COLOUR-REACTIONS OF LATEX AS A MARK OF IDENTIFICATION OF HEVEA CLONES*

THE presence of oxydases in the latex of *Hevea brasiliensis* is a well-known phenomenon. The differences in the discoloration of crepe-rubber, prepared of latex from individual trees, show, that the amount and also the quality of the enzymes in the latex from different trees varies. The crepe-rubber of the latex from some trees or clones may show a strong spontaneous discoloration, whilst the rubber from others will not, or only slightly discolour. Not only the intensity of the discoloration but also the nuance of the colour varies strongly.

The results of our first investigation on oxydases were published in 1924; in that article we treated the influence of various factors on the action of these enzymes. Attention was paid to the influence of various chemical compositions on the oxydases, and specially to the effect of calcium and magnesium salts on the activity of these enzymes. After addition of such salts, and more specially of chloride of calcium, nitrate of calcium and monophosphate of calcium, a striking strengthening of the activity of the enzymes was observed, which appears by a rapid and intensive discoloration of the latex. These salts, therefore, may be considered to be inorganic activators. The change in the colours of the latex, after the addition of calcium salts, differs for different trees, as well as to the intensity as to the nuances of the discoloration.

The manner in which the latex from the bark of different trees reacts upon the addition of calcium salts, may be elucidated by the following quotation:

"Latex from 21 different trees was collected separately. Nine of these show a spontaneous discoloration; and the latex thus contained a great amount of enzymes. However, the latex from the other trees did not discolour, but after addition of calcium salts the discoloration of the nine first mentioned latices was observed in the course of 5 to 25 minutes; further with latices which were poor in enzymes, it lasted much longer, before discoloration appears. With one tree 1½ hour; with two other trees 2 hours; with another tree 3½ hours; and with the remaining trees it lasted much longer; but on the following day all latices were more or less discolored."

From this it appears, that it lasts in most cases rather long, and even after addition of activators, before the enzymes in the latex from the bark grow active. The deportment of the latex in young organs (fruits, flowers, and young leaves) is quite different; almost immediately after being exposed to the air, and specially after addition of chloride of calcium, the latex begins to discolour. The intensity and the nuance of the discoloration varies also with such latex. In some cases an orange colour appears at first, which changes into black or bluish; and in other cases the orange or orange-red discoloration is very weak and is rapidly superseded by black or dark-blue.

The occurrence of intensive discoloration in latex from young organs is considered to be a general phenomenon. We wish, however, to point out, that such is not always the case, and that trees exist, which show none or almost no discoloration of the latex from young organs. This fact

* By W. Bobilioff in *Archief voor de Rubbercultuur* Vol. 15, No. 7, July, 1931.

appears to be of a certain interest from a general physiological point of view, since the presence of "oxydase-less" latex shows that these enzymes are not indispensable for the various processes in the latex vessels.

An example of the absence of oxydases, the trees CRS 45 and 6 (in the garden of the station) can be mentioned; the latex of the young organs of these trees does not discolour at all or very slightly. In the same garden also the trees 26, 60, and 75 are growing and they all react very intensively, so that the mentioned difference cannot be explained by variations of the soil.

The investigations on the occurrence of oxydases in the latex have in the last time been extended to a number of clones; we came to the conclusion that the enzymeous colour-reaction can be used as a mark of identification for the clones; the reaction is useful for the recognition of clones and for the examination of the purity of clones, either as an independent characteristic or as a supplement to the determination on habitus. The first mentioned application refers to such cases, where a determination on habitus is for some reason impossible or very difficult.

Characteristics of the Reactions.—The reaction is accomplished by adding some drops of and 1% solution of chloride of calcium to a few drops of latex. The latex is withdrawn from the stems of young leaves; these are cut with a sharp knife on the connection place; on the cutting plane drops of latex appear, which are collected in the hollows of a white colour tray made of porcelain.

It would be desirable always to excuse the reaction under the same conditions and thus always to add an accurately measured quantity of chloride of calcium to a certain quantity of latex. These simple requirements however cannot be satisfied, as the quality of latex obtained generally is very small and therefore is difficult to measure.

After the addition of chloride of calcium to the latex the first discolorations appear in a short time. These show a different intensity and all sorts of nuances, such as orange-yellow, light-violet, and red.

After a certain time, sometimes only a few seconds, other colours begin to appear, viz. violet, blue and now and then a greenish colour. The differences in intensity between these second colours are greater than between the first appearing colours; the discoloration becomes darker, until black, blue-dark and grey-black, which colours characterize the final phase of the reaction.

When executing the reaction the following points have to be taken into consideration:

1. The *intensity* of the reaction and, as a part of it, the intensity of each separate discolouration.

2. The *time*, which elapses between the adding of the $CaCl_2$ and the *beginning of the reaction* and the epoch, in which the first colour (orange, orange-red, red, yellow, violet) changes into the second (violet, blue, brown, grey).

