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The development of hyperhydric tissue on the stems of *Sambucus nigra* L.

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Abstract

Hyperhydric intumescences on the stems of *Sambucus nigra* arise in places where the stem lenticels are immersed in water. The hyperhydric tissue develops through the transformation of the multilayered phelloderm, the parenchyma of the cortex, endodermis and pericycle. The phellogen loses its meristematic properties and is either incorporated into the developing hyperhydric tissue or crushed.
The succesive stages of hyperhydric changes which depend on the intense growth of cells and on the ability to devide acquired by them are presented.

Key words: intumescences, lenticel, hyperhydric cells

INTRODUCTION

Many types of abnormal structures like intumescences, protrusions or spots, can form on plant organs. They are usually undifferentiated histologically and were named by Sinnott (1960) "amorphous structures". The formation of these structures is evoked by the action of various factors which disrupt the optimum environmental conditions for the plant, or, these structures can also be caused by internal factors. They can arise on the roots, shoots, rhizomes, flowers and even fruits of various plants. An example of such abnormal intumescences is the formation of distentions from developed hyperhydric tissues.

Küster (1925) describes hyperhydric tissue as being overly hydrated, arising as the result of intense water uptake under conditions not permitting water to be given up. Under experimental conditions, similar structures

can also be formed as the result of using gaseous ethylene (Sinnott 1960), ether or chloroform (Küster 1925).

Hyperhydric tissue can develop in direct contact with water or in an environment with a high level of humidity. On stems, hyperhydric intumescences always develop in places where there are either stomas or lenticels. The cells which form them have large dimensions with very large vacuoles. The cell walls of these cells are thin and large intercellular spaces are filled with air giving the tissue a snow-white luster. This tissue is unstable and those of its cells which find themselves on the outside of the plant's organ, easily disintegrate.

Information in literature on the formation of hyperhydric intumescences dates back from the beginning of the XXth century (Devaux 1900, Wóycicki 1910, Schilling 1915, Küster 1925), is fragmentary and not totally in agreement. It is known that hyperhydric tissue arises from above-average growth of cells. However, there is no consensus on the questions of if and how much cell divisions influence the formation of tissue mass, what is the role of phellogen and which tissues undergo hyperhydric transformations.

The aim of this study is to analyse the formation and development of the hyperhydric tissue formed on *Sambucus nigra* stems as the result of contact with water.

MATERIAL AND METHODS

The experiments were carried out on several year old *Sambucus nigra* L. stems. The stems, 20-30 cm in length, were placed for a period of 6-7 weeks in containers with water. Samples were taken at intervals of a few days beginning when the lenticels were observed to start enlarging. The excised stem samples were fixed in a mixture of chromic acid, glacial acetic acid and formalin (CrAF at a percentage ratio of 0.5:1:20). The observations were done on microtome paraffin cross sections stained with Ehrlich's hematoxylin. The chemical composition of the cell walls and contents during the development of the tissue were studied with standard, commonly used staining and microreaction methods.

RESULTS AND DISCUSSION

The first morphological changes in the lenticel sites, in the form of white protrusions on the parts of the stems immersed in water, were found after 5 days. After about 5-6 weeks, the intumescences, which were expanding

vertically and horizontally, had formed extensive distentions on the surface of the stem and a white, spongy ring had formed around its node (Fig. 1). Leaves and, sometimes, adventitious roots grew from so-changed nodes. The external layers of hyperhydric tissue are easily destroyed and fall off, sometimes along with layers of cortex.

Hyperhydric cells, at all stages of development of the tissue, are characterized by a very large, generally central, vacuole. The cytoplasm is parietal, the nucleus is suspended on protoplasmic bridges and surrounded by amyloplasts containing starch grains. The content of the cell and the chemistry of its cell wall do not change during the development of the tissue. The walls retain their pectin-cellulose structure.

