A new method for inducing "bud mutation" by colchicine injection.

(En ny metod för framställminjau "knoppmutation" genom colchicininjektion.)
av Holger Jensen (Ramlösa Nursery at Helsingborg); *Lustjärden* 1941: 27-34.

Translated by Lennarth Johnson

In early 1938 the first information from the USA about Blakelee and Avery's epochal development of doubled chromosome numbers with colchicine came to Europe. Here, there and everywhere one began making similar trials following the methods described in the Journal of Heredity, December 1937. Among these methods mentioned was the treatment of buds with colchicine-agar solution. This somewhat gelatinized liquid was fixed on the auxiliary buds and these buds successively absorbed the poison more or less. The consequence was that the new shoots showed stronger or weaker effects of the poison with the typical colchicine reactions, such as abnormal shapes of or crinkling of leaves, almost cessation of growth, etc. However, this method proved to have some disadvantages in my tests. The liquid dried out so rapidly that it became necessary to add drops several times a day to keep the buds constantly wet. In addition a proper dose was difficult to obtain because buds of different species and of different stages of growth had very variable capabilities for absorbing the solution. It often occurred that some buds were killed by too high a concentration being absorbed as well as other buds equally treated not being affected at all.

In the spring 1939 I modified the method so that small pockets or containers were fastened to the shoot below the buds being treated. The colchicine solution was then poured in the pocket so that all the buds were affected constantly by the colchicine liquid. It was necessary to repeat this twice a day to replace the quantity being absorbed by the bud or evaporated. This treatment was an improvement over the former but still many buds were killed while others showed too weak a reaction, that is, those which at first showed the symptoms resumed normal growth.

Of the rather large number of plants treated in the spring 1939 (about 60 different kinds with 3-5 treated buds each) some successful results were obtained such as the buds producing permanent "gigas"—shoots. However, we also observed that some untreated buds, close to those treated with concentrated solution, proved typically affected by colchicine. In these cases obviously the absorbed poison had been transported by the sap and internally affected the apical cells of close by buds. This fact gave me spontaneously the idea of the injection method that, in the spring and summer 1940, was practiced in large scale and, in many cases, successfully. As this method has also been taken up by interested visitors, I believe it is worth a description.

My idea was to supply the sap of a bough with a constant and continuous dose of colchicine. For this purpose I used small glass pipes with conically drawn-out lips (Fig. 1) through which the sap could be supplied with the colchicine solution.

For thick branches, 7-15mm (0.3-0.6″) across, a pipe with an inclined lip is used. With a 1 1/2-2mm (0.06-0.08″) drill, a hole is made on a slant through the branch from above downwards. The lip of the glass pipe is put into the upper opening of the hole and is firmly fixed. Both above and below the drilled hole the bark is polished for a total length of 2 1/2-3cm (1″ or somewhat more). Irregularities like warts, bark-chips, etc. are carefully cut off with a sharp knife. Then the polished part is covered by *collodium elasticum* [collodion—nitrocellulose, ether and 100% alcohol] in order that all small irregularities and cracks will be sealed. At the lower opening of the hole (Fig. 2, Left) a V-formed cut is made into the cambium. Around the branch, the lowest part of the lip, and the opposite V-cut, a piece of leucoplast [Parafilm per August Kehr] is firmly fixed (Fig. 2, Right). But the pocket of the V-cut must not be filled. Finally the bandage of leucoplast is waterproofed by another covering of *collodium elasticum*. The aim is thus to obtain a waterproof container with freshly cut surfaces through the wood from bark to bark including the cambium. Now you inject the colchicine solution in the glass pipe, and the pocket is thus filled completely by the liquid that upon being absorbed is continuously replaced by the stored quantity in the glass pipe.

When treating thinner branches only 3-6mm (0.1-0.2″) across, this method must be modified because a drilled hole might weaken and injure the branch too much. In the latter case a part of the bark is also polished as described above but the V-cut is made differently. The lower cut is made perpendicularly to about one-third of the branch diameter (Fig. 3, Compare with chip-budding). The upper cut is similarly made so that the cut surface in proportion to the diameter of the branch will be 1/2-1 1/2cm (1/5-3/5″) long. On the bark immediately above the upper cut a ball of waterproof putty is fixed so that it seals the space between the bark and the glass pipe when the lip reaches the lower cut (Fig. 3, Center). The puttyball will thus hold the pipe to the branch and fill the space between the branch, the glass pipe and the bandage of leucoplast. Pay attention that the putty is not pressed down over any cut surface and stop the pores. The fixed glass pipe and the branch are then finally wrapped with a piece of leucoplast (Fig. 3, Right) and all is covered by *collodium elasticum* analogous to above. If the bandage is carefully made without any leakage and no evaporation takes place—this easily made after some practice—the solution in the glass pipe will remain constant and can only be absorbed by the xylem, cambium and phloem. The glass pipe is kept filled during the treatment—refilling is easiest done by a pipette.

