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BARD

FINAL REPORT

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Biotechnological Control of Postharvest Fungal Storage Rots of Citrus Fruits

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Title of Research Project:

Biotechnological Control of Postharvest Fungal Storage Rots of
Citrus Fruits

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A. ABSTRACT

This research project explored the potential for developing biotechnological procedures for the control of the fungal storage rots of citrus fruits.

The studies in Israel involved Penicillium digitatum, P. italicum and Geotrichum candidum infections in oranges and mandarins.

Improved fruit treatment methodology was developed and used for a definitive study of wound depth as well as the effect of inoculum density, differences in pathogenic strains, effect of storage time and wound tissue recovery. These studies demonstrated the relative ineffectiveness of the current SOPP and TBZ fungicides. Improved methodology was developed for the isolation and evaluation of soil microorganisms producing antifungal antibiotic biochemicals active against these citrus pathogens. The important problem of resistance development was also studied and evaluation studies carried out using the antifungal-producing isolates and selected fungicides.

The studies in the U.S. involved P. digitatum and G. candidum infections in lemons. Considerable effort was directed toward improving the degree of G. candidum infectivity and some success was achieved using cycloheximide, glyphosate, blasticidin S, a pectinase preparation and dialyzed supernatant from a G. candidum culture. These treatments did not interfere with the effectiveness of etaconazole. The studies with P. digitatum involved the use of antagonistic microorganisms to reduce the amount of infection. Some degree of success was obtained with Trichoderma viride and a Rhodotorula sp isolated from the surface of lemon fruit.

B. OBJECTIVES OF THE RESEARCH

The purpose of this project was to initiate an overall research program with the potential of establishing the basis for the development of microbial antifungal antibiotic biochemicals for the effective control of fungal storage rots of citrus fruits.

BARD funding for this project was provided for only a 2-year period. In view of the broad scope of the project and the limited funding, the first 2-year period concentrated on the biological aspects of the program, with the chemical phases of the research goals to be emphasized in a continuing support period. This division of the research goals was necessitated not only by the scope of the program, but also by the need to have research support personnel of different capabilities available during the biological, and later the chemical phase of the program.

C. RESEARCH RESULTS

In general, the research program in Prof. Ziffer's laboratory followed the outline of the original research proposal. It was quickly recognized, however, that fruit treatment methodology, and the occurrence of fungal pathogen resistance to treatment compounds would be of major importance in achieving the potential project goals, and accordingly, considerable research effort was directed toward these two areas during the biological portion of the program.

The results of this portion of the research program are given in the very detailed report BIOTECHNOLOGICAL STRATEGIES FOR THE CONTROL OF THE POSTHARVEST FUNGAL STORAGE ROTS OF CITRUS FRUITS attached in Appendix No. 1. The data summarized in the report covered the following research activities:

1. Fruit treatment methodology
2. Fruit treatment studies
3. Pathogen resistance studies
4. Microbial culture isolation and evaluation

The research program in Prof. Eckert's laboratory also included studies in methodology, especially with regard to Geotrichum candidum infections, as well as alternative microbiological methods for the control of Penicillium digitatum infections of lemons.

The results of this portion of the research program are included in the PhD. thesis of Dr. Aristoteles Pires de Matos, who was partially supported by the BARD project. A copy of his thesis CHEMICAL AND MICROBIOLOGICAL FACTORS INFLUENCING THE INFECTION OF LEMONS BY GEOTRICHUM CANDIDUM AND PENICILLIUM DIGITATUM is attached in Appendix No. 2. The data summarized in this thesis covered the following research areas:

1. Inoculum additives to increase the susceptibility of lemons to Geotrichum candidum
2. Microbiological interactions involved in the infection of lemons by Penicillium digitatum

The scope of the research efforts and results described in these reports, supplementing procedures and data already developed in Prof. Ziffer's and Prof. Eckert's laboratories, enabled both laboratories to accomplish much more than would normally be reported for a 2-year period.

D. COOPERATION

The excellent cooperative efforts by both laboratories are evidenced by the results which complemented and reenforced each others approach to this problem.

Prof. Ziffer's laboratory concentrated on P. digitatum, P. italicum and G. candidum infections in oranges and mandarins, while Prof. Eckert's laboratory studied P. digitatum and G. candidum infections in lemons. Again, with regard to the objective of developing alternative controls for these storage rot fungi, Prof. Ziffer's laboratory used cultures isolated from soil for the production of antibiotic biochemicals antagonistic to these rot fungi, while Prof. Eckert's laboratory emphasized the use of antagonistic microorganisms from various sources, including isolates from the surface of lemon fruits. These approaches complemented each other.

Both laboratories recognized the necessity to improve methodology and devoted considerable efforts toward this purpose.

The cooperative efforts of both laboratories established the basis for eventually developing a solution to this important storage rot problem.

E. BENEFITS TO AGRICULTURE

The benefits to the postharvest problems of stored citrus fruits, and for that matter to other stored fruits and vegetables, will be considerable if the potential for the alternative control measures established in this BARD project are realized.

First, it is important to review the conditions now prevailing in the current commercial practice of preventing postharvest fungal storage rots. The procedures usually followed are the use of chemical fungicides which often are chlorinated compounds, and which eventually will probably be shown to be suspected carcinogens, or at best, toxicants which should not be used.

In citrus fruits, the recommended fungicides are SOPP and TBZ, which while not chlorinated compounds, have serious drawbacks. SOPP is especially phytotoxic to citrus fruits and was shown in these studies to be ineffective under postinfection conditions. The most serious problem with these fungicides, and especially TBZ, as well as with other systemic fungicides, is that the fungi become resistant to them so that the established control procedures become ineffective. This was recognized in the studies carried out in Prof. Ziffer's laboratory and considerable efforts were made to develop methodology in this area and to evaluate the biochemical antifungal candidates for the potential development of resistance.

Among the major accomplishments of Prof. Ziffer's group was the development of improved methodology to show the effect of wound depth on the postharvest infection process. While the conclusions drawn from these studies could be considered self evident, this was the first definitive study undertaken in this very important area.

The use of such methodology enabled the demonstration of inoculum density effects, differences in pathogen strains, the effect of the length of storage and fruit homeostatic defenses and the effect of wound tissue recovery. Finally, this methodology enabled the demonstration of the ineffectual protection afforded by the presently recommended postharvest citrus fungicides.

The development of methodology for the isolation and evaluation of

antifungal-producing microorganisms was a major contribution by both laboratories, and the potential shown by these studies, together with the demonstrated need for improved postharvest handling procedures to prevent fruit injuries and subsequent fungal infections, should be the basis for the development of effective alternative control methods.

F. LIST OF PUBLICATIONS

J. Ziffer and I.J. Kopelman. 1984.

The effect of wound depth on the postharvest *Penicillium* storage rots of citrus.

In Antimicrobials and Agriculture. M. Woodbine (ed.) Butterworths, London.

G. APPENDICES

Appendix No. 1

Jack Ziffer

Biotechnological Strategies for the Control of the Postharvest Fungal Storage Rots of Citrus Fruits.

Appendix No. 2

Aristoteles Pires de Matos

Chemical and Microbiological Factors Influencing the Infection of Lemons by Geotrichum candidum and Penicillium digitatum.