A *Sambucus nigra* stem which is a few years old, is covered by periderm with overlaying cells, a few layers of cork cells, phellogen and one layer of phelloderm. Within the lenticels, however, under the filling cells, there is also one layer of phellogen and directly underneath it there are several layers of living, overlaying cells, which it seems, are a multilayered phelloderm. The cortex parenchyma cells form distinct layers.

The first histological symptom of hyperhydric changes is the increase in the volume of the multilayered phelloderm and the gradual loss of the overlaying arrangement of these cells (Fig. 2). The cortex parenchyma cells also increase in size, which causes the border between the phelloderm and cortex to become increasingly less clear (Fig. 3). The increase in the volume of the parenchyma cells is accompanied by their divisions which initially take place on the border between the phelloderm and cortex. Most of the microscopic images showed that within the multilayered phelloderm, the growth of the cells precedes their division; within the parenchyma cells, on the other hand, the cells first divide, then intensely grow (Fig. 2).

After 6-8 days following the immersion of the stem in water, the phellogen within the lenticel also gradually changes. Most of the phellogen cells grow, but not all of them at once. Those which retain their original size are usually crushed by the neighboring, growing cells (Figs. 3, 3a). Concomitantly with the morphological changes in the phellogen, the cortex parenchyma cells increase in size and gradually turn into cells of the hyperhydric tissue (Fig. 4).

The hyperhydric tissue arises and grows not only due to the increase in cell size, but also through their divisions. Mainly, the cells of the growing multilayered phelloderm divide (Fig. 4, 4a) as well as do the cells of increasingly deeper layers of the cortex (Figs. 6, 6a, 6b). The divisions are usually periclinal, due to which the entire hypohydric tissue grows radially and causes the contents of the lenticel to be pushed out to the exterior of the stem (Fig. 6).

The development of the hyperhydric tissue also takes place, although to a lesser extent, tangently to the circumference of the stem. The cells

then squeeze themselves under the periderm or in between its cells. In addition to this, on both edges of the lenticel, those cells which are the external descendents of the phelloderm and whose cell walls have not yet been suberized, become highly extended radially (Fig. 5, long arrow). Cells with suberized walls do not change, but are pushed to the side by growing cells of the hyperhydric tissue. The growth of the tissue tangently to the circumference, in the beginning stage of its development, may even exceed its development in the radial direction. These are not, however, frequent cases and do not have significant meaning for the final development of the intumescence.

The more advanced stages of hyperhydric tissue development encompass increasingly deeper layers of cortex (Fig. 5). Underneath the cells whose shape is extensively modified, parenchyma cells with yet unchanged shape are dividing and will next increase in size and become incorporated into the mass of hyperhydric cells. In the final stages of development, the changes include all of the cortex parenchyma cells. Distinct pillars of cells which are still able to divide both in the central and side parts of the tissue, that is, near the cork, arise. The divisions are periclinal or slightly slanted (Figs. 6, 6b). The pillars of cells mentioned above, fall apart in the surface part of the intumescence; large spaces arise between them and the cells easily fall out.

The cells of the endodermis and pericycle are initially streched in the tangent direction, after which they too elongate radially and are included in the hyperhydric tissue and, as its other cells, can divide (Figs. 7, 7a).

Fig. 1. A stem with hyperhydric intumescences after 6 weeks of being submerged in water, slightly reduced

Figs. 2-4. Cross sections of developing hyperhydric intumescences arising at the sites of lenticels. CrAF, Ehrlich's hematoxylin. Fig. 2 — Enlarged cells of multilayered phelloderm (double arrow), cortex parenchyma cells after division (short arrows), phellogen (long arrow). 3 days submergence of stem in water. X 100. Fig. 3 — The enlarged phelloderm cells are losing their overlaying arrangement, the border between the phelloderm and external layers of parenchyma cells is beginning to disappear, phellogen (arrow). 6-8 days of submergence of stem in water. X 100. Fig. 3a — An enlarged phellogen cell (long arrow) becomes incorporated in the hyperhydric tissue, next to it is a crushed phellogen cell (double arrow). Fragment of Fig. 3. X 500. Fig. 4 — Most of the cortex parenchyma cells hyperhydrically changed, phellogen (arrow). X 100. Fig. 4a — Prophase in a cell of the hyperhydric tissue. X 500


