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FINAL REPORT

PROJECT NO. I-616-83

Development of Strategy for Control of Citrus Sour Rot

I. Barash, E.E. Butler

1987

Date April 1988

BARD

P.O.Box 6

Bet Dagan, ISRAEL

BARD Project No. I-616-83

Title of Research Project : Development of Strategy for Control of Citrus Sour
Rot

Names of Investigators :

Principal Investigator Isaac Barash

Cooperating Investigator Edward E. Butler

Name and Address of Affiliated Institutions :

Principal Institution : Dept. of Botany, Tel Aviv University, Israel

Cooperating Institution : Dept. of Plant Pathology, University of
California, Davis, USA

Project's starting date : September 1984

Type of Report : 1st Annual..... 2nd Annual..... Finalx.....

Signatures :

Principal Investigator

Institution's Authorizing Official

Isaac Barash

S. Sarid

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ABSTRACT

Geotrichum candidum is a damaging pathogen of citrus fruits and is a taxonomically complex fungus. The primary goal of the present project was to gain a deeper insight into the mechanisms of pathogenicity, ecology and taxonomy of this pathogen. It is anticipated that such information should be useful for identifying sensitive targets in the disease cycle which can then be manipulated for disease control.

The present report is composed of 6 sections, each of which describes an independent study. They are all interconnected and related to the main goal of the project.

A comparative study between virulent and avirulent strains of G. candidum was undertaken in order to understand mechanisms of virulence. At the beginning of these studies, virus-like particles, containing double-stranded RNA with an apparent MW of 3.6×10^6 daltons were detected in almost all virulent strains of G. candidum but not in avirulent strains. The virus particles were characterized and their possible association with virulence was investigated.

The second section describes a comprehensive study on the mode of infection of virulent as compared to avirulent strains. It was found that initial development of virulent and avirulent strains in wounded lemons, as expressed by colony-forming units was similar. Also, in both cases incipient maceration could be detected in advance to the penetrating hyphae. However, in contrast to the avirulent strains which exhibited a very limited maceration, progressive maceration was detected in the virulent strains. The possible involvement of active defence mechanisms was thoroughly examined by searching for differences in various parameters between virulent and avirulent strains, which are known to be characteristic to the resistance response. The negative results obtained suggest that active defence might not be involved. Additional studies described in this section suggest that the initial amount of polygalacturonase (PG) produced in vivo and the accessibility of the pectin in situ to this enzyme are the main factors that govern virulence of G. candidum on citrus fruits.

Ecological and pathogenicity studies described in section 3 included a survey of pathogenic and non-pathogenic isolates of G. candidum in 99 different locations and regions in Israel. Results presented clearly indicate that the citrus-pathogenic isolates are mainly adapted to the citrus groves. The perfect state of sour rot described by Butler (section 5) was found also in Israel (section 4). Preliminary studies suggest that the occurrence of the perfect state might be quite rare in Israel.

The fifth section describes the discovery and formal description of the sexual stage of the sour rot pathogen, i.e. Galactomyces citri-aurantii sp. Nov. E. E. Butler. Accordingly, the sour rot pathogen was removed from Geotrichum candidum L.K. and was assigned to Geotrichum citri-aurantii. Criteria for speciation including morphology, nutritional requirements, pathogenicity and ecologic isolation, were described.

Characterization of pectic enzymes produced by Penicillium digitatum in relation to the synergism exhibited by this pathogen with G. citri-aurantii is given in section 5.

OBJECTIVES OF ORIGINAL RESEARCH

The following objectives were originally proposed :

- I) Distribution, sources of inoculum and dispersal mechanisms of the sour rot pathogen
- II) Nature of sexuality and genetic variability of G. candidum
- III) Comparative phenotypic characters and biochemical components of the sour rot pathogen to related species, e.g. Galactomyces reessii and Galactomyces geotrichum
- IV) Mode of pathogenicity of G. candidum on citrus fruits
- V) Development of a sensitive and rapid procedure for quantitative estimation of G. candidum on citrus fruits

With the exclusion of Objective V, all other objectives have been either fully or partially covered in the report. Although we have initiated the production of antibodies against Geotrichum, the development of a suitable (relatively specific) preparation for ELISA procedure required much effort, more than we had originally anticipated. For various reasons we did not give it high priority and this study was dropped after a year.

As indicated in the Abstract and in the body of the Report, much effort has been directed towards Objectives I, II and IV. Objective III was actually combined with part of Objective I.

DESCRIPTION OF CO-OPERATION

During the last 3 years we have exchanged ideas, results and isolates of G. candidum by mail communication. Also, Dr. Barash visited Dr. Butler's laboratory during the summers of 1986 and 1987. It is noteworthy that the detection of the sexual state of G. candidum in Israel could not be achieved without the availability of mating types discovered by Dr. Butler. A very close co-operation was established in Israel between the laboratory of Dr. Barash and the Citrus Marketing Board, Israel. The latter was particularly expressed in the ecological studies performed in Israel.

EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE AIMS OF THE ORIGINAL RESEARCH PROPOSAL

The ultimate aim of the present proposal was to search for sensitive targets in the disease cycle which can be utilized for sour rot control. This goal could be best achieved by understanding of the interrelationship between the pathogen, the citrus environment and the susceptible fruit - all are subjects which were dealt with in the body of the research.

One of the major objectives and achievements of the present proposal was the discovery of the nature of sexuality of the sour rot pathogen (Section 5). The discovery of Galactomyces citri-aurantii Novo E. E. Butler, as the sexual stage of the sour rot pathogen clearly indicates that it constitutes a separate species. Geotrichum candidum Lk. ex Pers. comprises a complex of asexual fungi which are morphologically indistinguishable and can only be separated on the basis of the sexual stage. The latter discovery enabled Dr. Butler to assign all the citrus pathogenic strains of G. candidum to Geotrichum citri-aurantii, the asexual form of Galactomyces citri-aurantii. Criteria for speciation of G.

citri-aurantii based on morphology, vitamin requirements, pathogenicity, carbon and nutritional assimilation patterns, further established the sour rot pathogen as a separate species. The latter included a thorough comparative study with other known species of Geotrichum, i.e. Galactomyces reessii and G. geotrichum, two related species.

The distribution and sources of inoculum of G. citri-aurantii were extensively studied in Israel (section 3) as well as in the U.S. (Section 5).

On the basis of our work in Israel, as well as previous work elsewhere, it is now well established that the sour-rot pathogen is mainly limited to soils of the citrus planting areas. Furthermore, our ability to detect the pathogen in all the fruit flies which were examined, suggests that soil contaminants (including dust) in combination with insects, are the main sources of inoculum of sour rot in citrus planting and packing houses. These results re-emphasize the significance of sanitation as an essential method of controlling the disease. The availability of selective media for the pathogen, which were developed in the course of the present research (Sections 3 and 5) provides a useful tool to follow the contamination level in the packing house. The active involvement of the Citrus Marketing Board of Israel in this project resulted in an immediate recommendation for improving the handling of citrus fruits. A by-product which was stimulated by our direct involvement was also the introduction of the proper use of the guazatine as a successful fungicide against sour rot (results not included in this report).

Research on the nature of virulence constituted a major part of this project. A comparative study between virulent and avirulent strains was undertaken to elucidate factors involved in the infection process. Many efforts were aimed at detecting active resistance as a mechanism which might distinguish virulent from avirulent strains (Section 2 and unpublished results).

However, the negative results obtained suggested to us that active resistant mechanisms are at least uncommon in the present host-pathogen interaction. (These results, however, exclude wound healing which might be considered a resistant mechanism as demonstrated elsewhere). On the other hand, the "killing in advance" mechanism by the release of pectic enzymes gained support from our histochemical, ultrastructural and biochemical studies. These results may explain the common knowledge among plant pathologists that inoculation of citrus fruits by the sour rot pathogen is considerably more difficult than inoculations by the green and blue molds, for example. The latter pathogens appear to be more "parasitic" by nature than G. citri-aurantii and penetrate ahead of the release of pectic enzymes (for references, see Section 2). This explains why Penicillium italicum, P. digitatum and Phytophthora citrophthora facilitate the establishment of G. citri-aurantii. It appears that infection by G. citri-aurantii occurs only when the pathogen succeeds in establishing itself in the wound and in releasing sufficient enzyme to cause maceration. Consequently, the predisposing factors for infection by sour rot are more rigid than with the other mentioned pathogens and prevention can be obtained more easily.

The mechanisms of synergism between P. italicum and the sour rot has been investigated as part of the project. However, an insight into this mechanism requires knowledge on the pectic enzymes of P. italicum. Although considerable advancement was made (Section 6) this part has not yet been concluded.

The presence of ds-RNA virus in G. citri-aurantii may be linked to virulence (Section 1). However, more work is required to establish such a relationship, if it exists at all. It is noteworthy that the situation here is different than that with Endothia parasitica, where dsRNA was found to be associated with hypovirulence.

PUBLICATIONS

1. Mor, H., R. Steinlauf and I. Barash. 1984. Virus-like particles and double-stranded RNA in Geotrichum candidum, the causal agent of citrus sour rot. *Phytopathology* 74 : 921-924.
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4. Behr, L., J. Hershenhorn, I. Barash, T. Arzee. 1988. Mode of sour rot formation as inferred from comparative studies with virulent and avirulent strains of G. candidum. *J. Phytopathology* (submitted).
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6. Butler, E. E. 1988. Galactomyces citri-aurantii sp. Nov., the cause of sour rot of citrus fruit. *Mycotoxon* (in preparation).
7. Hershenhorn, J. and I. Barash. Purification and characterization of pectolytic enzymes produced by P. italicum (in preparation).
8. Hershenhorn, J. and I. Barash. The role of pectolytic enzymes of P. italicum in synergistic effect on sour rot production (in preparation).