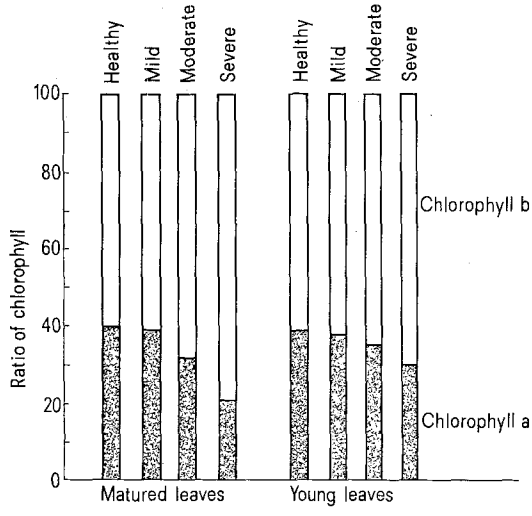
showed the ratio to be around 40:60. In the infected matured leaves, the ratio was reduced to 38.93:61.07, 32.34:67.66 and 20.51:79.49 respectively in leaves exhibiting mild, moderate and severe symptoms of the disease. Similar changes in the infected young leaves were observable. The ratio of chlorophyll 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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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-

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tion was measured in a Specol spectrocolorimeter at 474, 515 and 556 nm, against similarly treated reagent blanks in 10 mm glass cells. The readings were corrected for unspecific background colour by applying Ecorr. = $2E_{515}-/E_{474} + E_{556}/$[5].

The results of analysis (Table) indicated that the smallest content of estrogen-like substances occurs in unrooted dormant hyacinth bulbs, and that they are present only in leaves and inflorescence. In the non-cold-treated bulbs which were planted in soil and had grown all the time in the greenhouse, about 2-fold increase of the content of estrogen-like substances was observed in leaves and inflorescence during flowering time. However, estrogen-like substances were not found in the fleshly scales and roots.

Occurrence and distribution of estrogen-like substances in different organs of cold-treated and non-cold-treated hyacinth bulbs

Type of bulbs	Heel	Scales	Leaves	Inflore-scence	Roots
Dormant bulbs analyzed in November 1972	0	0	23	9	—
Non-cold treated bulbs analyzed at the flowering time (January 16, 1973)	0	0	42	21	0
Cold-treated bulbs analyzed at the flowering time (January 20, 1973)	0	27	62	33	21

Results are expressed in μg equivalent of estrone in 100 g of fresh weight plant material

In the cold-treated hyacinth bulbs analyzed at the flowering time, an increase of the content of estrogen-like substances in leaves and inflorescence was observed. These substances were also found in fleshly scales and roots in cold-treated bulbs.

It can be then summarized that as in the other species so far investigated the estrogen-like substances are present in hyacinth plants. After the cold treatment of hyacinth bulbs – which is necessary for normal growth of inflorescence and leaves – an increase in the level of estrogen-like substances took place. It is known from other data[6] that estrogens can replace a low temperature treatment in the flowering process of *Cychorium intybus*.

Zusammenfassung. Kälteeinwirkung auf Hyazinthenknollen, welche eine normale Entwicklung von Blütenständen verursacht, führt gleichzeitig zu einer allgemeinen Steigerung des Oestrogengehaltes in der Pflanze mit Höchstwerten in den Blättern und Blütenständen.

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Self-Inhibiting Extracellular Proteins from *Aspergillus oryzae*

Earlier we described the occurrence of both self-stimulating and self-inhibiting substances in cultures of filamentous fungi[1-3]. It was shown that the stage of action during the culture development of these organisms is of critical importance. This explains the curious response one very often gets in these cultures by different inoculum sizes. Using low concentration substrates (10 g/l carbon source) filtrates from very young cultures of *A. oryzae* when added at the stage of inoculation to fresh cultures showed inhibiting effects of growth which often had lasting effects up to later stages of growth. The implications in continuous culture operation are obvious. In fact there are great difficulties in maintaining a true equilibrium of mycelium content in homogeneous single stage continuous culture, and the range of dilution rates allowing continuous procedure, albeit with considerable variations in mycelium content, is very narrow.

With high substrate concentrations on media producing a slightly alkaline reaction we could now show that in continuous culture of *A. oryzae* self-inhibiting extracellular compounds occurred (when tested as above), although the concentration of these substances seemed out of phase with the variations of mycelium content. An example of the effect of small amounts of culture

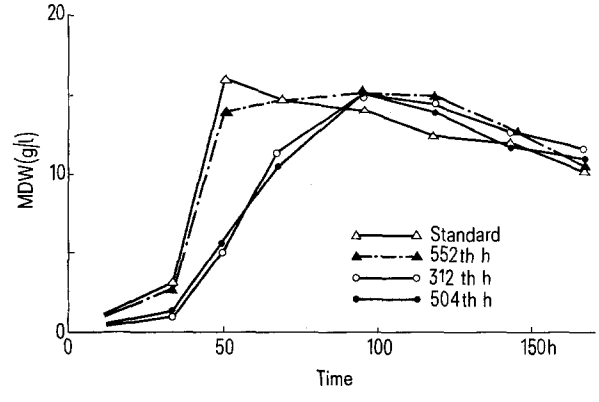

Fig. 1. Effect of culture filtrate (supernatant) from deep continuous culture of *A. oryzae* on fresh stationary cultures of the same organism. Experimental characteristics. Deep culture. Substrate: potato starch, 40 g; $(NH_4)_2SO_4$, 16 g; KH_2PO_4, 4 g; citric acid, 10 g; glacial acetic acid, 6.9 ml; $MgSO_4.7H_2O$, 0.6 g; $FeCl_3.6H_2O$, 0.1 g; $CaCl_2.6H_2O$, 10 mg; $ZnSO_4.7H_2O$, 5 mg; $CuSO_4.5H_2O$, 0.5 mg; $MnSO_4.4H_2O$, 0.5 mg; pH adjusted (NaOH) to 6.8; distilled water up to 1 l. Dilution rate, 0.04 h^{-1}; temperature 30 °C; mycelium separated by centrifuging.
Stationary culture. Substrate: glucose, 40 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4.7H_2O$, 0.3 g; $FeCl_3.6H_2O$, 0.003 g; Na_2HPO_4, 9.46 g; KH_2PO_4, 9.07 g; distilled water up to 1 l. 10 ml portions of sterile medium distributed in 100-ml sterile conical flasks; 1.5 ml conidial suspension in membrane-filtered supernatant (or sterile water as control) from deep culture added to each; inoculum size, 2×10^5 conidia/ml.

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