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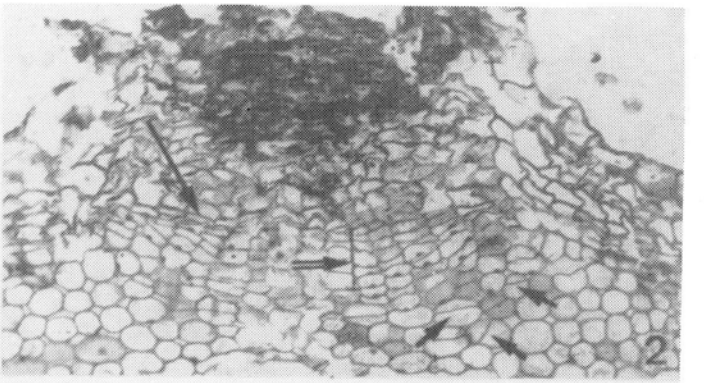
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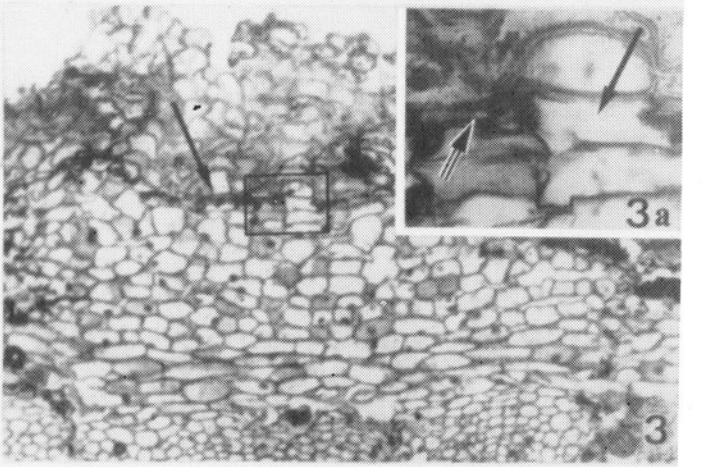
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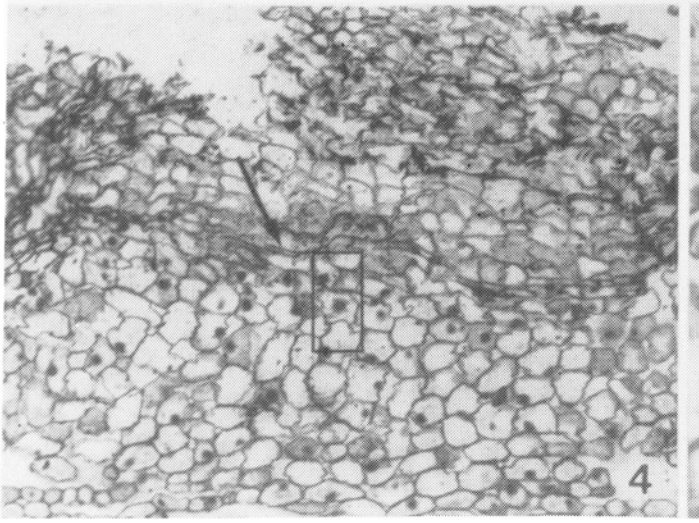
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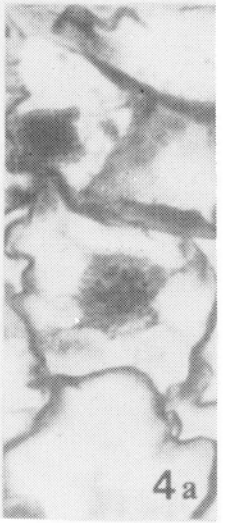
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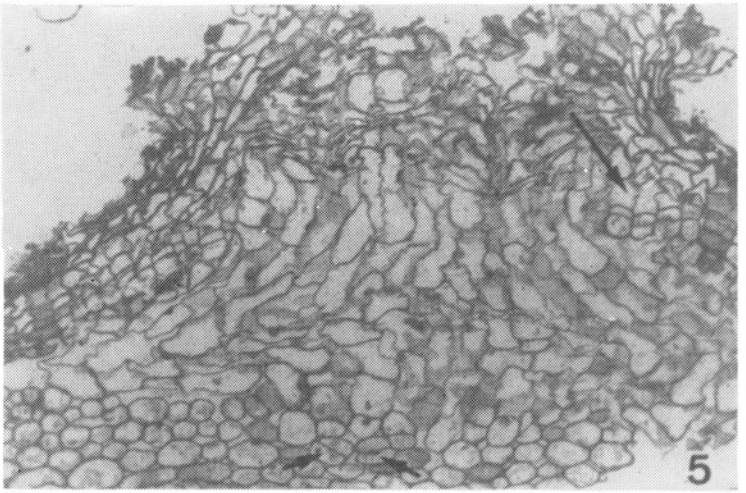

