

surface Ca while the other group was not rinsed. After 10 days there was no significant difference in color between rinsed and unrinsed fruit in any treatment. It does not seem possible to get sufficient uptake by the fruit to delay ripening, merely by applying Ca to the skin.

Considerable care was found to be necessary to ensure that all solutions and utensils used were free from microbial contamination. Preliminary experiments had considerable losses due to infections, obtained during the dipping process but, by insuring that adequate

sanitation procedures were carried out, this problem was overcome in the main experiments.

Literature Cited

1. Bangerth, F., D. R. Dilley, and D.H. Dewey, 1972. Effect of postharvest calcium treatments on internal breakdown and respiration of apple fruits. *J. Amer. Soc. Hort. Sci.* 97:679-682.
2. Bramlage, W.J., M. Drake, and J.H. Baker, 1974. Relationships of calcium content to respiration and postharvest condition of apples. *J. Amer. Soc. Hort. Sci.* 99:376-378.
3. Faust, M., and J.D. Klein, 1974. Levels and sites of metabolically active calcium

in apple fruit. *J. Amer. Soc. Hort. Sci.* 99:93-94.

4. Faust, M., and C.B. Shear, 1972. The effect of calcium on respiration of apples. *J. Amer. Soc. Hort. Sci.* 97:437-439.
5. Poovaiah, B.W., and A.C. Leopole, 1973. Deferral of leaf senescence with calcium. *Plant Physiol.* 52:236-239.
6. Scott, K.J., and R.B.H. Wills, 1977. Vacuum infiltration of calcium chloride — a method for reducing bitter pit and senescence of apples during storage at ambient temperatures. *HortScience* 11: 71-72.
7. Tingwa, P.O., and R.E. Young, 1974. The effect of calcium on the ripening of avocado (*Persea americana* Mill.) fruits. *J. Amer. Soc. Hort. Sci.* 99:540-542.

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Ethylene Evolution as Related to Changes in Hydroperoxides in Ripening Tomato Fruit¹

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Additional index words. *Lycopersicon esculentum*

Abstract. Hydroperoxide levels were determined in aqueous and lipid extracts from fruit of tomato (*Lycopersicon esculentum* Mill.) at 6 different stages of ripening. An increase in the levels of peroxides in both the aqueous and the lipid fractions was associated with the upsurge in ethylene evolution. The changes in peroxides in the lipid fraction corresponded to the changes in the activity of lipoxygenase. Peroxides may constitute some of the active oxygen forms occurring *in vivo* which are required for the synthesis of ethylene in fruit.

Several studies showed that the synthesis of ethylene *in vitro* is stimulated by peroxides (12, 14, 17). In the present work we show that the upsurge in ethylene evolution in intact ripening tomatoes is associated with a rise in hydroperoxides as occurring *in vivo* in fruit.

'Azes' tomatoes obtained from the Volcani Research Center, Bet-Dagan, Israel, at 6 stages of ripening including 1) green, 2) white, 3) breaking, 4) pink, 5) red, and 6) overripe, and were used for the determination of ethylene evolution, peroxide levels, and lipoxygenase activity.

Ten fruit weighing on the average 800 g were kept in a closed glass jar fitted with a septum. Gas samples of 1 ml were withdrawn from the head space at 15-min intervals, for 1.5 hrs. The ethylene concn in the gas samples was determined by comparing against known ethylene concn using a Packard 844 gas chromatograph. Ethylene evolution is expressed as $\mu\text{l}/\text{kg}/\text{per hr}$.

Following ethylene determinations,

tissue increments from the fruit pericarp were removed in duplicate for the determination of peroxides and lipoxygenase activity. Tissue portions weighing 100 g were homogenized in a water-methanol-chloroform mixture and filtered through a Whatman filter paper No. 1, and the filtrate was separated into aqueous and an organic phase (3). The organic phase, which contained the lipid fraction, was reduced to a few ml by vacuum and supplemented with acetone to 20 ml. Peroxides were determined in 20 ml samples from the aqueous and the lipid fractions, respectively, as outlined previously (4).

Lipoxygenase (EC 1.13.1.13) activity was determined in protein extracts from the fruit tissue obtained as previously described (8). The enzyme activity in the extract was determined by measuring the rate of O_2 uptake (7), and is expressed as rate of oxygen uptake/min/per mg protein. Protein was measured by the Lowry method (13) following precipitation with trichloroacetic acid, acetone wash, and resolubilization in 0.1 M NaOH.

