The crowded leaves were wet with water condensed from transpired water vapor, and from the center of the mass toward the outside the leaves became increasingly dry. The number of intumescences decreased toward the outside with the decrease in moisture.

Other experiments were made with the leaves well separated and with an abundant supply of $CaCl_2$ in the chamber. Intumescences did not develop on these leaves, which usually dried and withered in a few days.

To make sure that the leaves had an adequate amount of water given them through the stems, cut twigs were set in rubber tubes into which water was run from a tank eight feet above. The leaves were then enclosed in a chamber with $CaCl_2$, where they dried out completely in a few days without producing any intumescences.

Another set of twigs was treated as in the preceding test except that water was injected into them from a water tap. The leaves on these twigs behaved exactly as did those with water forced into them under a lower pressure.

It appears that leaves enclosed in a stagnant atmosphere will not develop intumescences unless the humidity of the atmosphere is high, no matter how much water may be supplied internally from the twig.

The results of forcing water into leaves enclosed in damp chambers

It has been shown by experiment that if the twigs are not supplied with water, the intumescences developed on the leaves are few and small. To discover whether or not an excess of water supplied through the twigs would increase the number, or the size, or the rate of development of the outgrowths, leafy twigs were set in tubes with water forced into them from a tank eight feet overhead, and the leaves were enclosed in a damp chamber. It could not be seen that the intumescences were in any way different from those on control leaves in damp chambers on twigs merely placed in water.

A repetition of the test with an increased pressure in tubes attached to a water tap gave the same result—from which it appears that twigs with their cut ends in water are able to supply a sufficient amount of water for the development of intumescences on leaves in damp chambers. An excess of internal water is not the cause of intumescences; nor is it even an aid to their development.

Since an excess of water supplied to the leaves, internally and externally at the same time, will not initiate intumescences, it is apparent that water plays a minor rather than a major role in their causation.

THE EFFECT OF LIGHT

Light has been considered an important factor in the growth of intumescences by a number of investigators. Some have found that low light intensity, resulting in a low rate of assimilation, is conductive to the abnormal growths; while others have discovered that full illumination was required for their development.

Most of the experiments described in this paper were performed in a laboratory in light from a north window, so that it was known, from the first discovery of the existence of the intumescences, that they would develop well in weak light. A number of experiments were made to test the effect of various light intensities, and from them, and from other experiments made primarily to study the effect of other factors, it was found that the growths appeared on leaves of *Populus grandidentata* and *P. tremuloides*, under all the following conditions: (1) in weak light in laboratory; (2) in darkness in laboratory; (3) in shade, twigs attached to tree; (4) in sunlight, twigs attached to tree; (5) in shade, leaves on tree, but rolled up by insects, or experimentally; (6) in sunlight, leaves on tree, but rolled up by insects, or experimentally; (7) in shade, leaves on tree, but stuck together by insects, or experimentally; (8) in sunlight, leaves on tree, but stuck together by insects, or experimentally; (9) in weak light, upper surface of leaves shaded, experimentally; (10) in sunlight, upper surface of leaves shaded; (11) in shade, paper fastened on surface; (12) in sunlight, paper fastened on surface.

Under all these conditions, which represent a great variety of light intensities ranging from full insolation to complete darkness, the intumescences were unvarying. No differences could be detected in their abundance, their structure, or the rate of their development which indicated that one light condition was more favorable to their initiation than another.

Inasmuch as the leaves in all cases seem to contain sufficient food to develop intumescences, even the indirect effect of light as a producer of food seemed to be of no consequence.

FOOD SUPPLY AND MINERAL NUTRIENTS

Early in the study of this problem it was demonstrated repeatedly that intumescences developed on detached leaves floated in water, either in light or darkness. Apparently the leaves did not need the extra food which might have resulted from photosynthesis in their tissues. Halves of leaves, even, contained sufficient food for a full development of intumescences.

However, it cannot be said that the relative abundance of food is of no importance in these growths, for if leaves attached to twigs are floated on water, the outgrowths are larger and more abundant than those on detached leaves. The twigs to which these leaves were attached were not examined to determine their starch content, but each was about four inches long and could have held an abundant supply of food for much more growth than is represented by a set of intumescences on the leaves. That the twigs contain an ample food supply is shown by the fact that experiments in which leaves attached to the tree were placed in closed compartments did not result in larger or more numerous intumescences than were developed on leaves attached to the four-inch twigs.

