THE EFFECT OF LOW TEMPERATURE ON PHOSPHATE ESTERIFICATION AND CELL MEMBRANE PERMEABILITY IN TOMATO FRUIT AND CABBAGE LEAF TISSUE*

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Summary

Exposure to 0°C for 4 weeks caused a threefold increase in cell membrane permeability of mature-green tomato fruits (susceptible to chilling injury) but had no effect on that of cabbage leaves (not susceptible). While tomato fruits chilled for 12 days lost two-thirds of their capacity to esterify phosphate at 20°C, a steady rise in this capacity occurred during chilling of cabbage leaves for 5 weeks. In tomato fruits the rate of phosphate esterification at the chilling temperature fell in 12 days to about one-half of the rate at the commencement of chilling. It is suggested that the characteristic symptoms of chilling injury in mature-green tomato fruits, viz. increased susceptibility to fungal attack and loss of the capacity to ripen normally, may result from an energy deficit caused by a chilling-induced reduction in the phosphorylative capacity of the tissue.

I. Introduction

Like many other fruits of tropical or subtropical origin the mature-green tomato (*Lycopersicon esculentum*) is highly susceptible to injury from exposure to non-freezing temperatures below about 10°C, either before harvest or during transit and storage. Chilling reduces the resistance of the fruit to attack by the rot fungus *Alternaria tenuis* and causes slower and inferior ripening. The injury becomes more apparent following transfer from chilling conditions to the warmer conditions normally used for ripening, and is more severe the longer the exposure and the lower the temperature.

On the other hand, many plant tissues withstand exposure to chilling temperatures for considerable periods without injury. For example, Parsons (1959) showed that cabbage (*Brassica oleracea* var. *capitata*) stored for periods up to 8 weeks kept better at 32 than at 36°C, and better at 36 than at 45°C.

This paper compares the effects of storage at low temperatures on cell membrane permeability and phosphorylative capacity of the tissues of mature-green tomato fruit and cabbage leaf.

II. Materials and Methods

(*a*) *Cell Membrane Permeability of Tomato and Cabbage Tissue*

Tomato fruits of an unnamed, leaf-mould-resistant strain were used. This strain was developed at Purdue University especially for greenhouse use, and was from the F_8 generation derived from (Ohio WR7 Globe × Vinequeen) × Ohio WR7.

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Matched groups of 15 freshly harvested mature-green fruits were prepared by grading the fruits into a number of different classes by weight, and then dividing the fruits in each class equally among all the experimental groups. From the outer wall tissue of fruits of one group disks were cut with a 1-cm cork borer; after washing in several changes of distilled water to remove sap from ruptured cells, duplicate 25-g samples of disks were placed in beakers containing 1500 ml distilled water. Each sample was gently agitated with a magnetic stirrer for 400 min, and the conductivity of the water surrounding the disks was determined at intervals using a Barnstead conductivity meter. The experiment was carried out in a constant-temperature room at 20°C. The rate at which conductivity increased was a measure of the rate at which electrolytes leaked from the cells. Identical determinations were carried out on four other matched groups following storage at 0°C for 1, 2, 3, and 4 weeks, respectively.

Similar measurements were made with disks cut with a 1-cm cork borer from the leaves of a cabbage (cv. Enfield Market) after storage at 0°C for 0, 1, 2, and 4 weeks. Duplicate 4-g samples of washed disks were placed in glass jars containing 350 ml distilled water. Agitation was maintained by a stream of air bubbles in place of magnetic stirring (which made temperature control difficult because of the heat generated by the motors). Measurements were made over a period of 360 min.