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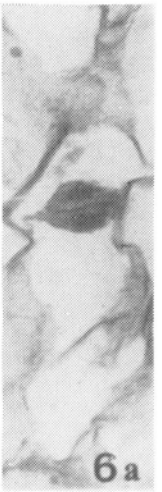

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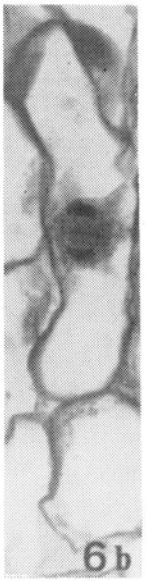

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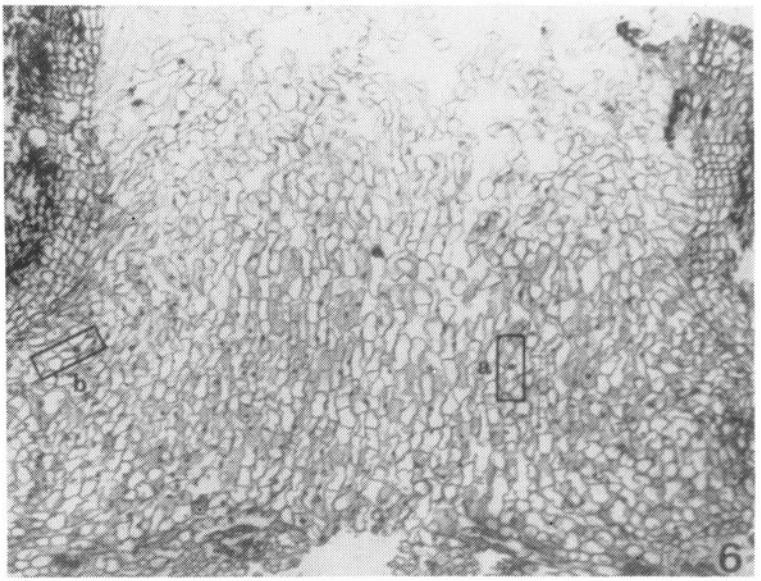

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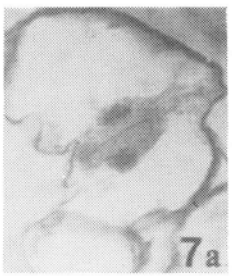

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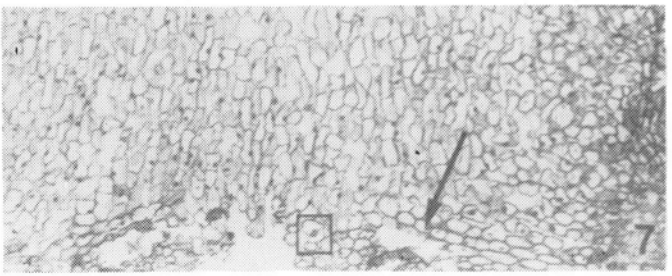

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Phloem cells (with the exception of fibers) usually also increase in size but do not acquire traits characteristic of hyperhydric tissue. No distinct changes were found in the xylem.

