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Evaluation of Strategies and Implementation of Biological Control of Fire Blight

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ABSTRACT

The main objective of this study was to develop data that would facilitate a consistently effective method of biological control of fire blight disease to be developed and to enable its implementation for disease control by ensuring its compatibility with variations in the biological, environmental, and chemical conditions present in pear orchards. As considerable information on the pathogen and biological control of fire blight was already gathered from studies in California and elsewhere, an emphasis was placed on investigating the genetics and ecology of *Erwinia amylovora*, the causal agent of fire blight in Israel. Studies of plasmid profile, virulence on several hosts, serological characteristics, as well as DNA fingerprints with selected primers all revealed *E. amylovora* strains in Israel to be homogeneous. Strains did vary in their resistance to streptomycin, with those from more northern locations being resistant while those in the southern coastal plain were all sensitive to streptomycin. Resistance appeared to be conferred by chromosomal mutations as in streptomycin-resistant strains in California. The biological control agent *Pseudomonas fluorescens* strain A506 colonized flowers of both the Costia and Spadona pear cultivars in Israel as well as Bartlett pear in California. Flowers that were open at the time of spray inoculation of trees subsequently harbored from 10^5 to 10^7 cells of strain A506 per flower, while those that opened subsequent to spraying developed population sizes of about 10^5 cells/flower within 5 days. The incidence of fire blight infections were reduced about 3-fold in several trials in which moderate amounts of disease occurred in the plot areas; this degree of biological control is similar to that observed in California and elsewhere. On two occasions warm

and moist weather that favored disease led to epidemics in which nearly all flowers became infected and which was so severe that neither *P. fluorescens* strain A506 nor chemical bactericides reduced disease incidence. A novel method for identifying antagonistic microorganisms for biological control of fire blight and other diseases was developed. A bacterial ice nucleation gene was introduced into *E. amylovora* to confer an Ice⁺ phenotype and the population sizes of this modified pathogen on flowers that had been pre-treated with potential biological control agents was estimated by measuring the freezing temperature of colonized flowers. Antagonistic strains that prevented the growth of *E. amylovora* in flowers were readily detected as those in which flowers froze at a low temperature. The method is both rapid and unbiased and several bacterial strains with substantial biological control potential have been identified using this method.

Objectives of the original research proposal:

The main objective of the study was to determine how effectively known biological control strains from the US would colonize cultivars specific to Israel and how climatic conditions unique to Israel would influence biological control of fire blight, and to determine if more effective biological control agents could be identified using a novel screening method developed during the course of the study. The more specific objectives addressed in this study included:

- 1) Characterize the variability of the pathogen population from various geographic locations in Israel and California. Pathogen strains were characterized for plasmid

with superior biocontrol potential. Two novel procedures for identifying antagonists were developed.

profile, virulence on several host, and serological characteristics, as well as DNA fingerprints with selected primers and streptomycin resistance. Results of this study enabled us to evaluate the heterogeneity of the *E. amylovora* population in the two countries and whether sufficient ecological differences exist among the target pathogen population to influence biological control using a single antagonist.

2) Determine the effects of plant variety on biocontrol agent. We studied the differential colonization of pear varieties with *P. fluorescens* A506 and addressed the question of whether it's ability to suppress the pathogen is similar on different cultivars.

3) Population dynamics of *P. fluorescens* A506 on pear flowers. We determined the distribution of A506 among pear flowers as a function of time after spray applications and as a function of different stages of tree development.

4) Study the mechanism by which *P. fluorescens* A506 suppresses *E. amylovora*. We addressed whether competition of A506 for limiting resources is the primary form of initial antagonism on the nectary and pistil and the role that early maturation of the flower plays in preventing the build-up of the pathogen inoculum and the infection process.

5) Selection of strains with biocontrol potential. Strain A506 is a very good colonist of pear blossoms and we expected it to be as effective in Israel as it is in California.

Although the weather conditions in the two countries are similar there is still a possibility that A506 would not survive well in Israel and would not achieve high population sizes on the common pear cultivars in Israel. The identification of additional bacterial agents from natural habitats in Israel and California enabled us to determine the efficacy of biocontrol with strain A506 relative to other strains and to identify strains

Relevant data, methodology, results and discussion

Nearly all of the studies conducted as part of this project have now been published, are in press, or are in preparation for publication. We therefore will report our results in the following 10 research publications. The last 2 publications (in preparation) summarizes the results obtained to date, but are not yet in a form ready for publication; we expect to submit these manuscripts soon. The relationship of the results reported in each publication to the original objectives of the project (as outlined earlier) should be obvious and will not be reiterated.