(*b*) *Phosphorylative Capacity of Tomato and Cabbage Tissue*

(i) *General Methods.*—Slices of tissue were first vacuum-infiltrated with $[^{32}P]H_3PO_4$ (10–50 μc/ml) and then washed rapidly in several changes of distilled water. Duplicate samples weighing about 1 g were then incubated at high humidity at a constant temperature for a given time. Following incubation the samples were immediately frozen and held at −10°C pending extraction and paper-chromatographic separation of the phosphates by the method of Loughman and Martin (1957). The distribution of radioactivity on the chromatogram was determined with a Forro radiochromatograph scanner equipped with a windowless gas flow counter with a 5-mm slit. The percentage of the original ^{32}P which had become esterified by the tissue was calculated from the area under those peaks of the distribution curve which corresponded to organic phosphorus and the total area under all the peaks of the curve.

(ii) *Selection of Incubation Period.*—For each lot of experimental material several samples of infiltrated tissue were incubated at 20°C for different lengths of time. These preliminary experiments, which enabled the selection of an incubation period which would permit appreciable esterification of phosphate to occur but would not allow equilibrium to be attained between organic and inorganic ^{32}P, showed that equilibrium was reached in 3–4 hr in cabbage (unknown variety) tissue, but had not been attained after 24 hr in tomato (cv. Tiny Tim) tissue.

(iii) *Effect of Incubation Temperature.*—Duplicate samples consisting of 3-mm square pieces of outer wall tissue of mature-green tomato fruits (cv. Tiny Tim) were incubated in the presence of ^{32}P at 0, 5, 12, and 20°C for 21 hr. Duplicate samples of leaf disks 5 mm in diameter from an unknown variety of cabbage were also incubated in the presence of ^{32}P at 0, 5, 11, and 20°C for 2 hr.

(iv) *Effect of Storage at Low Temperature.*—Slices of tissue from matched samples of mature-green tomato fruits (cv. Tiny Tim) which had been stored at 0°C for 0, 2, 5, 8, and 12 days were infiltrated with ^{32}P and the amount of esterification after 21 hr was determined at 0°C and at 20°C. The amount of esterification at 0°C was considered to be an indication of the rate at which esterification was proceeding immediately prior to removal of the fruits from chilling conditions. The capacity for phosphorylation retained by the tissue after transfer to a ripening temperature was indicated by the amount of esterification at 20°C. For comparison, disks 5 mm in diameter were taken at weekly intervals from the leaves of a cabbage (cv. Enfield Market) during storage at 0°C for 5 weeks. Duplicate samples of disks were incubated in the presence of ^{32}P for 0·5 hr at 20°C.

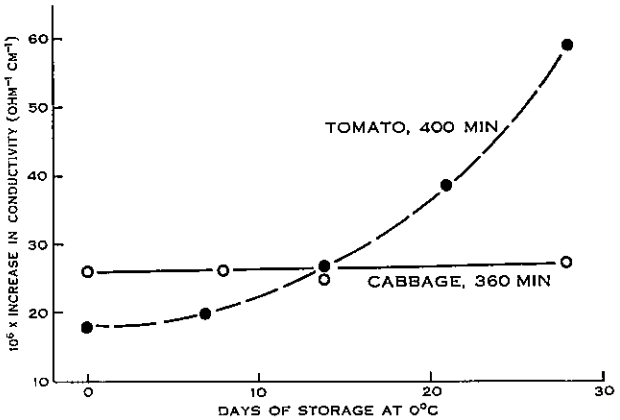

Fig. 1.—Effect of storage at 0°C on rate of leakage of electrolytes from tomato and cabbage tissue, measured as an increase in conductivity of bathing solution after periods of 400 and 360 min, respectively.

III. Results

(*a*) *Cell Membrane Permeability of Tomato and Cabbage Tissue*

There was a continuous increase in cell membrane permeability of mature-green tomato fruit tissue with increase in the duration of exposure to 0°C (Fig. 1). This increase in permeability was small during the first few days of chilling but became progressively more rapid with further exposure. After 4 weeks at 0°C cell membrane permeability was about three times that observed in unchilled tissue. As indicated in Figure 1, no appreciable change occurred in the permeability of the cell membrane of cabbage leaf tissue even after 4 weeks of storage at 0°C.