3. The *colour-nuance* of the reaction as a whole and of the different phases.—It is possible to speak about a red, an orange-red, a blue or a violet reaction, and also about a colourless one, which possesses a very weak reaction, appearing as a light greyish tinge after a long time.

4. *The colour of the serum.*—Till now such changes are mentioned, which occur in the flocculations. The serum also generally shows a colour-reaction, which however, can differ rather much from the reaction of the coagulum; sometimes the serum remains almost colourless, whilst the reaction as a whole is rather strong.

5. *Particularities.*—The structure of the latex after the addition of chloride of calcium can sometimes be used as a valuable mark of identification. After addition of chloride of calcium to the latex coagulation and curdling processes occur, which give a special character to the reaction; sometimes small coagula arise, which show a more intensive reaction than the environment. In that manner strongly coloured tips, small staves, and small islands are forming.

It is self evident that all the points mentioned above have to be taken into consideration when a certain latex has to be distinguished. Sometimes the differences are such, that there can be no doubt about the distinction; in other cases, however, the differences are not so clear, so that the subjective element will take up a large room in the appreciation.

The Influence of Various Factors on the Reaction.—In a biochemical reaction the external circumstances play a leading part. When executing the colour-reaction of the latex, it is not however possible to act under constant conditions.

As mentioned above, the quantity of latex, with which the reaction is executed, varies strongly. Moreover, it has to be taken into consideration, that the reaction progresses rather quickly and it is necessary to work as promptly as possible when executing the reaction on a number of trees, which have to be compared simultaneously with each other. The temperature and the exposure are not the same in the various moments of the reaction; and they exercise their influence on it. It is advisable to take certain precautionary measures and to proceed in the following manner, in order to obtain the best results with the reaction:

1. Only robust and healthy looking leaves have to be used when collecting the drops of latex.

2. It is preferable to cut the leaves from the intact tree and not to take them from cut suckers. This is easily done with young trees, but with older trees (e.g., from clone testing gardens of some years age) the suckers, which mainly occur at the top of the trees, have to be cut at first. It is undesirable to let the suckers lie for a long time, as the flow of the latex then strongly falls off.

3. The best time to execute the reaction is between 8 and 11 o'clock in the morning. If the reaction is performed earlier, the chances are that the latex drops will soon coagulate, resultant on the low temperature and mixing with dew-water. After 11 o'clock the temperature is generally too high and the reaction therefore runs irregularly.

4. The precaution has to be taken, that the reaction always is executed in the shade and never in the clear sun.

Reaction of Latex, Originating from Different Parts of the Tree.—The first investigations on oxydases were performed with latex from the bark. The reaction with such latex runs very slowly, even after addition of activators. As contrasted with this the latex from the fruit-wall gives in most cases an intensive discoloration, as soon as it is exposed to the air; when adding chloride of calcium the reaction, which is moreover extremely intensive, is still accelerated. The latex from the bark and from the fruit-wall of the same tree are two extreme cases and form a good example of a very slow and a very fast reaction; for our purpose these organs therefore are of little use. On closer investigation of the reaction of the latex from leaves in various stadia of development, it appeared that leaves of a certain stadium of development furnished the most adapted latex.

In the development of the leaves the following stadia can be distinguished:

1. Very young leaves, dark-brown, turned stiffly down below.
2. Very young leaves, brown, turned down below.
3. Young leaves, yellow-green (bronze-green) still limp.
4. Young leaves, light-green, still limp.
5. Half grown leaves, light-green, not limp.
6. Full grown leaves, dark-green, stiff.

The reaction of the latex, originating from leaves in the stadia 1, 2, and 3, is a little weaker compared with that from the leaves in the stadia 4 and 5, whilst full grown leaves (stadium 6) show a weaker and different reaction. Therefore, the leaves in the stadia 4 and 5 are used for the reaction; only if such are missing one has to shift with leaves in the stadia 1 to 3. Full grown leaves however are not usable for the reaction.

The reaction of the latex form different parts of a budding. The budding was about 5 metres high and had already formed 9 stories of leaves; during the investigation all the leaves of the 4 highest stories were still prresent. The reactions in these stories were as follows:

1st story from top, leaves in the fourth stadium; young, light green still limp leaves, typical reaction of clone AV 49.

2nd story from top, leaves in the fifth stadium; half grown, light-green, not limp leaves, reaction a little weaker than in the topmost story, yet still typical for AV49.

3rd story from top, full grown leaves (sixth stadium), no or weak reaction.

4th story from top, full grown leaves (sixth stadium), no or weak reaction.

From this it is evident, that full grown leaves are not usable for the reaction, whilst leaves in the fourth or fifth stadium are suitable for examination.

Besides, the reaction of the latex from the bark was traced, and this may be important, as we originally meant, that it should be possible to determine by means of the reaction to which clone budwood belonged. In the bark of the young budding 5 different stadia could be distinguished according to the colour of the stem, thus to the forming of cork.

The first (lowest) part of the stem is all corky; the latex is reactionless.

The second part of the stem has a light-brown, here and there still a somewhat green bark; forming of cork not complete; no reaction.