The changes in ethylene evolution as related to peroxide levels at different stages of tomato ripening are shown in Fig. 1. The upsurge in the evolution of ethylene commenced at stage 3, reaching a peak at stage 5, and declined afterward. By comparison,

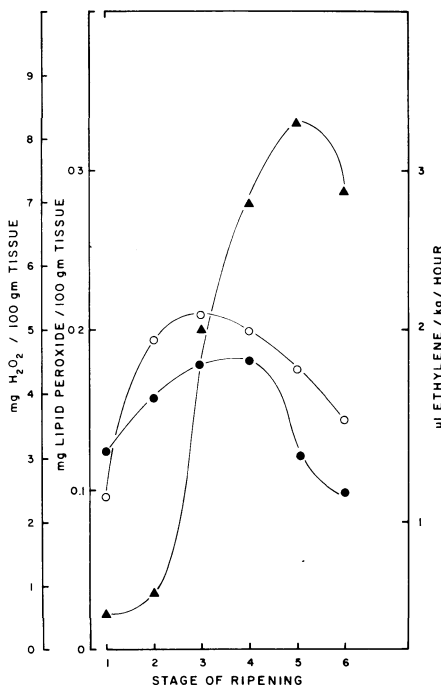


Fig. 1. Evolution of ethylene (▲) at different stages of ripening of tomato fruit as related to the changes in peroxides in fruit tissue extracts representing the aqueous fraction (○) and the lipid fraction (●).

the peroxide level in the aqueous fraction representing H_2O_2 showed an increase beginning at stage 2, reached a peak at stage 3, and declined afterward. Although the rise in the peroxides in the organic phase is consistent with that in the aqueous fraction, it appears to be somewhat delayed. However, it too precedes the upsurge in ethylene evolution. The present results agree with previous data (4) showing that the evolution of ethylene in pear parallels changes in peroxides either as occurring naturally in ripening fruit or as influenced by treatments which alter the peroxide concn in the fruit tissue.

The synthesis of ethylene *in vivo* is dependent on oxygen (5). *In vitro* studies suggest that the active oxygen species constitute reduced oxygen intermediates. Ethylene synthesis was stimulated by peroxides (12, 14) and, conversely, was inhibited following the degradation of H_2O_2 by the action of catalase (6). Additional studies (2) show that ethylene synthesis is

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also stimulated by the joint action of H_2O_2 and superoxide ions, leading apparently to the formation of active hydroxyl radicals, as described by the Haber-Weiss reaction (2, 9). By this view, the function of H_2O_2 , at least in part, is to promote the formation of active oxygen radicals leading to the release of ethylene from precursor metabolites. Although the mode of H_2O_2 action awaits further clarification, the peroxides appear to be associated with onset of ethylene synthesis *in vitro* (12, 14) in isolated chloroplasts (6) and in the present study, *in vivo* in intact fruit.

The role of lipid oxidation in the synthesis of ethylene is less certain. Lipid oxidation was viewed as leading to the release of intermediary metabolites in the pathway of ethylene synthesis (12), but the yield of ethylene originating from lipid fractions is too small to account for the natural production of the compound (1). However, the synthesis of ethylene was stimulated by fatty acid hydroperoxides *in vitro* (14) or following the application of these compounds to intact fig fruit (10). The primary function of lipid peroxides in the synthesis of ethylene may be to furnish peroxides, since the present study shows (Fig. 1) that the level of the lipid peroxides is not negligible, for they constitute about 5% of the peroxide in the aqueous fraction. The changes in peroxides in the lipid fraction correspond to the changes in the activity of lipoxygenase (Fig. 2) and may result from the oxidation of fatty acids as previously suggested (16). Lipid peroxidation may also result from the increase in H_2O_2 action by the Haber-Weiss mechanism (9) serving to

initiate the oxidation of unsaturated fatty acids (11). Tappel (15) suggested that animal senescence is accompanied by lipid oxidation. The present results indicate that lipid peroxidation also occurs during senescence in plants. In fruit the process may be related to the evolution of ethylene.

In summary, we propose that the increase in peroxides, including H_2O_2 and lipid hydroperoxides, may represent some of the active oxygen forms occurring *in vivo* which are required for the biosynthesis of ethylene at the onset of fruit ripening.

