

A. D. J. MEEUSE and E. C. J. OTT: The Occurrence of Chlorophyll in *Nelumbo* seeds (Hugo de Vries Laboratory, University of Amsterdam)

The green colour of the plumule in the seeds of *Nelumbo* has struck several observers in the past, but factual evidence regarding the chemical nature of the green-coloured substance was lacking. Before any physiological, ecological or phylogenetic significance can be attributed to the occurrence of a green pigment in an embryo of an Angiosperm, the chemical composition of the green matter must be established. To this end seeds of *Nelumbo lutea* (Willd.) Pers., kindly supplied by Dr. R. K. Godfrey, University of Florida, Tallahassee, were opened and the dark green plumules taken out. The plumules were ground up with sand and a little CaCO_3 in the smallest possible quantity of solvent (acetone or ether) sufficient to extract the pigments. After decanting, the residue was washed with a small quantity of the solvent and the rinsing fluid was added to the first extract. After centrifugation at about 3000 r.p.m. the supernatant was concentrated *in vacuo* to approximately one-fourth of its original volume. A reference solution was prepared in the same way from dried *Prunus* leaves. Both solutions were tested by means of paper chromatography. The experimental work involved was carried out by the junior author (Ott).

The green solution prepared from the *Nelumbo* plumules showed the typical red fluorescence of chlorophyll in UV light and the presence of chlorophyll was suspected. A comparison of the paper chromatogram obtained from both solutions on the same disc of filter paper showed that the extract of the plumules yields two spots corresponding with those produced by chlorophylls a and b of the reference solution in travelling distance, visual colour and relative intensity. A spot corresponding with that produced by yellow and brown pigments (xanthophyll, etc.) in the reference solution was also present, but carotene could not be detected in the *Nelumbo* chromatogram.

The identity of the green-coloured substances in *Nelumbo* seeds with chlorophyll a and chlorophyll b in the normal 1:4 ratio thus seems to be well established, but the absence of carotene is rather unexpected. The chlorophylls are formed in the seeds of *Nelumbo* in the dark, which is an unusual feature in an Angiosperm. The lack of carotene is another singular phenomenon and it is thought that *Nelumbo* seeds provide interesting material for studies of the formation of chlorophyll and most probably also of the biosynthesis of carotene (which substance is undoubtedly formed in the leaves after the germination of the seeds). The capacity of producing chlorophyll in the dark is not uncommon among Gymnosperms and the occurrence of this mechanism in *Nelumbo* may have some phylogenetic significance. In our opinion, these two reasons are sufficiently important to justify putting our findings on record so as to focus the attention on this remarkable case.