Botany. — *"Latex as a constituent of the cell-sap."* By F. A. F. C. WENT.

(Communicated at the meeting of February 27, 1926).

The number of investigations on the latex of plants has greatly increased during the last years, mainly due to the extension of rubber-cultivation. Yet it cannot be said that latex has lost much of its obscurity. Even the locality of the latex in the interior of the cell is not yet known with sufficient accuracy. This is conceivable because latex is to be found in long tubes or vessels, which will always be opened when the preparation is made. This can be no objection in fixed preparations, but when carrying on an investigation on the living plant, it will be almost impossible to prevent the injury of the cell, so that no safe deductions can be drawn about the cell contents.

The investigations of MOLISCH [1]), however, have made it probable that the chief elements of the latex are derived from the cellsap, that the solid particles are situated in the interior of the vacuole. MOLISCH came to this conclusion after he found vacuoles in the slime extruding from cut up leaves of Musa, and partly also after he examined sections of stems of Euphorbia and other laticiferous plants in which cases, however, the latex-tubes were hurt. This last circumstance makes his results rather doubtful though it must be conceded that the representation of MOLISCH looks very probable. Also an investigation of the extruding latex gives rise to objections because it may change during the extrusion.

Now, a short time ago, W. BOBILIOFF [2]) could show that in intact laticiferous tubes which he succeeded in isolating and cultivating separately, small particles of the latex are indeed to be found in the cellsap. Yet, it is perhaps of some interest to mention here a few of my own observations which were made three years ago during a stay of a month at the Raleighfalls in the interior of Surinam. The objects of these investigations were several species of Podostemonaceae.

When great plants of Mourera fluviatilis are cut, a white milky juice comes forth which has the same appearance as any other latex; with the smaller forms there can generally be no question of such an extrusion. Yet, similar substances may also be found there as I shall show in the next pages. The small particles of this sap are soluble in alcohol, so the

[1]) H. MOLISCH: Ueber Zellkerne besonderer Art. Botanische Zeitung. LVII. 1899, p. 177.
,, : Studien über den Milchsaft und Schleimsaft der Pflanzen. Jena 1901.

[2]) W. BOBILIOFF. Waarnemingen van melksapvaten in levenden toestand. Archief voor de Rubbercultuur in Nederlandsch-Indië. Mededeelingen uit het physiologie-fonds 9. 1925, p. 313.

investigator, who works with alcoholmaterial in Europe does not see much of the presence of this latex.

Yet, several former investigators have already pointed out that secretory tubes are present. First of all GOEBEL [1]) who describes that in cutting the leaves of living plants of Rhyncholacis macrocarpa „ein gelbes Sekret in ziemlicher Menge ausfliesst". Afterwards WÄCHTER [2]) has given a description of secretory channels in Weddelina squamulosa and a few years later we find some short remarks on this subject made by MILBREAD [3]) on several species from different genera of this family. A detailed description has been given by MATTHIESSEN [4]). The only trouble is that no investigator, except GOEBEL, has observed the living plants. Therefore, it may have some sense when I mention what I have observed in the living plants. Of course I made some complementary investigations on material which after fixation I brought with me to Europe.

I already made the remark that with the larger forms the outflow of the latex may be easily seen after making an incision, but that this is not the case with the smaller species. Notwithstanding this, the secretory cells or channels are conspicuous enough even there, because they strongly reflect the light, so that they may easily be seen as bright white spots or streaks on a dark lining ; even a microscope is not always necessary although it makes them more distinct.

I have already mentioned that the substance which reflects the light so strongly is soluble in alcohol. For this reason a search was made among the reagentia which I had taken along with me in order to know whether some of these would leave the secretory products untouched and yet might be used as a conserving fluid. It became evident that a strong solution of corrosive sublimate may be used as such and accordingly several of these plants were taken with me in this conserving fluid in order to investigate them more closely here in Utrecht.

