

(for example, *Coleus*) contain relatively small amounts of chlorophyll and are capable of only limited stomatal response (opening) to light (6). Guard cells of albino barley which were completely devoid of chlorophyll did not respond to light. The necessity of chlorophyll for the normal stomatal opening was not conclusively proved, however, because Shaw was unable to observe an opening of the stomata of albino barley leaves at any time. It is also well established that high concentrations of carbon dioxide in the guard cells or substomatal cavities give rise to stomatal closure.

The mode of action of atrazine has been studied by many workers, and Crafts presented a comprehensive review of the literature (7). The action of the chemical apparently is to inhibit CO<sub>2</sub> fixation in photosynthesis and to increase respiration (1). The tolerance of some species, such as corn, appears to be due to the ability of the plant to detoxify the herbicide as rapidly as it is taken up.

There is ample evidence that atrazine is translocated throughout a seedling in a short time (less than 3 hours). The material concentrates first at the leaf margins and accumulates basipetally (8).

Stomatal closure after treatment with atrazine would thus appear to be a result of increased CO<sub>2</sub> in the guard cells and substomatal cavities resulting from the inhibition of photosynthesis and increased respiration rate instituted by the herbicide. This is substantiated by the fact that other rapidly translocated photosynthetic inhibitors such as monuron [3(4-chlorophenyl)-1,1-dimethylurea] and diuron [3(3,4-dichlorophenyl)-1,1-dimethylurea] gave a similar response while herbicides with a mode of action not directly involving photosynthesis, such as dalapon (2,2-dichloropropionic acid) and amitrol (3-amino-1,2,4-triazole) caused no reduction in transpiration rate during the 24-hour test period. The transpiration reduction with the substituted ureas was similar to that observed by Minshall (9; 10).

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#### References and Notes

1. F. M. Ashton, G. Zweig, G. W. Mason, *Weeds* 8, 448 (1960).

2. N. Grobbelaar, *Science* 131, 1614 (1960).
3. W. T. Williams, *Ann. Botany (London)* 13, 309 (1949).
4. M. Shaw and G. A. MacLachlan, *Can. J. Botany* 32, 784 (1954).
5. O. V. S. Heath, *J. Exptl. Botany* 1, 29 (1950).
6. M. Shaw, *Can. J. Botany* 36, 575 (1958).
7. A. S. Crafts, *The Chemistry and Mode of Action of Herbicides* (Interscience, New York, 1961), pp. 115-123.
8. T. J. Sheets, *Weeds* 9, 1 (1961).
9. W. H. Minshall, *Can. J. Botany* 38, 201 (1960).
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### Peach Mesocarp Explant Enlargement and Callus Production in vitro

**Abstract.** Explants from fruits of the peach were stimulated to grow by cell enlargement, cell division, or both when placed on media containing kinetin. Callus cultures have been obtained, even occasionally from ripening fruits, long after cessation of normal cell division.

Tissues from fruits of deciduous orchard trees have not been grown commonly in culture. The cessation of active cell division within 4 weeks after bloom (1) suggests that cells of matur-

ing fruit tissue might be incapable of resuming active cell division. Letham, however, reported proliferation of apple fruit tissue from both young fruits and fruits which had been developing for 130 days (2).

Among evergreen fruit species, Schroeder (3) and Schroeder and Spector (4) have reported culturing tissue from the pericarp of the avocado, *Persea americana* Mill, and the mesocarp of citron, *Citrus medica* L. Also, small fruits of the cucurbit, *Echallium elaterium* Rich., have proliferated callus in vitro (5).

The purpose of our investigation (6) was to determine the behavior of explants from the mesocarp of fruits of the peach, *Prunus persica* (L.) Batsch. Particular attention was given to changes in behavior with advancing fruit age and to the relation of plant growth-regulating substances to length of explant life and the initiation of callus proliferation.

