ing activity on root growth: the other fractions stimulate more than controls (Figure 3).

From the after-ripe embryos, 5 fractions were isolated: only the fraction 3 exhibited a moderate depressing effect on seed germination. The fractions, when assayed on hypocothyl growth, showed a very high stimulating activity (significant values) but lower than GA_3 solution (Figure 2). The bioassay on lettuce root showed that the fraction 3 strongly depressed while other fractions stimulated growth (Figure 3).

Discussion. The behaviour of wheat seed during ripening is the result of effects caused by growth substances which are acting either on its embryo or endosperm. Their relationships regulate a lot of enzymatic reactions which lead to the accumulation of photosynthetic products, the utilization of storage products and seedling growth. The results obtained confirm the existence of a variation, during the seed ripening, in the regulation capacity on seed germination and growth by the embryos.

In the milky-ripe embryos, no fraction which depressed germination was detectable. On the contrary, they contain substances which stimulate hypocothyl and root growth. Yet, since these substances appear to have stimulating efficiency lower than the assayed gibberellin, but are capable of inducing a strong stimulating effect on the root growth, they might contain cytokininlike products[10]. However, at this ripening phase, the wheat caryopsis, being capable of germination, exibits a reduced seedling growth. Since the lack of a detectable inhibitory activity in the extract from unripe embryos was detected, they may be unable to utilize storage products which are accumulating[11,12]. The waxy-embryos show some fractions (the 2nd and 5th) which moderately depress the initial phases of germination but are capable of stimulating the hypocothyl elongation.

It appears that, just before the wheat seeds attain their maximum dry weight, the embryos possess a lot of stimulating substances which are not opposed by others having depressing effects. When the seeds reduce their water content, the embryos exibit a fraction (the 3rd in the fully-ripe seed) which depresses the seed germination and root growth but is capable of highly stimulating the hypocothyl growth which depends principally on distension. As the auxins rapidly disappear when the wheat grains get ripe[11], the effects of the fraction could be due to the relative concentration of active substances present in it, giving different or opposite effects in the assayed tests[13].

The presence of germination depressing substances in the diffusates from embroys during the ripening phases has been detected[4]: similar substances do not diffuse from the endosperms. From our experiment it appears that the capacity to depress germination increases, when the seed is ripening, reachs its maximum in the ripe seeds and decreases during the after ripening phase.

The germination-depressing activity of fraction 3 from fully-ripe embryos appears to be similar to specific inhibitors like ABA or Cis4. These, on the contrary, exibit a depressing activity on seedling root growth, lower than that detected for the fraction 3.

The different and opposite effects – stimulating or depressing – due to the fraction 3 could be the consequence of the simultaneous presence of different active products in this fraction which, when isolated from ripe and after-ripe embryos, appear to be the same. A preliminary investigation to identify the active products by reagents for gibberellins and auxins did not show the presence of such promotors in the fraction 3. Different procedures are being tried to evaluate the presence of growth substances in these extracts.

Summary. Bioassay of fractions from wheat embryos revealed that, at all ripening phases, they posses natural substances which stimulate hypocothyl growth. The fully-ripe and the after-ripe embryos exibited a very effective fraction for depressing seed germination and seedling root growth; the other fractions stimulate root growth. The results are discussed in relation to current hypotheses of physiological balance between antagonist growth substances.

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[10] A. W. Wheeler, Ann. appl. Biol. *72*, 327 (1972).
[11] A. A. Abdul-Baki, Crop Sci. *9*, 732 (1969).
[12] C. Floris, G. Bot. ital. *103*, 59 (1969).
[13] K. W. Bailiss and T. A. Hill, Bot. Rev. *37*, 437 (1971).

Coniferyl Alcohol from Callus of *Castanea sativa* Cultured in vitro

In vitro culture is being used increasingly in studies of the metabolism and morphogenesis of forest tree species, but little has been done on the Fagaceae. Some authors[1–4] reported the successful culture of *Quercus* sp., *Fagus sylvatica* L. and *Castanea sativa* Mill. callus from cambial tissue, but none of them made studies of the metabolism of the callus.