The third part of the stem is predominately green; weak reaction, generally irregular.

The fourth part of the stem is all green (not corky); reaction rather intensive, yet weaker than in the latex from young leaves.

The fifth part of the stem has a light-green bark; reaction of the latex equal to that from young leaves.

It appears from this, that the reaction restricts itself to the young and not yet full grown part of the branches, and that as soon as these have turned corky, the intensity decreases strongly.

Owing to this, determination of budwood by means of the reaction is not possible.

In order to obtain a clear reaction it is therefore necessary to have young leaves at one's disposal, which hinders the investigation somewhat, as such leaves are not always present.

The age of the tree, from which the leaves originate, is without importance, save one case, (namely the first story of a budding). The latex from a budding one year old, gives, e.g., the same reaction as the latex from a tree of four years. We will demonstrate this by means of two examples only:

AV 50-budding, about 5 years old, from a test-garden (Tjiomas). Marks of reaction: intensity very strong, beginning after $\frac{1}{2}$ minute, finished after $15\frac{1}{4}$ minutes, first discoloration orange to orange-red.

AV 50-budding, about 1 year old, from multiplication-garden (Tjiomas). Marks of reaction: intensity very strong, beginning after $\frac{1}{2}$ minute, finished after 15 minutes, first discoloration orange to orange-red.

Tjir 1-budding about 4 years old, from a test-garden (Tjiomas). Marks of reaction: intensity strong, beginning after $\frac{3}{4}$ minute, finished after $6\frac{1}{4}$ minutes, first discoloration red-orange with violet tinge.

Tjir 1-budding, about 1 year old, from multiplication-garden (Tjiomas), Marks of reaction: intensity strong, beginning after $\frac{3}{4}$ minute, finished after 5 minutes, first discoloration red-orange with violet tinge.

It is evident from the above mentioned examples that the reaction is the same in old and young buddings of one clone.

An exception is formed by the first story of young buddings. This gives sometimes no reaction, and sometimes a weak and irregular one. The investigation has shown that the reaction of the first story is not fit for the determination of buddings.

The preliminary result of the investigation is, that only young, preferably half-grown leaves are fit for the determination, and that the reaction is not dependent on the age of the tree, from which the leaves originate, except those from the first story of buddings, and finally that latex from bark, that is already corky, is not suitable for the reaction. Therefore, testing budwood by means of the reaction is not possible. Efforts to find other activators, which would accelerate the action of the latex from the bark, have failed till now.

SUMMARY

1. The enzymes present in the latex of Hevea, are activated by salts of calcium and magnesium. The best results were obtained with and 1% solution of chloride of calcium. The activity of the enzymes utters itself as a discoloration of the latex and of the serum.

2. The discoloration, resultant on the mentioned reaction, is an individual property of the trees.

3. The discoloration can be used as a mark of testing clones on their purity.

4. In one clone the reaction is fairly constant, small variations, however, may occur.

5. When executing the reaction, it is advisable always to work under the same external conditions.

6. The reaction is made on a few drops of latex from the connecting place of the leaves.

7. Only latex from young leaves will give a typical reaction. The same reaction, however, is obtained with latex from green, still young bark. Latex from bark, already corky, is not usable for the reaction.

8. A description of the reaction of various clones is made. Composing a determination-table is however impossible, since the appreciation of the colours is somewhat subjective, by what such a table would possess only a relative value.

JELUTONG*

THE production of jelutong in Malaya and Borneo continues to increase the bulk of the output being taken by the U.S.A., where it is used as a substitute for chicle in the manufacture of chewing-gum. Chicle, the coagulated latex of the Sapodilla (*Achras sapota* L.) derived principally from Central America, commands a higher price than jelutong. The high relative cost of collection of chicle, combined with the fact that supplies are rapidly diminishing as a result of destructive tapping methods, renders it probable that the demand for jelutong will continue to increase. Borneo jelutong is derived from *Dyera Lowii* Hk.f., a tree confined to the swamps. In Malaya the product is derived from *Dyera costulata* Hk., f. and *Dyera laxiflora* Hk. f., two species occurring chiefly on flat land and low hills at altitudes below 1,500 feet. There does not appear to be any intrinsic difference in the jelutong of these three species though the Malayan product, owing to better methods of preparation, is generally preferred and commands an enhanced price. It has been found from experimental work in Malaya that the exercise of certain precautions in coagulation and in the agents used as coagulants results in a markedly superior product. Iron, from the use of rusty tins, is now known to cause oxidation and subsequent deterioration of the finished product. Consequently attempts are being made to induce collectors to refrain from using as coagulating vessels, iron receptacles or wooden boxes caulked with clay, which also contaminate the product.

It is estimated that in America, where the consumption of chewing-gum is steadily increasing, the markets are capable of absorbing about 5,000 tons of jelutong annually. The use of chewing-gum is stated to be on the increase in parts of Europe and also in the north of England among factory hands.

---

* From *Bull. of Mis. Inf.*, No. 10, 1930.