It was found that intumescences developed on leaves floated in Pfeffer's solution, Benecke's solution, and Robbins's solution. They developed also on nutrient agar made up with these solutions, but in no case did it appear likely that any substance in these nutrient solutions supplied any need not met by the store of materials in the twig or even in the leaf itself.

The effect of sugar solutions on the growth of intumescences

At the same time as the preceding tests were made, another set of leaves was placed in Pfeffer's solution with the addition of 4 percent dextrose, and on agar made up with this solution. Other leaves were floated in Robbins's solution with 2 percent dextrose added, and others were placed on agar made with that solution. Without exception, the nutrient solutions, and the nutrient agars which contained dextrose, brought about a more rapid and a more extensive development of outgrowths than was found on the corresponding solutions without sugar. Quantitative results could not be secured easily, but it was evident that the development on nutrient agar containing dextrose was superior even to that found on leaves still attached to the tree. This is not to be attributed to a lack of food in the tree, but rather to the fact that the leaves left attached to the tree, but enclosed in a damp chamber, begin and even complete the formation of an abscission layer before the intumescences have reached their fullest extension. After abscission, which seems to be stimulated by the enclosure of the leaves in the damp chamber, the leaves contain too small a food supply to compete with richly fed leaf segments on a dextrose agar. It should be noted that the nutrient agar used was kept sterile, and the leaf segments were sterilized with "Zonite," so that fermentation of the agar was prevented on a number of the plates, though some developed colonies of yeast, and mold.

THE PARTS PLAYED BY CARBON DIOXID AND OXYGEN

By the time the discovery was made that neither an excess of water nor any special condition as regards food supply could account for the initiation of intumescences, it became rather more apparent that the inclosure in a chamber was responsible in some way for the phenomenon. The respiration of the enclosed leaves must have had the effect of increasing the concentration of CO_2, and of lowering the oxygen tension; conceivably, either of these changes might result in the formation of the outgrowths.

It so happens that conditions which surround the leaf with an atmosphere saturated with water vapor also are usually conditions which lead to a stagnation of the atmosphere with the consequences just noted. Leaves rolled up by insects or stuck together by web-spinning insects are the only ones which have developed intumescences in the woods under natural conditions. Under these circumstances, closed or partially closed chambers are formed. Under the discussion of the role of water the rather large number of ways in which leaves were inclosed experimentally have been described. All of these serve equally well in securing an unventilated atmosphere about the leaves with subsequent decrease of oxygen and increase of carbon dioxid.

The behavior of leaves in ventilated damp chambers

An experiment was devised to allow of change of air, and at the same time to keep a water-saturated atmosphere about the leaves. Air, charged with water-vapor by bubbling it through four wash bottles containing water, was drawn by an aspirator through the closed chamber containing the leaves. After the experiment had been run for four days with leaves of *P. grandidentata* and *P. tremuloides*, long enough to produce intumescences under ordinary conditions, only four intumescences could be found on a total of 33 leaves subjected to the experiment. The control leaves in a chamber identical to that used in the experiment, but unventilated, bore hundreds of intumescences.

A repetition of the experiment gave only three intumescences on 33 leaves, and hundreds on the control leaves. A third test on 25 leaves showed that three had small scattered intumescences, and two, which had lain close against the wall of the chamber, had an abundant crop of the growths. Another trial, in which the leaves were crowded closely together, so that the air may not have passed freely among them, showed numerous outgrowths on a few leaves and none on the others. Two other tests, in which care was taken to prevent leaves from being pressed closely together, did not produce a single intumescence. In all these experiments care was taken to see that the atmosphere about the leaves was thoroughly saturated with water. The control leaves almost without exception produced great numbers of outgrowths. From these results, and those on the effect of water above, the conclusion must be drawn that the stagnation of the atmosphere is more important in the production of intumescences than high humidity can be.

A further confirmation came from trials in which a rather small number of leaves were placed in a large chamber of which the atmosphere was saturated with water. These trials always resulted in the development of a few intumescences on the leaves just above the water in the bottom of the chamber, and none in the upper part of the chamber where the CO_2 content was probably less.