(*b*) *Phosphorylative Capacity of Tomato and Cabbage Tissue*

(i) *Effect of Incubation Temperature.*—Figure 2 illustrates the linear increase in rate of phosphate esterification with increase in incubation temperature for both tissues.

(ii) *Effect of Storage at Low Temperature.*—Exposure of mature-green tomato fruit to 0°C quickly initiated a steep decline in the capacity of the tissue for phosphorylation at 20°C (Fig. 3). This decline continued as chilling was prolonged, until after exposure to 0°C for 12 days only about one-third of the original capacity remained. Chilling also brought about a slower but significant decrease in the rate of phosphorylation at 0°C. After 12 days at this temperature the activity was only about one-half of that shown by the fruit prior to chilling. On the other hand, there was a continuous increase in the capacity of cabbage leaf tissue for phosphorylation at 20°C, and after 5 weeks of storage at 0°C this capacity was 50% greater than in unchilled tissue.

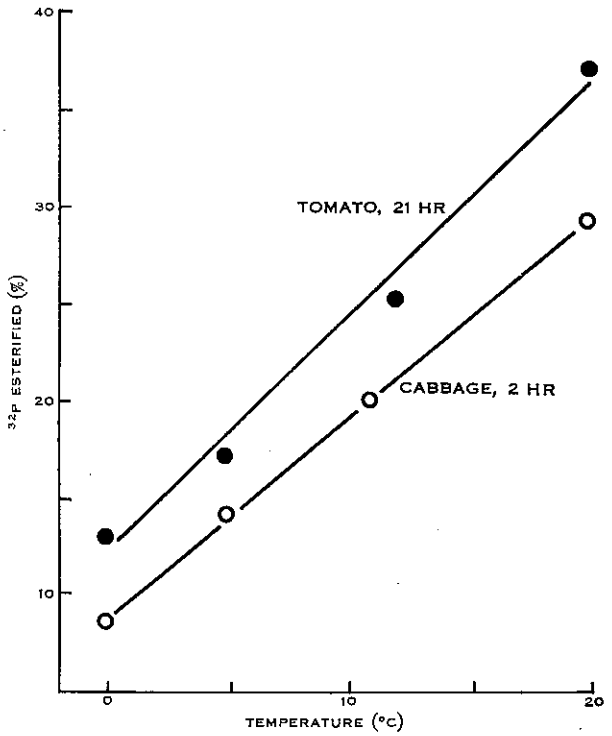

Fig. 2.—Influence of temperature on rate of ^{32}P esterification. Tomato tissue incubated for 21 hr prior to assay, cabbage tissue for 2 hr.

IV. Discussion

An increase in cell membrane permeability has been shown to occur when mature-green tomato fruits undergo relatively brief chilling treatments, but the cell membrane permeability of cabbage leaf tissue was unaffected by much longer chilling treatments. Chilling has previously been shown to elicit similar rapid responses in two other tissues which are injured by chilling, viz. mandarin endocarp (Pantanelli 1919) and sweet potato root (Lieberman *et al.* 1959). The rapidity of this response, coupled with the absence of an effect in the chilling-resistant cabbage leaf tissue,

suggests that this increase in permeability may be related to the process most directly affected by chilling.

Figure 2 shows that there is no alteration at or below the threshold temperature for chilling injury in the slope of the straight-line relationship between esterification rate of ^{32}P and incubation temperature. Thus it appears that temperatures in the chilling range do not exert any unusual influence on the rate of phosphorylation in freshly harvested mature-green tomato fruits. Moreover, the lack of any significant difference between the slopes of the straight-line relationships for tomato fruit and cabbage leaf suggests that injury does not arise from a simple lowering of the rate of phosphorylation at chilling temperatures.