This investigation made it clear that these secretory cells are present in almost every part of all the species of Podostemonaceae which were examined. This is best shown by the following list :

1. Mourera fluviatilis. In the so-called "gills" secretory cells are found which are not differentiated from other cells by their form. They are never found in the epidermis, but lie subepidermal. In other parts of the leaf these cells are generally somewhat stretched in the direction of the longer axis of the leaf, in the stem they become long laticiferous channels of which the description though was already given by MATTHIESSEN ;

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1) K. GOEBEL. Pflanzenbiologische Schilderungen II. Marburg. 1893. p. 346.

2) W. WÄCHTER. Beiträge zur Kenntniss einiger Wasserpflanzen. Flora Bd. 83 1897. II. Weddelina squamulosa Tul. p. 382.

3) J. MILBREAD. Beitr. z. Kenntn. der Podostemonaceen. Inaug. Diss. Berlin 1904.

4) FRANZ MATTHIESSEN. Beiträge zur Kenntnis der Podostemaceen. Bibliotheca botanica Heft 68. Stuttgart 1908.

here they are found more especially in the neighbourhood of the vascular bundles. In the flowers the secretory cells are found more especially in the spathella, the stamens and the wall of the ovary, whereas the ovules are free from them. In the haptera secretory cells are also to be found.

2. Oenone Staheliana. (This is a new species of which the description will shortly be published by me.)

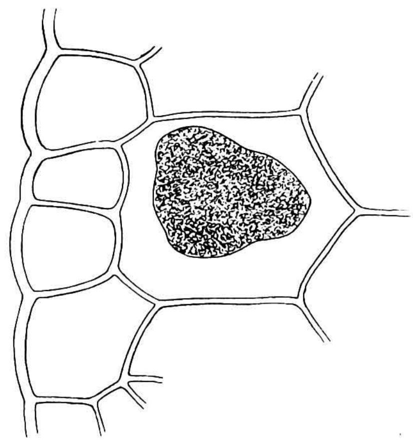

Fig. 1. Young leaf of Oenone Staheliana with subepidermal secretory cell in glycerine. Magn. 720 ×.

Here the secretory cells in the "gills" are mostly subepidermal as will be seen from figure 1, but in the leaf they may be found in other spots. In the stems the subepidermal position is very striking but besides they also accompany the vascular bundles. These are surrounded by starch-containing parenchyma and on the outer border of this tissue a certain number of long laticiferous tubes may be found.

3. Oenone Richardiana. About this species almost the same can be said as about the former one. In the "gills" a great many secretory cells are to be found, especially in the layer of cells bordering the epidermis, but also in other parts of the leaf and in the stem. In the flower they are present not only in the spathella and in the filaments but also in the wall of the ovary and in the stigmata.

4. Apinagia perpusilla. The same may be said about this species as about the last one. Especially in the leaves the secretory product is easily to be detected. One often gets the impression of it being some thick sticky liquid; so that, when cutting, it is often spread over the sections with the razor.

5. Tristicha hypnoides. Here the secretory cells are found more particularly near the margin of the leaf, as may be seen in figure 2. The small teeth of this margin consist of cells without chlorophyll, everyone of them containing a deposit of silica. These are bordered very often by secretory cells, which also contain chlorophyll; sometimes these are lying

in a single row, or this row is broken by ordinary cells as in our figure. A row of secretory cells is also present on both sides of the midrib.

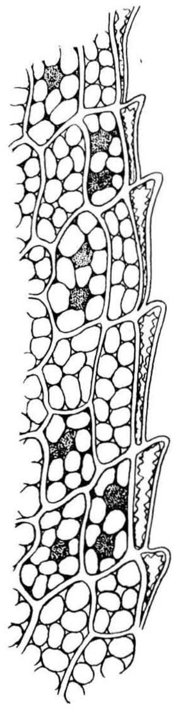

Fig. 2. Margin of a leaf of Tristicha hypnoides. In the teeth of the margin silica deposits; all the other cells have chloroplasts, but some have got besides these also the secretory product, which here has a brown colour. Magn. 1080 ×.