Results with leaves in air of low oxygen content and in rarefied air

A test was made of the effect of lowering the oxygen tension by placing a tube of alkaline pyrogallol in a chamber tightly closed, and with a water-saturated atmosphere. No intumescences developed in this test, or in a repetition of it. Most of the leaves were dead after 10 days but some remained normal.

Another set of leaves was put in a chamber from which the air was pumped out by a rubber bulb operated by hand, and then forced through a series of wash bottles filled with pyrogallol before being returned to the chamber. This procedure resulted in injury, due to oxygen starvation apparently, for in three days most of the leaves were dying. No intumescences had even been initiated except on four leaves which lay against the side of the chamber, where they may have held a certain amount of oxygen inclosed.

The concentration of the air was decreased in two experiments by enclosing leaves in a rubber-stoppered glass jar of one-gallon capacity from which air was exhausted by an aspirator on a water tap in the laboratory. Though the leaves were alive after 10 days under such conditions, no sign of any abnormal growths could be found on either of the species, *P. grandidentata* or *P. tremuloides*. Control leaves, in ordinary damp chambers, showed the usual abundance of intumescences.

Further evidence was gained by placing a set of leaves in a closed jar in which another set of leaves had been enclosed previously for three days. The first set of leaves had produced abundant intumescences, but the second group put into the stale air did not develop a single intumescence. This test was made on leaves of *P. grandidentata*, but a similar one made on *P. tremuloides* developed intumescences very abundantly in four days, a rather short time for their full development in that species. A study of the situation to explain this anomalous result showed that in this instance only a small number of leaves had been confined in the chamber before the second lot of leaves was put in.

The water in the bottoms of the jars used in the two preceding experiments became rather foul before the second set of leaves was added. To remove the effect of foul, and possibly toxic, water the experiment was repeated, but the second set of twigs was placed in a bottle of fresh water which was then set in the bottom of the chamber. Leaves of *P. grandidentata* were used in this test and the same as in the one where the twigs were in the polluted water.

It seems that in the experiments where leaves of *P. grandidentata* were used, the first set of leaves was a rather large one, and it used up the oxygen to such an extent as to leave the atmosphere too poor to allow any growth of the second set of leaves. The experiment with leaves of *P. tremuloides* was made with only a small number in the first set, which did not lower the oxygen tension too much, but just sufficiently to initiate a rapid development of intumescences on the second set.

There is good evidence from these experiments that oxygen plays an important part in the growth of intumescences, and that when the oxygen tension is too much lowered no outgrowths are formed, even if the leaves are surrounded by an atmosphere charged with CO_2 and water vapor. It is rather to be expected that any great reduction of the oxygen would not leave a sufficient supply to allow of any growth whatsoever. For this reason, apparently, submerged leaves are unable to develop intumescences though they may remain alive for many days. Of course, they may be prevented by their position from receiving the stimulus which initiates outgrowths on the unsubmerged leaves in the same container. On the other hand, the disturbance of respiration due to an excess of CO_2, or a lack of O_2, might be the stimulus to development of outgrowths. So far as the evidence gained by the preceding experiments is concerned, the one might serve as well as the other. Accordingly some trials were made to determine, if possible, the part which CO_2 might play in the process.

Results with leaves kept in an atmosphere free of carbon dioxid

Several experiments were made in which leaves of *Populus grandidentata* were enclosed in a water-saturated chamber in which NaOH or KOH was exposed to absorb the CO_2. None of the leaves developed intumescences, but they soon became pale and unhealthy in appearance and the lack of outgrowths might as readily be attributed to an abnormal condition of the leaves as to the lack of CO_2 in the chambers.

A trial was made with soda-lime to absorb the CO_2. The leaves on five twigs of *P. grandidentata* had no outgrowths, but those on two twigs produced numerous intumescences. The leaves on these two twigs may have been crowded together so that the CO_2 was trapped between them, so another test was made with five bell jars set over vessels containing soda-lime. Leafy twigs of *P. grandidentata* were introduced into the jars. The leaves in two of these jars became very dry, but those in the other three jars developed intumescences.

To be sure this result was not due to the failure of the CO_2 to reach the soda-lime, another set of leaves of *P. grandidentata* was enclosed in a water-saturated chamber from which the air was drawn, pumped through a train of bottles containing soda-lime, then through wash bottles containing water, and finally returned to the chamber. The air was pumped by a rubber bulb at intervals of about two hours during the day. After 30 hours under these conditions the leaves became very unhealthy in appearance and were covered with black spots which exuded drops of water. The control leaves were green and covered with incipient intumescences. Just why the soda-lime or the NaOH and KOH should have injured the leaves is not clear, but it was evident that these substances were injurious to such a degree as to render them unfit for further use in experiments.

