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Regulation of *Colletotrichum Gloeosporioides*
Latency in Avocado

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C. ABSTRACT

The resistance of unripe avocado fruits to attack by postharvest pathogens was associated with the preformed antifungal compound called diene. Previous results led to the belief that diene decrease was a result of increased lipoxygenase activity. Activity of this enzyme was regulated in the fruit peel by the decrease in concentration of the flavan 3-ol inhibitor, epicatechin and not due to changes in the enzyme protein content. The differential decrease of epicatechin concentration after harvest was used as a criteria for classifying resistance of avocado cultivars to postharvest disease. Exogenic compounds that inhibited lipid peroxidation activity of lipoxygenase were tested as possible inhibitors of diene peroxidation and were found to delay the development of anthracnose and stem end rot.

D. INTRODUCTION AND ORIGINAL OBJECTIVES.

Colletotrichum gloeosporioides infections of avocado are the cause of the vast majoritys of losses due to fruit decay in Israel and most foreign markets. *Colletotrichum* spores germinate on the surface of the unripe fruit and produce appressoria, but further development of the fungus is delayed until the fruit ripens after harvest. The resistance of unripe avocado fruits peel to fungal development was found by Prusky et al. to result from the presence of the antifungal compound 1-acetoxy-2-hydroxy-4-oxo-12,15-heneicosas-diene (1). The concentration of this compound in avocado peels decreased during ripening, concomitantly with resumed growth of previously quiescent *Colletotrichum* infections.

Since the antifungal diene shares the *cis,cis*-1,4-pentadiene system of linoleic acid, the possibility that the diene may be a substrate for plant lipoxygenase and that lipoxygenase may be responsible for decomposition of the antifungal compound during avocado fruit ripening was investigated. It was observed that lipoxygenase activity extracted from fruit peel increased by 80% during ripening, coincident with increased respiration and evolution of ethylene, and followed by the appearance of fungal disease symptoms (2). It was observed as well that a partially purified fraction of avocado lipoxygenase oxidized the antifungal diene from avocado *in vitro* suggesting that lipoxygenase might be involved in the breakdown of the antifungal diene. The question that was asked was how is lipoxygenase activity regulated during fruit ripening. Experiments with some lipoxygenase inhibitors suggested the possibility that the decrease of the diene compound in avocado fruits during ripening may be linked to increased lipoxygenase activity (2). Tocopherol acetate infiltration of whole fruits inhibited lipoxygenase activity in extracts from the fruits and delayed the development of disease symptoms in the fruit *in vivo*. Furthermore, α -tocopherol acetate and butylated hydroxy anisole inhibited lipoxygenase-mediated oxidation of the diene compound by 42 and 89% respectively, *in vitro*. Tocopherol acetate, however, did not inhibit growth of the fungus in culture, indicating that its effect on decay development is via plant metabolism, probably as an antioxidant.

Based on that initial data, the objectives of the original research were:

1. The isolate natural inhibitor(s) of avocado lipoxygenase and determine the possible relationship with the latency of *Colletotrichum gloeosporioides*.
2. To determine if the changes of lipoxygenase-inhibitor concentration or either the increase in specific activity of the enzyme are regulating the breakdown of the antifungal diene.
3. To determine the relation of inhibitor concentration and cultivar susceptibility to *C. gloeosporioides* after harvest.
4. To evaluate antioxidants as possible agents for prevention of decay development during fruit ripening and under prolonged storage

REFERENCES

Prusky, D., N.T. Keen, J.J Sims, and S. Midland 1982: Possible involvement of an antifungal compound in latency of Colletotrichum gloeosporioides in unripe avocado fruits. Phytopathology 72: 1378-1382.

Prusky, D., N. T. Keen, and I.L. Eaks, 1983. Further evidence for the involvement of a preformed antifungal compound in the latency of Colletotrichum gloeosporioides in unripe avocado fruits. Physiological Plant Pathology 22: 189-198.

E. REPORT

1. Isolation of natural inhibitors of avocado lipoxygenase and their relationship with the quiescence of C. gloeosporioides.

2. Involvement of epicatechin in the regulation of antifungal diene concentration during activation of quiescent Colletotrichum gloesporioides infections of ripening avocado fruits.

This study was designated to test wheter an increase in lipoxyge nase activity during ripening indeed results from degradation of its endogenous inhibitor epicatechin or from an increase of the amount of enzyme in the fruit. The use of a immunological technique for quanti tation of lipxygenase in crude extracts was developed and for this purpose was needed the purification to homogenity of avocado lipox ygenase. We report here the process of purification of avocado lipox ygenase and the use of an immunological technique to determine the relationships among lipxygenase activity, lipxygenase amount and its inhibitor during fruit ripening.

F. DESCRIPTION OF COOPERATION.

The cooperation between the Department of Storage of Agricultural Products, ARO and the Department of Agricultural Botany, Hebrew University was very active during the whole project. The work of the Department of Plant Pathology, University of California, Riverside, was tremendously significant for the identification of the inhibitor of avocado lipoxygenase and a new antifungal compound from avocado still not reported. A continuous exchange of ideas and results occurred during the whole project between the US and Israeli laboratories. Dr. Keen visited the Volcani to revise current work and to present a talk before the Bat Sheva de Rothschild, Host-Parasite interaction Conference. Dr. Prusky visited twice the Department of Plant Pathology, where he was involved in the purification of the lipoxygenase-inhibitor. It can be summarized that each lab had his significant contribution needed for the success of the project, while all the data was concentrated by the PI in the Department of Storage of Agricultural Products.

G. RESEARCH ACHIEVEMENTS.

The research achieved all the original aims of the project. The regulation of the mechanisms of fruit resistance in harvested avocado fruits was suggested and based on this results a commercial treatment was proposed. The development of this idea will continue in the next years, where several experiments in packing houses will be carried on.

H. LIST OF PUBLICATIONS

1. Prusky, D., Kobiler, Ilana, Jacoby, B., Simms, J.J. and Midland, Sharon, L. 1985. Inhibitors of avocado lipoxygenase: their possible relationship with the latency of Colletotrichum gloeosporioides. Physiological Plant Pathology 27: 269-279. 1.9
2. Marcus, L., Prusky, D. and Jacoby, B. 1988. Purification and characterization of avocado lipoxygenase. Phytochemistry 27: 323-327. 1.1
3. Marcus, L. and Prusky, D. 1987. Effect of particle size of activated charcoal on separation of triton X-100 from protein, liver cytosol, and lipoxygenase extracts. Analytical Biochemistry 163: 112-116. 2.06
4. Karni, Leah, Prusky, D., Kobiler, Ilana, Bar-Shira, Einav and Kobiler, D. 1988. Involvement of epicatechin in the regulation of lipoxygenase activity during activation of quiescent Colletotrichum gloeosporioides infections of ripening avocado fruits. Physiological and Molecular Plant Pathology: Submitted.
5. Prusky, D., Kobiler, Ilana and Jacoby, B. 1988. Involvement of epicatechin in cultivar susceptibility of avocado fruits to Colletotrichum gloeosporioides after harvest. Phytopath. Z.: In Press. 2.3
6. Prusky, D. 1988. The use of antioxidants to delay the onset of anthracnose and stem end decay in avocado fruits after harvest. Plant Disease 72: 381-384. 0.7