genese durch intensive Akkumulation des Pentahydroxychalkons markiert. Ob das Tetrahydroxy- und das Tetrahydroxy-3-methoxychalkon ähnliche Kinetiken zeigen, ist noch offen.

Mit diesen Untersuchungen konnte nachgewiesen werden, dass bei Synthese mehrerer, im B-Ring verschieden substituierter Flavonole entsprechend substituierte Chalkone in bestimmten Stadien der Organentwicklung akkumuliert werden. Damit kann freilich noch nichts über den biogenetischen Zusammenhang der einzelnen Komponenten ausgesagt werden. Die vollständige Übereinstimmung in der Substitution lässt es jedoch als wahrscheinlich erscheinen, dass bei dem hier untersuchten Objekt das Substitutionsmuster der Flavonole schon auf der Chalkonstufe festgelegt sein dürfte.

Summary. During the development of anthers in *Tulipa* cv. 'Apeldoorn' the following chalcones were isolated: 2′, 3, 4, 4′, 6-pentahydroxychalcone as the main component and 2′, 4, 4′, 6′-tetrahydroxy-3-methoxychalcone as well as 2′, 4, 4′, 6′-tetrahydroxychalcone in a very small amount.

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Studies on Yellow Mosaic Disease of Soybean. I. Effect of Virus Infection on Plant Pigments

The adverse effects of virus infection on the plant pigments have been studied[1–3]. The various types of yellowing and mosaic patterns associated with virus diseases may reflect the extent to which the plant pigments are affected. The effects of yellow mosaic disease on the pigments of soybean are reported in this paper.

The young and matured leaves of soybean showing mild, moderate and severe symptoms of yellow mosaic disease, were collected in the mornings. Comparable controls of healthy leaves were also obtained. The contents of chlorophyll a, b and total chlorophylls were estimated by the method of YOSHIDA et al.[4] and expressed as mg of chlorophyll/g of leaf. The carotenoids were extracted by the method of HARDER et al.[5] and the intensity of the colour was measured at 460 nm. The comparative carotenoids contents of diseased leaves were calculated, taking the content of healthy leaves as 100.00.

The chlorophyllase activity of the healthy and diseased samples was determined by modifying the method of WILLSTÄTER and STOLL[6]. The chlorophyll content of the solution used as substrate was determined by the method of YOSHIDA et al.[4]. After an incubation period of 24 h, the amounts of chlorophyll left in the solution were determined for different samples and the amounts of chlorophyll destroyed were calculated by substraction.

The chlorophyll contents of infected leaves decreased progressively with increasing intensity of symptoms. The reduction in the contents of chlorophyll fractions a, b and total showed similar trends. In the case of matured leaves infected by the virus, the percentage of decrease over healthy progressively increased from 9.69 to 79.66, indicating a heavy reduction in the chlorophyll a contents of severely infected leaves (Table I). The young severely infected leaves also showed such a remarkable reduction of 68.06% over healthy leaves. The effect of virus infection on chlorophyll b is less, as compared to chlorophyll a. Similar progressive reduction of this fraction was also evident as the intensity of the symptoms increased. Maximum reduction in chlorophyll b contents was observed in severely infected young and matured leaves.

The ratio of chlorophyll a to chlorophyll b was altered due to virus infection. Both young and matured leaves

[1] V. S. TRIPPI and J. R. MESIAS, Revta ind. agric. Tucuman. *41*, 29 (1957).
[2] P. NARAYANASAMY and K. RAMAKRISHNAN, Proc. Indian Acad. Sci. B. 62, 130 (1965).
[3] R. JEYARAJAN and K. RAMAKRISHNAN, Madras agric. J. *58*, 217 (1971).
[4] S. YOSHIDA, D. A. FORNO and J. H. COCK, *Laboratory Manual for Physiological Studies of Rice* (International Rice Research Institute, Philippines 1971), p. 61.
[5] D. E. HARDER, J. W. MARTENS and R. I. H. MCKENZIE, Can. J. Bot. *49*, 1783 (1971).
[6] R. M. WILLSTÄTER and A. STOLL, *Investigations on Chlorophyll* (The Science Press Printing Co., Lancaster 1928). p. 385.

Table I. Chlorophyll and carotenoids contents of healthy and diseased soybean leaves

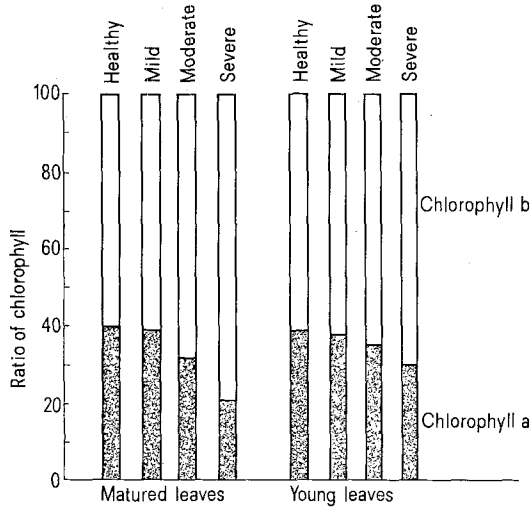
Nature of samples	Chlorophyll fractions (mg/g of leaf)			Carotenoids (parts)
	Chlorophyll a	Chlorophyll b	Total chlorophyll	
Matured leaves				
Healthy	1.332	1.976	3.308	100.00
Mild	1.203 (9.69)[a]	1.887 (4.51)	3.090 (6.29)	84.99 (5.01)
Moderate	0.808 (39.34)	1.690 (14.48)	2.498 (24.49)	81.66 (8.34)
Severe	0.271 (79.66)	1.045 (47.07)	1.316 (60.22)	71.66 (18.34)
Young leaves				
Healthy	1.440	2.221	3.661	100.00
Mild	1.300 (9.72)	2.122 (4.46)	3.422 (6.51)	89.64 (10.36)
Moderate	0.811 (43.69)	1.475 (33.59)	2.286 (37.56)	84.47 (15.53)
Severe	0.460 (68.06)	1.085 (51.15)	1.545 (57.80)	70.68 (29.32)