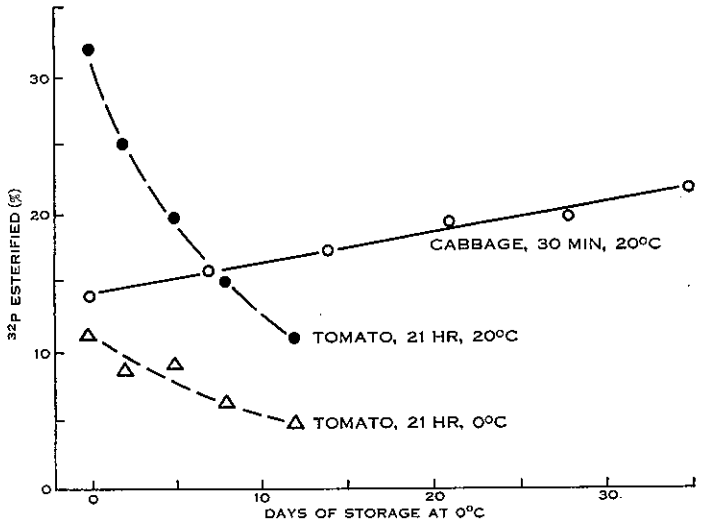

Fig. 3.—Effect of storage at 0°C on capacity of tomato and cabbage tissue for esterification of ^{32}P. Tomato tissue incubated for 21 hr at temperatures indicated prior to assay, cabbage tissue for 30 min.

However, the initial rate of phosphorylation in freshly harvested fruits placed at 0°C is not maintained, since exposure to this temperature quickly initiates a steep decline in the phosphorylative capacity of the tissue. Lieberman *et al.* (1959) observed that a decline in oxidative and phosphorylative activity commenced in sweet potato only after the roots had been held at 7·5°C (an injurious temperature) for about 4 weeks. They suggested that the apparent delay in response to chilling might be due to a masking effect of the injury sustained by the mitochondria in the normal isolation procedure. With tomato fruit tissue slices, on the other hand, the effect on phosphorylative capacity is immediate and even more marked than the effect on cell membrane permeability. This fact, together with the observation that chilling of cabbage leaf tissue results in an increase, rather than a decrease, in its phosphorylative capacity, suggests that the process of phosphorylation may be connected with that process which is primarily affected by chilling.

The progressive decline on chilling in the capacity of mature-green tomato fruit tissue for phosphorylation at the chilling temperature may lead to a shortage of energy needed for the maintenance of cellular organization. This would eventually lead to the symptoms that are observed after prolonged chilling in cases where fruits are not transferred to warmer conditions for ripening, namely, a dull, greyish appearance of the fruit, together with a loss of resistance to disease. Transfer to ripening conditions, even after much shorter periods of chilling, is followed by rapid development of fungal rot and by slow, blotchy, incomplete ripening. The more rapid development of injury symptoms following transfer to ripening conditions may perhaps be explained in the following manner. Lewis (1956) has shown that respiration rates of mature-green tomatoes following chilling are as high as, or higher than, those existing prior to chilling. While the respiratory process, and perhaps other catabolic processes, are taking place at the higher temperature at rates comparable with those obtaining before the fruit was chilled, the capacity for phosphorylation has been reduced by the chilling treatment. Because of the resultant energy deficit, a net breakdown of complex cellular components such as proteins occurs, with a consequent loss of cellular organization. This would give rise to the observed increase in cell membrane permeability, and to a lowering of the resistance of the tissue to fungal decay. The energy shortage would also prevent the normal occurrence of synthetic reactions, such as lycopene formation, which contribute to the ripening process, and which have been shown by Marks, Bernlohr, and Varner (1957) to require energy from oxidative phosphorylation. Thus the two most important symptoms of chilling injury in mature-green tomato fruits may perhaps be explained on the basis of an energy deficit arising from a chilling-induced reduction in the phosphorylative capacity of the tissue.

V. Acknowledgments

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