Species have highly varied capability of absorbing the colchicine solution. In many cases the absorption is high and is kept constant for a long time. In other cases the absorption is considerably slower and can completely cease after a couple of days, probably due to dead cells in the cut surface and choked-up pores. This is why it is impossible to give any general rules for the adequate time of treatment and the concentration of the colchicine solution because of the different reactions among species to this treatment. Doses too strong for one species prove to be without any effect with others. So you have to find the proper concentration for each species.

We have managed by this method to induce polyploidy by using colchicine concentrations from 0.1% to 1% over a period of 2 to 14 days and even longer! In the spring and summer 1940 we injected about 125 different plants, mainly trees and shrubs of importance for forestry

Figure 1—Injection pipes of suitable forms, the bigger ones of an internal diameter about 7 mm (about 1/4") and the smaller ones of about 4.5 mm (1/8").

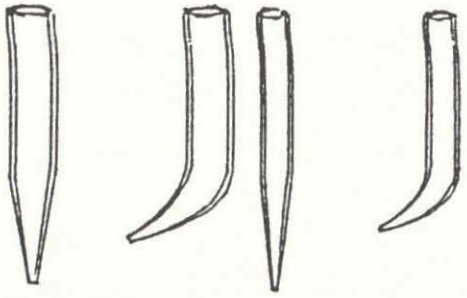


Figure 2—Left: A larger branch with a cut pocket and with dashed lines marking the drill hole through the branch. Right: The same branch with a fixed pipe and the leucoplast bandage.

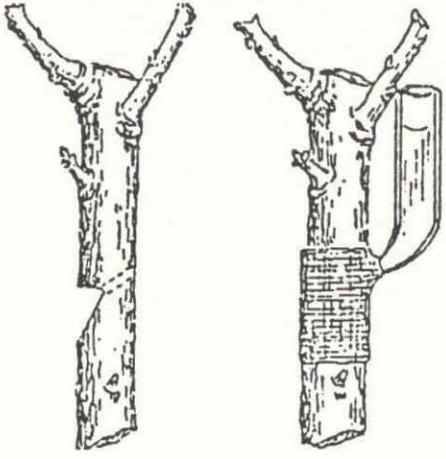
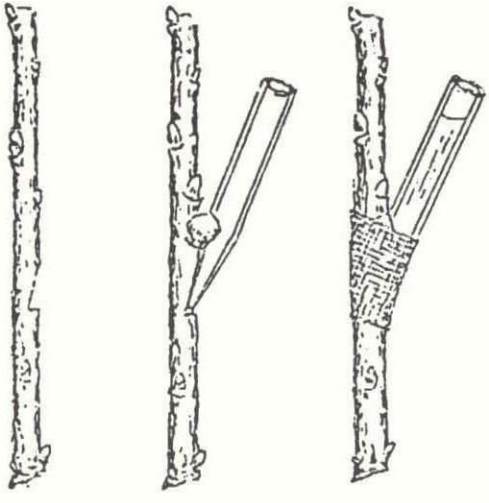


Figure 3—Left: A thinner branch with the cut but no drill hole. Center: The glass pipe fixed at the branch by putty. Right: The leucoplast bandage in place.



and gardening as well as some herbs. The results induced about 50 plants with buds having putatively doubled chromosome numbers. In some cases the induction seems to have given uniform cells with doubled chromosome numbers. In others it is obvious that affected cells are mixed with unaffected ones. These latter mixtures of all types are thus so called mosaic or chimera types and it is necessary to secure independent individuals from the buds putatively induced by vegetative propagation, that is, chip-budding.

Finally, something about the recognition of putative polyploidy, which, of course, is important but not always as easy as one may think. An affected plant usually shows more or less increased leaf size and amount of chlorophyll, coarser leaves, thicker branches, etc., but this is not always the case. You have to observe the putative plants throughout the vegetation season both the treatment year and the following year to find any abnormal symptoms on, for example, the leaf margins and stipules, indumenta, any resin-warts and lenticels, etc. First of all it demands a keen observation that can only be obtained by experience. However, you must not limit your observations to shoots close to the injection point as the effect may sometimes be far away. The poison moves unpredictably and is effective where it for some reason attains a proper concentration. We have found cases when putatively affected shoots are more than 30cm (1') from the injection point and sometimes even on another branch. If you wish to determine a positive effect you may study the stoma of the leaf but only a study of the chromosome number can conclusively prove whether the number is doubled.

The above described injection method has been successful for the following families: Coniferae, Salicaceae, Betulaceae, Fagaceae, Ulmaceae, and Oleaceae.