Description of the cooperation:

A very close cooperation has been established during the course of this study. This collaboration has been facilitated by frequent communication via electronic mail, through exchange of laboratory personnel, and through exchanging genetic materials. Dr. Manulis spent 6 months working in the laboratory of Dr. Lindow in Berkeley during the summer of 1996. Dr. Lindow spent several days during the summer of 1994 visiting the laboratories of Dr. Manulis and Zutra to discuss research results. The Israeli and Berkeley scientists also had the opportunity to meet and discuss results during the International symposium on Molecular Plant-Microbe Interactions held in Edinburgh Scotland in the summer of 1994 as well as at the American Phytopathological Society meeting in 1995. These frequent meeting permitted constant discussions and generation of ideas and experiments throughout the course of this study.

Evaluation of the research achievement:

The work conducted in this project was far-ranging. Our work was the first to characterize the nature of the fire blight pathogen in Israel. This important new disease had been relatively unstudied and we provided very useful data that showed that the pathogen is relatively homogenous both genetically and ecologically. This finding has great implications for future approaches to either chemical or biological control since it suggests that such strategies will not have to contend with heterogeneity in the pathogen population as in most other diseases. The data gathered in Israel was both basic and practical in nature. For example, in addition to genetic characterization of the pathogen populations, considerable data was generated that addressed the practicality of biological control of fire blight disease with antagonistic bacteria, and in the relative virulence of different strains of the pathogen and the susceptibility of different pear cultivars. The results of the biological control investigations reveal that the efficacy of biological control of fire blight on Israeli pear cultivars and under typical Israel field conditions was about 70%, similar to that observed with the same strains in the United States and elsewhere. In addition, we developed a very powerful new method, based on an ice nucleation screening approach, to identify antagonistic microorganisms that can reduce the population size of plant pathogens, and hence confer biological control. This approach was shown to be both quite efficient (hundreds of potential antagonists could be rapidly screened) as well as unbiased (no prior assumptions of mechanism of biological control must be invoked). During the development and demonstration of this screening method we also identified several antagonistic bacteria that appear to have superior biological

control abilities. Such strains should prove useful in future efforts to improve upon the already successful biological control agents that were evaluated in this study. Our data was sufficiently voluminous and of sufficient quality to generate 9 publications. We thus feel that we have thoroughly met the original objectives of the project, and through these studies have elucidated important features that enable us to evaluate the potential for biological control of fire blight disease, as well as having developed a very powerful new method by which antagonists of other diseases can be identified.

Conclusions:

A significant conclusion of the investigation into the biological control of fire blight disease in Israel was that this process operates in a similar fashion and to the same degree as in California and elsewhere. One of the limitations of biological control of other diseases has been the frequent observation that biological control is site-dependent. Often features of the pathogen population or the environment at different sites limited the effectiveness of antagonistic microorganisms that were developed at another site. This has led to inconsistent results in biological control. We saw little evidence that such factors will limit the effectiveness of biological control of fire blight in Israel. In fact, the biological control organism *P. fluorescens* strain A506 exhibited very similar population dynamics on pear trees and conferred very similar levels of biological control of fire blight disease as was observed in numerous tests in various sites in the United States. The finding that the population of *E. amylovora* in Israel is very homogeneous (even more homogeneous than the already rather homogeneous population of this pathogen elsewhere), also bodes well for future efforts for biological and chemical control of this pest. The degree of biological control of fire blight in Israel, about 3-fold, was very similar to that observed in studies elsewhere. This degree of biological control is useful, and may be sufficient in those sites where disease pressure is modest. Like all control measures, including chemical bactericides, control is incomplete and supplemental measures such as combinations of chemical and biological control as well as surgical removal of infections will need to be practiced to successfully manage fire blight in Israel, just as in other parts of the world where this disease has appeared. Future implementation of biological control of fire blight with strain A506 or

another antagonistic bacterium will need to address the compatibility of such strains with chemical bactericides such as antibiotics which will likely also be used for control. The widespread resistance of *E. amylovora* to streptomycin that was documented in this study indicates that streptomycin will not likely be an effective chemical control material in most locations and that other bactericides such as Starner or others will need to be evaluated for their compatibility with biological control agents such as strain A506. The compatibility of strain A506 with both streptomycin and Terramycin is one of its features that has led to the widespread adoption of this bacterium for biological control of fire blight in the United States, since it can be rather easily integrated into the management practices of most growers. Such adaptive research would need to be extended to Israel to verify that other pesticides applied to pear trees would not interfere with the biological control of fire blight by antagonistic microorganisms.

