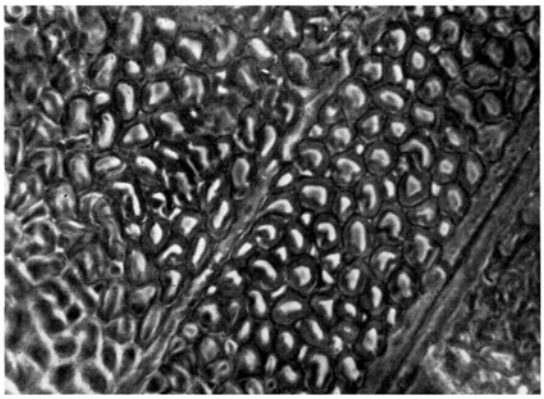

Fig. 1.–Cross-section of ash-tree containing tension wood (300 ×).

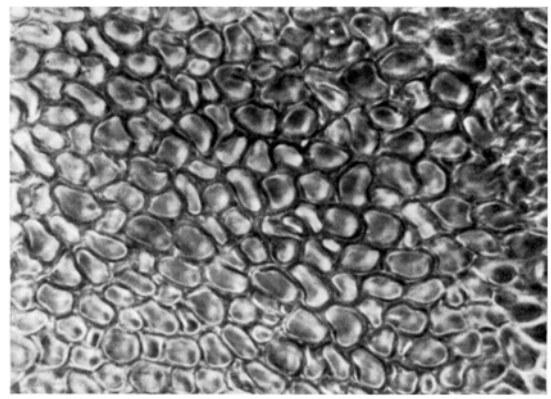

Fig. 2.–Cross-section of ash-tree containing no tension wood (300 ×).

tension wood fibres in the upper side of the branch under investigation. Cross-sections measuring 15 μ were made without pre-boiling the wood, the sections dropped into water. The results of the three staining methods applied to the cross-sections of ash wood were as follows:

(1) *Chlor-zinc-iodine.* There was no trace of violet rings. The fibre walls were stained totally yellow.

(2) *Phloroglucin-hydrochloric acid.* The entire fibre wall is stained light pink. Uncoloured rings are not observed.

(3) *Safranin-fast green.* The secondary layer stains green and the other layers red.

From these staining reactions no reliable conclusions can be drawn as to the occurrence of tension wood in ash.

The methods (1) and (2) show that no "gelatinous" ring is present and that in the cross-sections no fibres of tension wood occur.

The staining method (3) is indicative of a very thick layer of cellulose.

On account of these results, the three staining methods as such may not be used to indicate so-called tension wood. The cell wall of the tension wood fibre is likely to have a particular chemical constitution making it impossible to reveal the ring in a reliable way by means of the three staining methods. Hence, up to the present, no investigator has succeeded in determining tension wood in ash-trees[1]. Tension wood in ash remains invisible if we confine ourselves to the use of staining methods. Consequently we have made an attempt at demonstrating the occurrence of tension wood in ash quite differently, in a physical way. For this purpose we used the phase-contrast microscope.

As starting material we used cross-sections measuring 5 μ as described above and as control test material cross-sections of the wood growing at the lower side of the branch. The latter sections normally have no tension wood.

The cross-sections were examined in a suitable embedding material with the aid of the phase-contrast microscope. Figures 1 and 2 show the results, Figure 1 giving a cross-section containing tension wood. The darker ring is identical with the so-called gelatinous layer having a substance with a refractive index of about 1.536.

The use of the phase-contrast microscope actually enables us to make visible a ring in the tension wood fibre of ash-trees, it is even possible to determine the refractive index of the ring-material. Moreover, it ei obvious that the substance of the ring is not homogen-s ous, revealing a coarse grained structure, which cannot be demonstrated by staining methods or may be taken for an artefact. Figure 2 shows cross-sections containing no tension wood. The gelatinous layer is not present in this case.

The white shadow connected with the cell wall is the "halo", an artefact inherent in phase-contrast microscopy.

Conclusions. It is not possible to demonstrate visually tension wood in ash-trees by staining methods.

With the aid of the phase-contrast microscope it proves possible to reveal tension wood in the fibre wall of ash in a very simple way provided the method mentioned above is taken into account.