As can be seen from the description given above, hyperhydric changes in the stem of *Sambucus nigra* encompass the phellogen, multilayered phelloderm, all of the layers of the cortex and pericycle. The changed phellogen loses its meristematic properties. The formation of such a large mass of cells is the result of both increasing the volume as well as the numerous divisions of cells forming the hyperhydric intumescence.

The results obtained in this study are in agreement with the observations made on many tree species by Devaux (1900) and Küster (1925), from which it results that mainly the phelloderm and parenchyma of the cortex are involved in hyperhydric changes. The phellogen, however, does not play the role of an active meristematic tissue as claimed by Schilling (1915). Also, a special meristematic layer within the changed phelloderm or cortex parenchyma does not arise, as had been observed by Devaux (1900) in the stems of the poplar and willow. Our results also do not agree with the claims of Sinnott (1960) and Hejnowicz (1973) that the hyperhydric intumescence is the result of only proliferation of the cells filling the lenticel. In *Sambucus nigra*, these cells are pushed outside of the stem early in the development of the intumescence by the growing and dividing parenchyma cells found under the phellogen. It can not, however, be excluded that in different species, the development of hyperhydric intumescences can take place in a somewhat different manner.

The hyperhydric intumescence on the stem of *Sambucus nigra* always forms in places where lenticels are found and only on parts submerged in water, thus, due to the direct influence of water.

Cross sections of developing hyperhydric tissue, CrAF, Ehrlich's hematoxylin

Fig. 5. Development of hyperhydric tissue on both sides of a lenticel, changed, nonsuberized cells descending from phellogen (long arrow), divided cells of deeper cortex parenchyma layers (short arrows). 3 weeks submergence in water. X 100. Fig. 6. Highly developed hyperhydric tissue encompassing the entire cortex mitoses in various points in this tissue. 6 weeks submergence of stem in water. X 80. Figs. 6a, 6b. Telophases in hyperhydric cells, enlarged fragments of Fig. 6. X 500. Fig. 7. The same intumescence as on Fig. 6, the next section. Unchanged endodermis cells (arrow). X 80. Fig. 7a. Telophase in a hyperhydrically changed endodermis or pericycle cell. Fragment of Fig. 7. X 500

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Rozwój tkanki hiperhydralnej na łodygach Sambucus nigra L.

Streszczenie

Badano rozwój i budowę tkanki hiperhydralnej tworzącej narośla na częściach łodyg bzu czarnego (*Sambucus nigra* L.) zanurzonych w wodzie. Obserwacje prowadzono w ciągu 7 tygodni, pobierając materiał w odstępach kilkudniowych poczynając od czasu, w którym stwierdzono powiększanie się przetchlinek. Stosowano utrwalacz CrAF (w stosunku procentowym 0,5:1:20), barwiono hematoksyliną Ehrlicha.

Stwierdzono, że hiperhydralne narośla powstają zawsze w miejscu występowania przetchlinek. Tkanka rozwija się z przekształcenia wielowarstwowej fellodermy, miękiszu kory pierwotnej, endodermy i pericyklu. Fellogen traci zdolności merystematyczne i zostaje włączony w tkankę hiperhydralną lub zgnieciony. Zmiany hiperhydralne komórek obejmują najwcześniej fellodermę, a następnie coraz to głębsze warstwy miękiszu kory. Powiększanie masy komórkowej tworzącej hiperhydralną narośl jest wynikiem silnego wzrostu komórek i nabytym przez nie właściwościom dzielenia się.