The finding that biological control of fire blight disease can be successfully implemented in Israel should be tempered with the observation that environmental conditions in Israel will occasionally be so conducive to disease that no control measure for fire blight may be successful. The several extremely severe outbreaks of fire blight that have occurred in the few years since the introduction of *E. amylovora* into Israel resemble the history of culture of pear in California, a state with a climate similar to that of Israel. In the early parts of this century pear was grown widely in all parts of California, including the Central Valley where warm early spring temperatures are common. The occasional coincidence of warm temperatures and spring rain, however, caused a series of devastating epidemics of fire blight to pear, which eventually largely led to the abandonment of pear culture in the Central Valley and its culture being

moved to cooler regions in northern valleys and the Sacramento River delta. While there is insufficient history of pear culture in the presence of *E. amylovora* in Israel, early signs suggest that in some areas of the country the coincidence of warm spring temperatures and moisture are too frequent to allow the culture of pear. The lack of control of fire blight even with effective bactericides under highly favorable weather conditions present a severe constraint to culture of pear under such conditions. Clearly, control of fire blight under cooler, drier conditions where the number of flower infections are far more limited is possible by either chemical or biological control agents, it may be difficult to maintain orchards in more conducive regions in those occasional years when disease is not manageable by existing methods.

The new method for identifying potential biological control agents that was developed in this study appears to be very robust and to be a powerful new approach by which antagonists of a variety of bacterial disease as well as fire blight can be identified. A major limitation in the selection of antagonistic microbes to be developed for biological control is that they are often selected under rather artificial culture conditions on plants in a rather unnatural way. For example, many antagonists are initially selected (or potential antagonists are de-selected) based on their ability to produce antibiotic-like compounds that inhibit the pathogen in culture media, or when injected into immature fruits in the case of antagonists of fire blight disease. Clearly the normal site of interaction of antagonists and the pathogen is not culture media and there is strong evidence that the chemical and physical environment of flowers or leaves where such interactions occur are very different from that in culture. The method that we developed in this study therefore overcomes the limitations inherent in pre-screening antagonists in

such conditions, and instead allowed us to determine directly which antagonists, when pre-inoculated onto pear flowers, prevented the growth of *E. amylovora* in the flowers themselves. By estimating the population size of an ice nucleation active derivative of *E. amylovora* we were able to directly quantify the effectiveness of potential antagonists while both the antagonist and the pathogen were in the normal site of infection on the plant. Because of the ease of estimating pathogen numbers using a flower freezing assay, we found that this method was sufficiently rapid that hundred of potential antagonists could be assayed for biological control potential, thereby abolishing the need to "pre-screen" antagonists for those which might be most effective. Because of this, we did not need to "bias" the selection method by scoring the antagonists for any trait exhibited in culture, and instead scored them directly of their ability to inhibit the pathogen growth *in planta*. We have found that all bacteria into which we have introduced the ice nucleation gene from *Pseudomonas syringae* can express ice nucleation activity. It therefore should be possible to adapt the plant-freezing assay for use with a variety of other bacterial plant pathogens. Indeed, there has been considerable interest in this new method since we published our results, and numerous researchers have requested the cloned ice nucleation genes to develop a similar selection scheme for pathogens for which biological control agents have not yet been identified. We found several antagonistic bacteria that appear to have biological control potential that is superior to that of *Pseudomonas fluorescens* strain A506. While the survival of such strains under the variable physical environment of pear orchards will need to be documented, some of these may prove to be commercially important in future efforts in biological control.

List of publications:

Lindow, S.E., G. McGourty, and R. Elkins. 1996. Interactions of antibiotics with *Pseudomonas fluorescens* strain A506 in the control of fire blight and frost injury to pear. *Phytopathology* 86:841-848.

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Patents

None