We obtained callus tissue from cotyledons of *Castanea sativa* Mill. which have been maintained in subculture for a year. This paper describes a chemical study of exudates which appear on the surface of callus in the course of their development, one component of which has been identified as coniferyl alcohol.

Material and methods. Callus tissue of *Castanea sativa* which originated from cotyledon tissue was sub-cultured every 7–8 weeks. 7-month-old stock tissue was used for experiments. The basal medium contained the Murashige and Skoog[5] inorganic components plus benzyladenine, 0.5 ppm; indole-3-butyric acid, 1 ppm; m-inositol, 500 ppm; vitamin B_1, 1 ppm; Ca-pantothenate, 0.5 ppm; sucrose, 3% w/v and 0.7% agar. In other experiments kinetin (0.5 ppm), but not benzyladenine, was added. The pH of the medium was adjusted to 5.6 before autoclaving. The cultures were grown at 25 °C in the dark. For histological studies the explants were fixed in FAA and microtome sections were processed in the conventional manner using safranin-fast freen as the stain. For insuring localization of lignified tissues, polarized light microscopy was performed.

[1] C. Jaquiot, C. r. Acad. Sci., Paris *231*, 1080 (1950).
[2] C. Jaquiot, C. r. Acad. Sci., Paris *243*, 510 (1956).
[3] J. Grente, C. r. Acad. Sci., Paris *252*, 2441 (1961).
[4] G. Borrod, C. r. Acad. Sci., Paris *272*, 56 (1971).
[5] T. Murashige and F. Skoog, Physiologia Plant. *15*, 437 (1962).

After 7 weeks of culture, small drops of exudates appear to be present on the surface of callus grown on the benzyladenine medium but not on the kinetin one. The exudates were taken off with a micropipet, eluted with 2 drops of water and essayed for two dimensional paper chromatography (PC). The solvent benzene-acetic acid-water BzAW (6:7:3 upper layer) was used for the first dimension and 2% acetic acid (2% HOAc) for the second. The chromatograms were sprayed with diazotized *p*-nitroaniline to visualize phenolics. Standard compounds were used for comparison, and the identity of coniferyl alcohol was confirmed by UV spectral analysis in the presence of diagnostic reagents.

Results and discussion. Two dimensional PC of crude exudates showed the presence of 4 major phenols. They were successively purified by PC and one of them showed identical Rf values in 5 solvents when co-chromatographed with standard coniferyl alcohol. The Rf values are: BzAW: 0.60. HOAc: 0.67. Isopropanol-ammonia-water (10:1:1): 0.77. Butanol-acetic acid-water (4:1:5 upper layer): 0.82. Propanol-ethyl acetate-water (7:1:2): 0.93.

Also both standard and natural substances absorb at short UV lamp and give the same UV spectrum: a maximum at 265 nm in methanol; and in alkaline medium, a maximum at 290 nm and a shoulder at 315 nm. The compound was identified as coniferyl alcohol.

Sections of the tissues grown on kinetin medium contain a large number of nodules of tracheids as revealed by safranin-fast green and polarized light microscopy. Tissues grown on benzyladenine were much less lignified. These results seem to be in accordance with those of SARGENT and SKOOG[6] who reported an increase of lignin biosynthesis in tobacco tissue culture when kinetin was added to the medium.

The occurrence of coniferin in the sap of many gymnosperms helped to establish a theory that lignin is formed from its aglycon coniferyl alcohol and it is apparent that the oxidase responsible for the dehydrogenative polymerization of lignin precursors is exclusively peroxidase[7]. Numerous studies on lignification have demonstrated a direct relation between peroxidase levels and lignification[8]. On the other hand, in experiments with tissue cultures grown with growth regulators it can be seen that the medium on which the cultures are grown can change the pattern of enzymes responsibles of lignification[9,10].