Explants were aseptically cut from peach fruits after the epidermis and a few underlying cell layers had been peeled and discarded. In early experiments, explants were approximately 3 mm<sup>3</sup> in size. In later tests, explants consisted of a cylinder 8 mm in diameter and 8 mm long. Tissue ad-

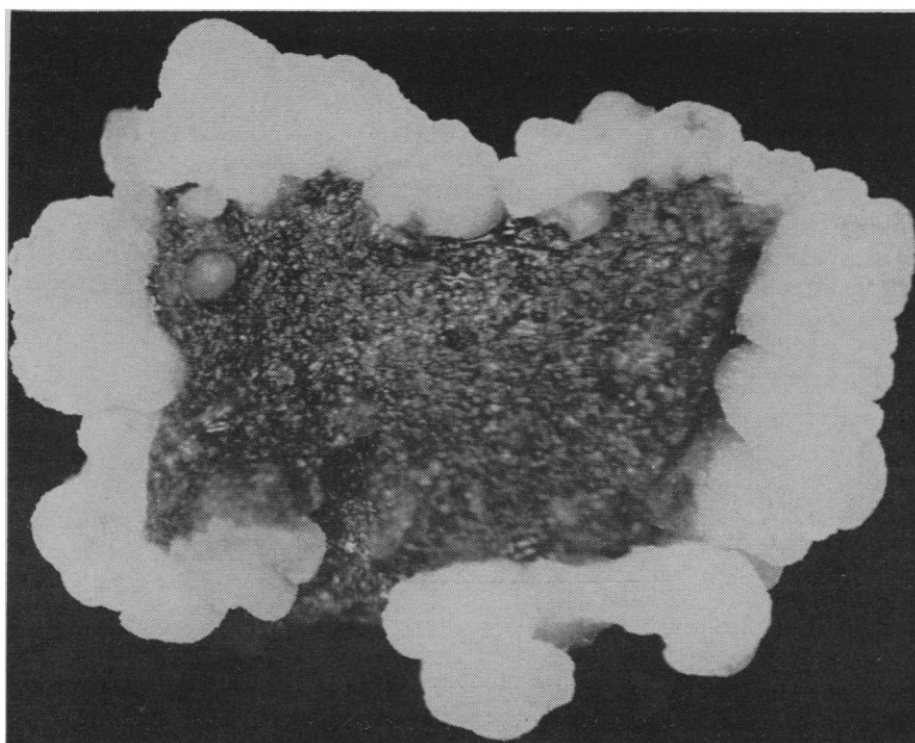


Fig. 1. Callus production by an explant of Miller's Late peach mesocarp obtained about 4 weeks before the fruit would have been ripe. The photograph was made after 6 weeks in culture. Actual size approximately 5 by 9 mm.

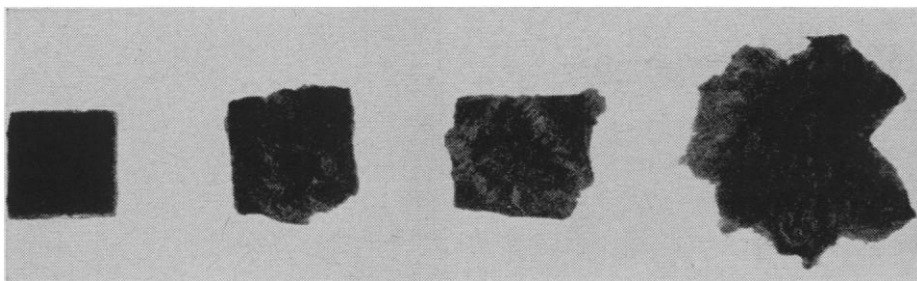


Fig. 2. Swelling of Miller's Late peach mesocarp explants obtained about 3 weeks before the fruits would have been ripe. Left, little or no swelling; middle, with prominent growth cracks; right, with many growth cracks causing a "popcorn" appearance.

jaacent to the peel was utilized in all tests.

The explants were grown on White's medium as modified by Skoog and Cheng (7) to which the following additions, in milligrams per liter, were made: glycine, 2; thiamine hydrochloride, 0.1; nicotinic acid, 0.5; and pyridoxine, 0.5. To this basic medium were added varying concentrations of kinetin, gibberellin (potassium gibberellate), naphthaleneacetic acid, and coconut milk. The medium was solidified with 0.5 or 0.7 percent Bacto agar, and the pH was adjusted to about 7.0. The naphthaleneacetic acid and gibberellin were filter sterilized and added separately when their precise concentration was a critical part of the test.

With explants from the cultivar Miller's Late, obtained about 1 month before the fruits were ripe (about 170 days after bloom), a dichotomy in growth response was observed. Some explants exhibited definite callus proliferation within 2 weeks, accompanied by only minor swelling of the explant (Fig. 1). Other explants enlarged and developed growth cracks, with little evidence of callus production (Fig. 2). The internal enlargement of the latter explants continued, with growth cracks occurring repeatedly, to produce a "popcorn" appearance. In a few cases callus eventually developed from these explants.