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Forest Products Research Institute T.N.O. and Central Laboratory T.N.O., Delft (Netherlands), June 13, 1955.

Zusammenfassung

Es ist nicht möglich, Zugholz in Eschenholz mittels Färbungsmethoden erkennbar zu machen. Mit Hilfe des Phasenkontrast-Mikroskops kann man die Anwesenheit von Zugholz in der Faserwand von Eschenholz auf einfache Weise zeigen, wenn man die angegebene Methode genau einhält.

[1] H. E. Dadswell and A. B. Wardrop, *Structure, properties, and formation of tension wood.* Paper to be presented to sub-section 13b. For. Anatomy. 8th Internat. Congress Botany, Paris (1954).

Developing Wheat Embryos Excised from Ovaries Cultured *in vitro*

Uttaman[1] failed in rearing proembryos of maize, and Ziebur and Brink[2] did not succeed in developing barley embryos 0·3 mm or less in diameter. Ovaries of various plants (tomato, gherkin, bean, tobacco and strawberry) have been cultivated *in vitro* by Nitsch[3]. With gherkins, he found that only ovaries excised on the fourth day after pollination yielded seeds of which 6% germinated. Seeds developed in ovaries excised earlier, proved to be incapable of germinating.

[1] P. Uttaman, Current Sci. *18*, 215 (1949); Ref.: Ber. Wiss. Biol. *68*, 196 (1950).

[2] N. K. Ziebur and R. A. Brink, Amer. J. Bot. *38*, 253 (1951).

[3] J. P. Nitsch, Amer. J. Bot. *38*, 566 (1951).

The mineral nutrient solution used in our wheat-ovary cultures was the same which we had specially elaborated for embryo culture[1], supplemented with 3% of sugar, 0·8% of agar, and with vitamins. An addition of 5% of the juice of maize in the milky stage benefited the differentiation.

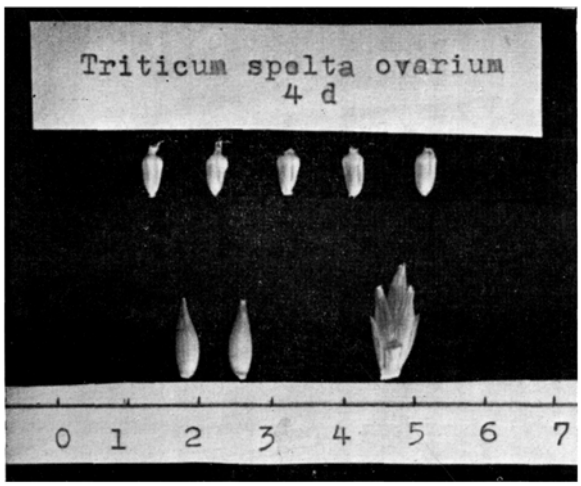

Fig. 1.–Wheat ovaries (upper row), flowers and spikelet (lower row) on the fourth day after anthesis when the culture was started.

After natural pollination occurred, the spikes were cut off, treated with a 10% calcium hypochlorite solution for 10–15 min, and washed thoroughly with sterile water. Ovaries, flowers, or spikelets with quite small rachillae, taken only from the middle third part of the spikes, were used for the cultures (fig. 1). The age of the ovaries was determined as beginning with the day on which the anthesis of the two main flowers of the spikelets was recorded. Development was the better the more of the rachillae and paleae remained attached to the ovaries. If ovaries deprived of all other maternal tissues were planted on the nutrient medium, the embryos seldom continued to develope. Ovaries enveloped by two paleae ordinarily promoted the differentiation of the embryo, even though the caryopsis grew but little in size. The endosperm discontinued developing normally, grew but feebly, and formed a glassy, jelly-like substance.

Having cultured the ovaries for 8–12 days, the embryos were excised and transferred to a fresh medium of the same composition, but supplemented with 0·5% of casein hydrolysate. They continued to develope, and a few days later germination began. From ovaries cultured for more than a fortnight a decrease in the number of viable embryos was observed. Later, the embryos germinated were planted in pots. They throve well developing into fairly normal plants.

In a single case, embryos capable of germinating were obtained even from in vitro cultivated ovaries excised on the second day after pollination. From ovaries excised on the fourth and sixth day, respectively, the embryos could be developed into seedlings quite regularly.