Literature Cited

1. Abeles, F. B. 1966. Ethylene production from linolenic acid. *Nature* 210:23-25.
2. Beauchamp, C., and I. Fridovich. 1970. Mechanism for the production of ethylene from methionol. *J. Biol. Chem.* 245:4641-4646.
3. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid fraction and purification. *Can. J. Biochem. Physiol.* 37:911.
4. Brennan, T., and C. Frenkel. Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiol.* 49:411-416.
5. Burg, S. P. and K. V. Thimann. 1959. The physiology of ethylene formation in apple. *Proc. Natl. Acad. Sci. U.S.A.* 45:335-344.
6. Estner, E. F. and J. R. Konze. 1974. Light-dependent ethylene production by isolated chloroplasts. *FEBS Letters* 45: 18-21.
7. Eskin, N. A. M., and H. M. Henderson. 1974. Lipoxygenase in *Vicia faba minor*. *Phytochemistry*. 13:2713-2716.
8. Frenkel, C. and C. E. Hess. 1974. The aqueous extraction of protein from plant tissue with the use of electrophoresis. *Can. J. Botany*. 52:1611-1614.
9. Haber, F. and J. Weiss. 1934. The catalytic decomposition of hydrogen peroxide by iron salts. *Proc. Roy. Soc. London A* 147:332.
10. Hirai, J., N. Hirata, and S. Horiuchi. 1967. Effect of deoxygenation on hastening the maturity of fig fruit. V. Effect of metabolic products in oxidative process of fatty acid on fruit maturity. *J. Jap. Soc. Hort. Sci.* 36:380-384.
11. Kellog, E. W., and I. Fridovich. 1975. Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by a xanthine oxidase system. *J. Biol. Chem.* 250:8812-8818.
12. Lieberman, M. and L. W. Mapson. 1964. Genesis and biogenesis of ethylene. *Nature* 204:343-244.
13. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with folin reagent. *J. Biol. Chem.* 193:265-275.
14. Mapson, L. W. and D. A. Wardale. 1972. Role of indole-3-acetic acid in the formation of ethylene from 4-methyl-mercapto-2-oxobutyric acid by peroxidase. *Phytochemistry* 11:1371-1378.
15. Tappel, A. L. 1973. Lipid peroxidation damage to cell compounds. *Feder. Proc.* 32:1870-1874.
16. Wooltorton, C. S., J. D. Jones, and A. C. Hulme. 1965. Genesis of ethylene in apples. *Nature* 207:999.
17. Yang, S. F. 1967. Biosynthesis of ethylene. *Arch. Biochem. Biophys.* 122:481-487.

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Late Blight in Mature Tomatoes and Transplants in Southern Georgia in 1976 and Possible Chemical Control¹

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Abstract. Late blight, caused by *Phytophthora infestans* (Mont.) d By. was epiphytotic in several fields of tomato transplants and mature tomatoes in southern Georgia in late May of 1976. This is the first time the disease has been observed in transplant fields in southern Georgia since the 1946 late blight epidemic in the eastern United States. Preliminary fungicide tests indicated that a combination of chlorothalonil and experimental compound from Ciba-Geigy GA-1-82 at 1.12 or 0.42 kg/ha gave excellent control for late blight. GA-1-82 very closely resembles *N*-(2, 6-dimethylphenyl)-*N*-(2-furanylcarbonyl)-*L*-alanine methyl ester (CGA-38140).

The last major epiphytotic of late blight in the eastern U. S. on tomato occurred in 1946 (3, 4, 10, 11, 14).

In that year, tomato losses due to late blight exceeded 50% in states from Florida to New York, and were as high as 25% in some midwestern states (11). Losses due to late blight were still widespread in many states in 1947 and 1948, but were lower than in 1946 due to a spray program, the use of late blight-free southern tomato transplants and possibly unfavorable environmental

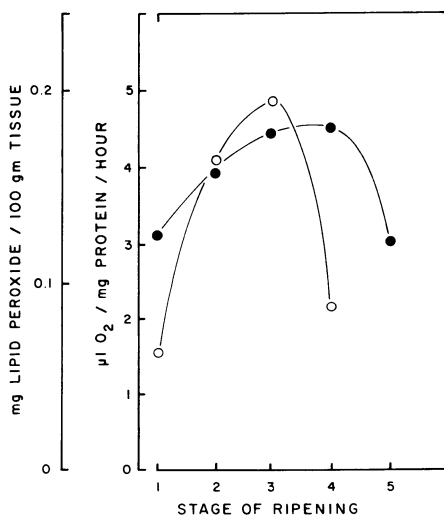


Fig. 2. Changes in lipid peroxides (●) as related to the activity of lipoxygenase (○) in tomato fruit at different stages of ripening.

¹Received for publication December 4, 1976. Mention of a pesticide in this paper does not constitute a recommendation by the USDA nor does it imply registration under FIFRA.