Abundant callus production was observed only where kinetin or coconut milk was added. With kinetin this occurred at a concentration of 2  $\mu\text{g/ml}$ ; a concentration of 10  $\mu\text{g/ml}$ , however, was toxic to the explant. In the presence of kinetin (2  $\mu\text{g/ml}$ ), naphthaleneacetic acid (1 and 10  $\mu\text{g/ml}$ ) appeared to hasten the appearance of callus, but proliferation developed in some explants lacking naphthaleneacetic acid in the medium.

Centers of proliferation occurred at the explant surface and appeared to be associated with vascular bundles. Similar observations were made by Letham with apple fruit tissue (2). The demonstration by Bradley and Crane of extensive endopolyploidy in many parenchyma cells of maturing fruits of a *Prunus* species suggests that only comparatively small parenchyma cells associated with the vascular bundles can be easily stimulated to divide (8).

In the absence of callus proliferation, explant swelling has usually accompanied the maintenance of a living condition for a month or more. Swelling and continued splitting of explants resulted in considerable fragmentation within 2 weeks (Fig. 2). Accompanying this growth, in most cases, was the development of a brilliant red color. In chromatographs of extracts, cyanin was found to be the most abundant anthocyanin pigment.

Explant expansion without callus production evidently is due primarily to cell enlargement. Parenchyma cells from explants that had developed repeated growth cracks often exceeded twice the size of larger parenchyma cells of the original explant. The swelling was further illustrated by differences in fresh weight. In a typical test, explants weighed  $387 \pm 11$  mg when obtained from the fruit. After 4 weeks, surviving explants with only slight swelling weighed  $425 \pm 8$  mg; those with one or more large growth cracks,  $742 \pm 18$  mg; and those with many growth cracks, resulting in a "popcorn" appearance,  $1097 \pm 39$  mg. Average dry weight values were  $19.6 \pm 0.6$  mg for explants with only modest expansion,  $41.5 \pm 2.5$  mg for those with large growth cracks, and  $55.9 \pm 1.5$  mg for those with multiple growth cracks and the "popcorn" appearance. These differences in dry weight demon-

strate that the growth observed is not due merely to water uptake.

Factors leading to growth by cell enlargement have not been fully evaluated. As expected, moderate amounts of naphthaleneacetic acid (1 or 10  $\mu\text{g/ml}$ ) appeared to stimulate enlargement, while high concentrations (100  $\mu\text{g/ml}$ ) appeared to be toxic. However, similar expansive growth was sometimes observed without added auxin. Furthermore, considerable variability in the amount of explant expansion occurred within treatments. The source of variability has not yet been determined.

The response to media constituents by explants from ripening fruits is of interest in studies of postharvest physiology. This is particularly true if the ripening process can be slowed, halted, or reversed. Consequently, several tests included the cultivars Late Elberta and Sullivan No. 2 at various stages of ripening as determined by Munsell color (9) and by flesh firmness as measured by a Magness-Taylor pressure tester (10) with a 5/16-inch tip. In the case of both cultivars, a few explants from fruits exhibiting a vivid yellow color and a flesh firmness test of approximately 12 pounds pressure enlarged slightly, and some of these eventually exhibited some callus production if in the presence of kinetin.

It is evident from the experiments reported here that at least some peach mesocarp cells retain for some time the ability to be stimulated to cell division after ripening has been initiated.

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#### References and Notes

1. R. M. Addoms, G. T. Nightingale, M. A. Blake, *New Jersey Agr. Expt. Sta. Bull. No. 507* (1930); C. H. Ragland, *Proc. Am. Soc. Hort. Sci.* 31, 1 (1934).
2. D. S. Letham, *Nature* 182, 473 (1958).
3. C. A. Schroeder, *Science* 122, 601 (1955).
4. — and C. Spector, *ibid.* 126, 701 (1957).
5. F. Nystrak, *Compt. rend.* 250, 1906 (1960).
6. We thank L. L. Creasy for anthocyanin pigment determinations and L. R. McKinnon for photographic assistance. This investigation was supported, in part, by AEC contract No. AT(11-1)34, agreement 73.
7. F. Skoog and T. Cheng, *Am. J. Botany* 35, 782 (1948).
8. M. V. Bradley and J. C. Crane, *ibid.* 42, 273 (1955).
9. D. Nickerson, Munsell Color Company, Inc., Baltimore, Md. (1957).
10. M. H. Haller, *U.S. Dept. Agr. Circ.* 627 (1941).

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