Barium hydroxid was tried next as a means of removing CO_2 from the chamber, and an experiment was made exactly like the preceding one except that a solution of barium hydroxid was substituted for the injurious soda-lime. The leaves remained healthy and all developed intumescences. A repetition of the experiment gave the same result. The possibility that the accumulation of CO_2 during the seven hours of the night, when the air was not circulated, might have initiated the development of the abnormal growths still existed.

Since the barium hydroxid did not harm the leaves, an experiment was set up with twigs of *P. grandidentata* in a jar of water which was set inside a larger jar filled nearly to the top of the inner jar with barium hydroxid. This brought the leaves very near the surface of the barium hydroxid solution. The top of the jar was covered with a glass plate sealed in place with vaseline. Under these conditions a heavy scum of barium carbonate soon formed on the surface of the hydroxid solution. In four days nearly all the leaves were covered by intumescences; even those which dipped in the solution had growths on the unsubmerged parts.

To secure a certain circulation of air about the leaves a set was placed in a chamber from which the air was drawn out and passed through a series of bottles containing barium hydroxid before it was returned to the chamber. But in this experiment, unlike some of the preceding ones, the air was pumped by a device developed by Osterhout (14), which kept the air in constant circulation and carried the CO_2 out of the enclosed chamber as fast as it was formed. As in most of these experiments, *P. grandidentata* leaves were used. Control leaves were enclosed in a chamber without circulation of air. Many leaves in the experiment, and also many among the control leaves, had no outgrowths, but there were a number under each of the conditions which bore a considerable number of intumescences. Quite as many intumescences were developed in the experimental lot as in the controls. Since the leaves were rather crowded in the experimental chamber, it was impossible to be sure that the intumescences had not been developed on leaves pressed against the walls of the chamber, or against each other, so that the CO_2 was trapped around them. A new experiment was planned to obviate this defect.

The problem of enclosing a number of leaves on twigs set in water, in a chamber in such a way as to keep each leaf in full contact with the air and entirely separated from the others and from the walls of the chamber, required the use of a special device. Finally the idea of using the metal-wire screening known as "hardware cloth" suggested itself. Two cylinders were made of this material, one of which fitted inside the other with sufficient space between to keep the leaves attached to them from touching at any point. Twigs of varying lengths were selected so that the leaves on them could be spread over the cylinders, while the bases of the twigs were submerged in water. The leaves were spread over the metal fabric and fastened in place with segments of a small tinned wire so that none of the leaves overlapped but nearly all the surface of each of the cylinders was covered. The larger cylinder was then placed around the smaller one and both were set on a glass plate and covered with a tall bell jar lined with saturated filter paper. Water was then poured into the bottom of the chamber thus formed so that the ends of all the twigs were in water, and the filter-paper lining of the chamber was kept wet. The leaves were now entirely exposed to the air except for the very small amount of surface in contact with the wires, beneath which very little CO_2 could accumulate. An inlet tube into the chamber was fitted with four outlets above the leaves to distribute the incoming air. The exhaust tube was lowered into the chamber so that its opening rested just above the water level, and CO_2 could not accumulate in the bottom of the chamber. The air was pumped continuously from the chamber, bubbled through four bottles containing barium hydroxid solution, and through two bottles of water, and returned to the chamber. Osterhout's device was used to secure a constant flow of air during the whole duration of the experiment.

A set of control leaves was prepared in exactly the same way except that the barium hydroxid solution was omitted, and the circulating air was passed only through bottles of water.

At the end of five days all the leaves were examined, and very numerous and very well-developed intumescences were found on nearly every leaf of the experimental set, and of the control group as well.

A repetition of this experiment gave the same result. In both these tests no difference could be seen between the experimental and the control leaves. No CO_2 could have accumulated in the experimental chamber, as the barium hydroxid solution was changed at intervals and the last of the train of bottles of this solution never showed any precipitate of barium carbonate. No CO_2 was removed from the control chamber except such as was absorbed in the water in the wash bottles. The evidence is conclusive that the accumulation of carbon dioxid around the leaves plays no part in providing a stimulus for the development of intumescences.