As far as we know this is the first instance reported of obtaining viable plants from *in vitro* cultures of wheat proembryos by subculturing embryos excised from ovary cultures.

G. RÉDEI and Mrs. G. RÉDEI

Institute of Genetics, Budapest, June 19, 1955.

[1] G. RÉDEI, B. GYÖRFFY, and Mrs. G. RÉDEI, Acta Bot. Acad. Sci. Hung. *2*, (1955) (in press).

Zusammenfassung

Es wurden 2–4 Tage nach dem Anthesis Weizenovarien *in vitro* in Kultur geführt. 8–10 Tage später wurden die Embryonen präpariert und wieder künstlich gezüchtet. Die so erhaltenen lebensfähigen Keimpflanzen entwickelten sich nach späterer Auspflanzung zu samenbringenden Pflanzen. Es ist uns kein Fall bekannt, wo aus Weizen Proembryonen *in vitro* Keimlinge gezogen wurden.

Role of Cystine Chelation in the Mechanism of Fusarium Wilt of Cotton

There has been much difference of opinion on the mechanism of pathological wilting of plants by pathogenic Fusaria[1]. GÄUMANN[2] and his school of workers working on the tomato wilt by *Fusarium oxysporum* f. *lycopersici* (SACC.) have attributed the wilt symptoms to an irreversible destruction of the osmotic pre-requisites for turgor by the systemic toxin lycomarasmin. WALKER[3] and his school of workers, who formerly advanced the vessel plugging theory against the systemic toxin theory, have subsequently reported typical wilt symptoms in tomato with fungal extracts rich in pectin methyl esterase activity and claimed that pectic enzymes could reproduce many of the wilt symptoms[4]. While it has been established that resistance or susceptibility to Fusarium wilts is only with reference to the fungus and not to the action of the active toxic principle[2], little is known of the mechanism of *in vivo* detoxication in the resistant varieties particularly in cotton.

Extensive chromatographic studies made by the author recently (employing the circular macro-[5] and micro-[6]techniques developed in this connection) on the distribution of α-amino constituents (positive to ninhydrine) in roots, shoots and leaves of susceptible and resistant varieties of cotton (Karunganni 2, Cambodia and Madras Uganda), have revealed the interesting fact that the resistant varieties of cotton, both the shoots and roots, particularly the latter contained considerable amounts of cystine besides the major constituents as asparagine, glutamine and other minor amino acids, while the susceptible ones did not reveal any cystine. It was therefore considered worthwhile to investigate if cystine was in any way related to resistance to wilt both in the presence and in absence of ferric iron, particularly in view of the earlier reports that the antibiotic activity of clavacin was due to its chelation to sulfhydryl group in plant saps[7].

Cut shoots of cotton (Susceptible variety Karunganni *2-Gossypium arboreum*) 10 to 12 days old, grown in normal garden soil were treated to neat dialysed culture filtrates of *Fusarium vasinfectum* Atk.[8] (grown in

[1] K. LAKSHMINARAYANAN, Proc. Indian Acad. Sci. *41B*, 132 (1955). – T. S. SADASIVAN and C. V. SUBRAMANIAN, J. Indian bot. Soc. *33*, 162 (1954). – C. V. SUBRAMANIAN, Curr. Sci. *24*, 144 (1955).

[2] E. GÄUMANN, Adv. Enzym. *11*, 401 (1951).

[3] R. P. SCHEFFER and J. C. WALKER, Phytopathology *43*, 116 (1953).

[4] S. S. GOTHOSKAR, R. P. SCHEFFER, J. C. WALKER, and M. A. STAHMANN, Phytopathology, *43*, 535 (1953). – N. N. WINSTEAD and J. C. WALKER, Phytopathology *44*, 153 (1954).

[5] K. LAKSHMINARAYANAN, Arch. Biochem. Biophys. *49*, 396 (1954).

[6] K. LAKSHMINARAYANAN, Arch. Biochem. Biophys *51*, 367 (1954).

[7] G. MIESCHER, Phytopath. Z. *16*, 369 (1950).

[8] R. KALYANASUNDARAM and K. LAKSHMINARAYANAN, Nature *171*, 1120 (1953).