[a] The percentages of decrease over healthy are given in parentheses.

showed the ratio to be around 40:60. In the infected matured leaves, the ratio was reduced to 38.93:61.07, 32.34:67.66 and 20.51:79.49 respectively in leaves exhibiting mild, moderate and severe symptoms of the disease. Similar changes in the infected young leaves were observable. The ratio of chlorophyll a to chlorophyll b in the young leaves exhibiting mild, moderate and severe symptoms were 37.98:62.02, 35.91:64:09 and 29.77:70.23 respectively (Figure).

Table II. Activity of chlorophyllase in healthy and diseased soybean leaves

Nature of samples	Chlorophyll destroyed (mg)
Matured leaves	
Healthy	0.23035
Mild	0.14160
Moderate	0.09115
Severe	0.07305
Young leaves	
Healthy	0.05035
Mild	0.38150
Moderate	0.33915
Severe	0.14160



The ratio of chlorophyll a to chlorophyll b in healthy and diseased soybean leaves.

The carotenoid contents of both matured and young infected leaves registered a reduction due to infection by the virus. The maximum reduction in the contents of these pigments was observable in severely infected leaves (Table I).

The variations in the chlorophyll contents of infected plants indicated that the chlorophyllase activity may be induced by virus infection. In the case of young leaves, the activity of chlorophyllase was appreciably increased soon after infection. The activity was reduced as the substrate in the leaves, showing moderate and severe symptoms, began to drop rapidly. In the matured leaves also, a similar trend in the activity of chlorophyllase could be observed in the leaves showing different intensity of symptoms, though the activity in the infected tissues was below the level of healthy leaves. It is reasonable to conclude that the appreciable reductions in the different chlorophyll fractions of the infected tissues may be due to the accelerated destruction of the pigments by the chlorophyllase, since this enzyme is known to play a significant role in the destruction of chlorophyll in virus-infected tissues[2,3,7].

Zusammenfassung. Es wird gezeigt, dass die Abnahme des Chlorophyllgehalts in den von der gelben Mosaik-Krankheit befallenen Soyabohnenblättern auf eine Stimulierung der Chlorophyllase-Aktivität im Frühstadium der Erkrankung zurückzuführen ist.

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Coimbatore-3 (India), 23 March 1972.

[7] P. D. Peterson, and H. H. McKinney, Phytopathology *28*, 329 (1938).

Estrogen-Like Substances in Dormant and Cold-Treated Hyacinth bulbs (*Hyacinthus orientalis* L.)

The estrogens have been isolated from many plant tissues[1]. It has also been found that estrogens, in developing neutral, long- and short-day plants, appear at the time of flower bud formation and reach a maximum at the time of their expansion[2–4].

The present experiment concerns the effect of cold-treatment on the occurrence and distribution of estrogen-like substances in different organs of hyacinth bulbs. The bulbs of *Hyacinthus orientalis* L., cv. Delft Blue with circumference of 14 cm, were used as plant material. The level of estrogen-like substances was determined in: 1. stored unrooted, dormant hyacinth bulbs, analyzed in November 1972, 2. bulbs which were potted in October 1972 and then kept in the greenhouse at 25 °C until flowering time and analyzed on January 16, 1973 (these bulbs growing in unnatural conditions showed abnormal development of inflorescence and leaves), 3. bulbs which were potted in October 1972, then treated with low temperature (4 °C) until the end of December 1972; at this date bulbs were transferred to the greenhouse (25 °C) where normal growth took place, and then samples were taken for analysis at the flowering time (January 20, 1973).

Samples of the following bulb organs were analyzed simultaneously: roots, heel, fleshy scales, inflorescence and leaves. Frozen material was homogenized in methanol and the homogenate filtered. The filter pellet was extracted in a Soxhlet apparatus with a benzene methanol mixture 3:1 v/v for 6 h. Methods of extraction and chromatography were the same as described previously[2]. For the quantitative determination of the estrogen-like substances the Kober colour reaction was applied[5] and light absorp-

[1] H. Singh, V. K. Kapoor and A. S. Chawla, J. scient. ind. Res. *28*, 229 (1969).
[2] J. Kopcewicz, Phytochemistry *10*, 1423 (1971).
[3] J. Kopcewicz, New Phytol. *71*, 129 (1972).
[4] J. Kopcewicz, Z. Pflanzenphysiol. *67*, 373 (1972).
[5] W. Nocke, Biochem. J. *78*, 593 (1961).