On the basis of our results, the known role of peroxidase in lignification as well as the role of growth regulators on enzymatic activity, a mechanism may be present for which, in the callus of *C. sativa* grown on benzyladenine medium, lower production of peroxidase must occur than in those grown on kinetin, because the polymerization of coniferyl alcohol with benzyladenine treatment appears to be retarded.

The next step of our work will be the study of perodixase activity in the callus of *C. sativa* when they are grown on both kinetine and benzyladenine.

Resumen. Alcohol coniferílico fue identificado de los exudados producidos por callos de *Castanea sativa* Mill. cultivados in vitro, originados de cotiledón.

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[6] J. A. SARGENT and F. SKOOG, Plant Physiol. *35*, 934 (1960).
[7] J. M. HARKIN and J. R. OBST, Science *180*, 296 (1973).
[8] D. S. VAN FLEET, *Handbuch der Histochemie* (Gustav Fischer Verlag, Stuttgart 1962), vol. 7, p. 2.
[9] T. T. LEE, Can. J. Bot. *50*, 2471 (1972).
[10] Y. P. S. BAJAB, M. BOP and S. BAJAB, Phytomorfology *23*, 43 (1973).
[11] Acknowledgment. Thanks are given to Prof. J. W. BRADBEER, Department of Botany King's College, London (U.K.) for correction of the manuscript.

Parthenin: A Growth Inhibitor Behaviour in Different Organisms

Parthenin, a sesquiterpene lactone of the pseudoguaianolide class isolated from *Parthenium hysterophorus* Linn., (Asteraceae), is an inhibitor of seedling growth in a crop plant *Eleusine coracana* (Linn.) Gaertn. Var. Poorna[1]. The present study deals with its behaviour in certain phases of two fungi such as – sporangial germination and zoospore motility in *Sclerospora graminicola* (Sacc.) Shroet. and conidial development in – *Aspergillus flavus* Link., in order to find out whether this inhibitor does exercise the same property in others. – Parthenin samples were obtained from KANCHAN[1] who has isolated them in her recent work. Parthenin was dissolved in 0.1% ethyl alcohol which was not toxic to test organisms. Appropriate media for test organisms and concentration of parthenin adopted in the experiment were used as given in the Table. Leaves of *Pennisetum typhoides* S. and H., infected by the downy mildew fungus *S. graminicola* were collected, surface sterilized with 0.2% chlorine water and washed in sterilized distilled water. The leaves were cut into pieces, floated on water with their abaxial surface upwards in a petridish lined with moist filter paper and kept overnight. Next morning, sporangia were scraped from the abaxial surface of leaf bits while zoospores were obtained by sowing the sporangia in distilled water for $^1/_2$ h. Sporangia and zoospores were seperately transferred to a distilled water drop on micro glass slides kept for control and experiment. In the experiment, they were tested against parthenin. For culturing *A. flavus*, methods cited by GARBUTT and BARTLETT[2] were followed. Medium with parthenin was sterilized before inoculation of the fungus. Room temperature was between 23 and 30 °C while conducting these experiments. In control *S. graminicola* zoospores were released from the sporangia after 30 min. The zoospore swam for 20–30 min and subsequently gave rise to germ tubes. Zoospores were released from the sporangia, swam for 2–3 min but eventually disintegrated. The results reveal that parthenin at the concentration of 500 mg in 1 ml of ethyl alcohol + 1,000 ml of distilled water inhibits sporangial germination in $^1/_2$ h and zoospore motility in 2–3 min. At the same concentration and even more (i.e., upto 1,000 mg), it never produced any visible inhibitory response in the conidial development of *A. flavus*. The results were further confirmed by

[1] S. D. KANCHAN, Curr. Sci. *44*, 358 (1975).
[2] J. W. GARBUTT and A. J. BARTLETT, micro-organisms, in *Teacher's Guide*, (Butterworths, London 1972), p. 233.