THE EFFECT OF VOLATILE SUBSTANCES GIVEN OFF BY THE LEAVES

It was noticed in the first tests in which considerable numbers of leaves were kept in a closed chamber that a strong odor could be detected in all the chambers after the leaves had been confined therein for a few days. The odors differed slightly with different species, so that one could distinguish readily the chambers in which a given species had been kept. *P. deltoides* always produced a stronger odor than any of the others, and *P. alba* gave scarcely any distinct poplar odor, but rather the usual smell of the leaves of most plants kept under such conditions. If the volatile substances which produce the odors were the stimulating factors, one might suspect that *P. deltoides* would develop intumescences on its leaves, which it never does; although, of course, it is not known that the same substances are involved in producing these different odors.

While there is a possibility that these volatile substances may in some way initiate the formation of the abnormal growths, it does not seem probable that this is the case. Positive evidence that they are not the only stimulus was gained from the experiments in which a second set of leaves was introduced into a chamber in which one set had already been kept for some days. These chambers always manifested a strong odor, but the second set of leaves did not develop intumescences, though the odor in the chambers was intensified by their presence.

The odors were equally noticeable in chambers from which the air had been exhausted by an aspirator and in those in which the oxygen tension was lowered by pyrogallol, and under these conditions intumescences did not appear. It is possible that these volatile substances may be combined with a lowered oxygen tension in stimulating the leaves to abnormal growth, but it seems more likely that they are noticeable only because they are confined in the stagnant atmosphere and that they are only a part of an unusual complex of environmental conditions, but like carbon dioxid ineffective as a stimulating factor.

From the foregoing results it appears that oxygen is needed for the growth of intumescences, and that if too little of it is present an internal decline of the leaves results and no growth is possible. On the other hand, the oxygen content of the air around the leaves must be lowered before the stimulus, whatever it may be, which initiates the intumescences can be developed. The hypothesis which appeals to the writer as the most tenable of those which he has considered in connection with the data yielded by his experiments is that some product, or combination of products, of anaerobic respiration provides the required stimulus.

DISCUSSION

There is no doubt that intumescences and similar abnormal growths may be initiated by more than one type of stimulus. Haberlandt, Smith, Harvey and Rose, Doubt, Wallace, Von Schrenk, Douglas, and others have shown that chemical stimulation may give rise to such abnormalities, and the number of different chemicals used by these investigators is great enough to suggest that many others might produce similar effects.

Contrasted with the effect of chemicals studied by the above workers one finds a number of investigators who have believed that intumescences were initiated by physical factors. That direct injuries could cause intumescences on cabbage leaves has been shown by R. B. Harvey and Wolf, who studied frost injury and injury by wind-blown sand, respectively. The writer (13) found that punctures induced intumescences in leaves of *Hieracium venosum*.

Atkinson, Douglas, Dale, Viala and Pacottet, and Sorauer all have emphasized an excessive water supply and low transpiration as agents in the process. One must admit the possibility that these two components of the immediate environment of the plants were sufficient to cause these abnormalities in certain plants. In the present paper the writer has shown that neither excessive water supply nor reduced transpiration would cause intumescences in poplar leaves, nor would the two of them in combination effect this result. One is led to suspect that this may be true of some other plants in which the appearance of intumescences has been attributed mainly to excess of water. Steiner, for example, kept the leaves on which intumescences appeared in a damp atmosphere. It may be that this meant also that the air was confined around them. Douglas enclosed her experimental plants in bell jars and considered that the intumescences which appeared were due to excessive moisture. She made no mention of any provision for ventilation of the bell jars, and unless they were ventilated, the idea must be entertained that changes in carbon dioxid and in oxygen content may have had an important effect.

Küster found that when leaves of *Populus tremula* were floated on water the development of intumescences was greatest on the side of the leaf next the water. On the same side of the leaves the oxygen supply obviously would be most limited. Some, though not all, of Dale's experiments were made under conditions which would have allowed only a limited oxygen supply to the leaves. Likewise the water around the roots on which Hahn, Hartley, and Rhoads found lenticular outgrowths may have cut off the oxygen from the roots. The paper by Viala and Pacottet is too brief to give full details, but in it they state that intumescences on grape leaves were found immediately under the glass of greenhouses. The writer found in the course of the studies described above that even in ventilated chambers poplar leaves which were pressed closely together or against the walls of the chamber developed intumescences, while all the others remained normal. Perhaps the grape leaves which bore the abnormalities studied by Viala and Pacottet were not only close to the glass, but actually pressed against it in such a way as to form closed chambers.