A more detailed study shows that in these cases we always have to do with living cells, also the long secretory channels are formed through the stretching of one single cell.

These cells may be stained red with borax carmine, brown with Iodine-solution. They show a positive Millon's reaction, and also a biuret-and a xanthoproteic reaction; consequently proteins appear to be present. With fixed preparations it can easily be shown by means of stains, e.g. heamatoxylin, that a number of nuclei are present as may be seen in figure 3, which refers to Oenone Richardiana. Hence in this respect those

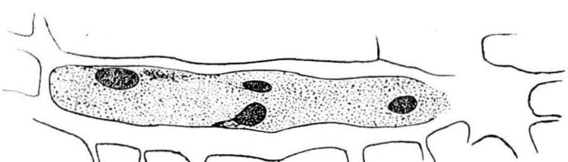

Fig. 3. Secretory channel in a stem of Oenone Richardiana with many nuclei. Fixation with Juel, staining with haematoxylin. Magn. 725 ×.

laticiferous tubes behave in the same way as other ones which have been investigated. This behaviour was first described by MELCHIOR TREUB.

The secretory product is insoluble in water, but soluble in alcohol, chloroform, acetone, etc.; it may be coloured with alkanetpigment and with Sudan III. Consequently it was supposed that we have to do with some fatty or resiniferous substance. The green colour acquired a few days after treatment with copper acetate, makes it probable that it is some kind of a resin. But on account of our being so very ignorant about these plant products, it may be of some use to mention here the reactions which these resins did show after treatment with those reagents which were at our disposal in the jungle. The secretory product is soluble in glacial acetic acid, also after some time in concentrated sulfuric acid and in a 33 % solution of chromic acid; also caustic soda solution and ammonia act as a solvent. In a 60 % solution of chloral hydrate no real solution takes place, only the oily drops become more transparent. In preparations which had long been kept in glycerine, oily drops were found with a great number of cristalline needles showing double refraction. The sap extruding from plants of Mourera did distinguish itself by a strong resinous flavour.

Lastly, I must draw attention to the curious hairs which are to be found on the surface of the stems and leaves of Mourera; these hairs sometimes contain little drops in their cells which give the same reactions and generally look like the contents of the resiniferous cells. I could not say for certain whether these substances really are identical, more especially because they ought to be studied in the living state, which study can only be carried on in the jungle.

Now, concerning the principal question it can be said that a study of the living cells as well with Mourera as with the two species of Oenone, with Apinagia and Tristicha has yielded the same results. More especially the small cells of the "gills" or warts were investigated, because these parts could easily be brought under the microscope without much dissection.

Figure 4 represents such a secretory cell; a parietal layer of proto-

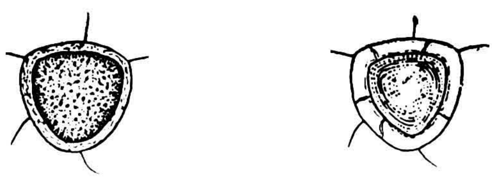

Fig. 4. Cell out of a young leaf of Oenone Staheliana; on the left hand the parietal layer of protoplasm is to be seen and the central vacuole with little particles or drops. On the right hand this same cell is plasmolysed in glycerin; the contents of the vacuole look like an oily fluid. Magn. 200 ×.

plasm may be seen and a mass of the secretory product in the centre of the cell. After treatment with glycerine plasmolysis did occur, as may be seen in the rigth hand part of the figure. The protoplasm has now contracted but the peripheral part is still to be seen, partly as thin threads. The secretory product has the appearance of a transparent oily mass.