Wisniewski and Schilling produced outgrowths on twigs by coating them with paraffin and vaseline. These authors were not included among those who used chemical means of stimulation in producing abnormalities because it seems to the writer that these stimuli were physical rather than chemical. In fact, Smith insists that all the chemicals by which he caused abnormal growths were physical rather than chemical in their effect. Schilling believed that lowered transpiration was the main cause of the response in the twigs which he studied, but he suggested that lowered oxygen tension also might have been concerned. No other investigator, so far as the writer can determine from the literature on intumescences, has even referred to the possibility that deficiency of oxygen might serve as a stimulus to renewed growth in plant organs. It is not unreasonable to assume that in some of the instances cited above, where the possibility of an oxygen deficiency was not eliminated, this factor may have been more important than the one most emphasized.

It is possible that the apparently diverse stimuli which result in intumescences are not really different in their final effect on the cells. Little is yet known as to the changes which are induced in the cells before they begin to increase in size, or to divide. R. B. Harvey suggested that the growth stimulus in frozen cells of cabbage leaves was due to a partial precipitation of the proteins with a consequent increase in the permeability of the cells to water, and in the ability of the cells to hold sugars. This hypothesis seems to agree well with the development of intumescences in poplar leaves in which the cells increase in volume but do not divide. The precipitation of the proteins Harvey believes to be due to an increase in hydrogen-ion concentration in the affected cells. He found such a change in pH in frozen cabbage leaves, although later (10) he discovered that the actual tumors on castor bean, beet, and *Bryophyllum* were less acid than the surrounding tissues. Incomplete studies on the pH of poplar leaves kept in closed chambers indicate that the pH is lowered by this treatment.

The cause of the changes in the pH of poplar leaves may possibly be found in the products of the anaerobic respiration necessitated by the low oxygen tension in closed chambers. Thomas (21) found that when the oxygen concentration of storage chambers falls below 10 percent the apples in these chambers begin anaerobic respiration and produce alcohol and acetaldehyde. In apples the accumulation of acetaldehyde causes storage scald. These, or other products of anaerobic respiration, such as oxalic acid or other organic acids, may account for a change in hydrogen-ion concentration in the cells, and ultimately for the initiation of abnormal growths.

At present no explanation can be given of the fact that a great many species of plants do not produce intumescences when placed in closed chambers were deficiency of oxygen must soon check aërobic respiration (12). It is even more difficult to understand why only three species of poplars produce these growths, and all other species of the genus tested thus far fail to do so. A more intimate knowledge of the respiration of these plants in atmospheres deficient in oxygen may explain these differences in the behavior of plants so closely related.

SUMMARY

1. Leaves of *Populus grandidentata* and *P. tremuloides* produce intumescences under any condition which causes them to be surrounded by stagnant, moist air.

2. Submerged leaves do not produce outgrowths, apparently because of lack of oxygen.

3. Leaves in the open air remain normal even though constantly sprayed with water for a period of several days' duration.

4. Leaves injected with water under pressure do not develop intumescences in the open air, nor in dry air in closed chambers.

5. A moist atmosphere and an adequate supply of internal water are required for the production of intumescences. Injection of water under pressure does not increase the growth, or the number of intumescences on leaves surrounded by moist air.

6. Leaves kept in ventilated moist chambers do not produce intumescences.

7. Intumescences are not produced in closed chambers in which the air is kept dry.

8. An excessive water supply, internal or external, or both internal and external, does not initiate intumescences in poplar leaves.

9. An atmosphere free of oxygen, or very low in oxygen content, does not allow intumescences to develop.

10. The oxygen content of the air around the leaves must be reduced below that of normal air before intumescences are initiated. The extent of the reduction required has not been determined.

11. An accumulation of carbon dioxid in the chambers which contain the leaves is not required for the production of intumescences. Removal of all carbon dioxid from the chambers does not prevent the formation of these outgrowths.

12. It is suggested that some product, or combination of products, of incomplete oxidation, due to lowered oxygen tension, stimulates the leaf cells to renewed growth which results in the production of intumescences.

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