This phenomenon which is very often observed in plasmolysis cannot be the result of glycerine entering the vacuole ; for when accidentally a cell in a preparation is opened so that the secretory masses lie free, these show no alteration on the addition of glycerin. Generally, the conclusion could be drawn from these and other similar plasmolytic experiments that the secretory masses are lying in the cellsap ; they are small drops, which can flow together and combine when they are drawn together.

In order to get a better insight into these phenomena, very young stages were investigated mostly in the spathella or in the "gills", so that as little handling as possible was necessary. In all cases, whether I used Mourera fluviatilis, Oenone Staheliana, Oenone Richardiana or Tristicha hypnoides, the result was always the same.

The first indication that one has to do with a secretory cell is the appearance of a few very small particles or little drops in the cellsap, which show a lively Brownean movement. Gradually, the number of these particles increases and at the same time their movements decrease till at last they stop entirely ; it looks as if these small masses gradually flow together.

In figure 5 some stages of this development are shown in cells of

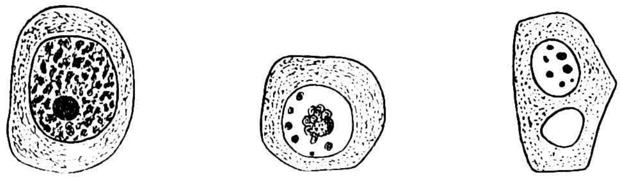

Fig. 5. Young cells from the warts of Mourera fluviatilis. On the right-hand side with two vacuoles, one without and the other with the beginning of the accumulation of secretory product ; in the middle cell somewhat more of this substance has been formed, on the left hand a somewhat older stage. Brownean movement visible in every cell. Magn. about 400 ×.

Mourera fluviatilis. The figure on the right-hand side shows a very young stage, where two vacuoles may be seen in one cell ; only in one of these a few particles of secretory product can be seen, the other one has only got watery contents. The middle figure shows a somewhat older stage, in which the Brownean movement was well visible. The same may be said of the left-hand figure which represents a somewhat older cell.

It was already said that in plasmolysing the small drops flow together. Very rarely the impression was given as if a new precipitate would arise during plasmolysis ; but I rather think that this is generally not the case. It is much more probable that these secretory products take their origin in the cytoplasm and that afterwards they have to pass the tonoplast. When explanations are sought for the semipermeability of the plasmamembrane and more especially of the vacuolar membrane it will always be necessary to account for the fact that oily or resinous drops can pass these membranes.

The question might be put whether it is permitted to call the mass found in the secretory cells of the Podostemonaceae latex. Perhaps we would not do this so easily if no milky juice flowed out of the larger forms after some wound has been made. I agree at once that this fluid is not in every respect comparable with the latex of Hevea, but the question might be put if this same remark would not hold true for other milky fluids? Has not every latex some peculiarity of its own not to be found in that of other plants? A short time ago ULTEE [1]) has given a summary of our knowledge about the composition of latices. In accordance with his view I should like to retain the general name of latex as long as we are so extremely ignorant about the part which latex plays in the life-history of the plants; this name originated from the general custom of the language and it tells nothing about its significance. Then also the milky juice of the Podostemonaceae cannot be left out of consideration when we deal with latex. Consequently, in generalizing it may be said that the particles of the latex are lying in the cellsap, now that this has been made probable or proved for the laticiferous tubes or vessels of the Musaceae, of Euphorbia, Ficus, Carica and the Podostemonaceae. This does not exclude that in the outflow of latex there will certainly also be some protoplasm which extrudes and mixes with the cellsap.

At all events the position of the little drops or small particles in the interior of the cellsap makes it extremely improbable that these would afterwards again play a part in the chemism of the plant so that we are justified in considering them as excretory products.

Utrecht, February 1926.

Botanical Laboratory.

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[1]) A. J. ULTÉE: Melksappen. Pharmaceutisch Tijdschrift voor Nederlandsch-Indië. 2e Jaargang N^{0}. 12 1925 p. 515.