BARD Final Scientific Report Cover Page

Date of Submission of the report: December 22, 2004

BARD Project Number: US-3165-99R

Project Title: The Involvement of Antioxidant Enzymes and Genes in the Resistance Mechanism to Postharvest Superficial Scald Development

Investigators

Institutions

Principal Investigator (PI):

Chris B. Watkins

Cornell University

Co-Principal Investigator (Co-PI):

Susan Lurie

ARO, Min. Ag.

Collaborating Investigators:

Amnon Lers

ARO, Min. Ag.

Patricia L. Conklin

SUNY, Cortland

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.
apple, storage, physiological disorder, 1-methylcyclopropene

Abbreviations commonly used in the report, in alphabetical order:
1-MCP, APX, CAT, GR, POX, SOD

Budget: IS: \$150,000

US: \$150,000

Total: \$300,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	0	3	2	5
Submitted, in review, in preparation	1	2	0	3
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Sunita Kochhar0

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	0	2	3	5
Longer Visits (Sabbaticals)	0	0	0	0

Description of Cooperation:

Our cooperation has comprised of regular contact between the Israeli and US scientists during the course of this project by e-mail, discussions at scientific meetings and two visits to the US by Lurie. Interactions have included optimization of technical approaches, decisions about direction of the project, and a joint publication (in prep.).

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				0
Issued (allowed)				0
Licensed				0

Abstract

The objective of this research project was to evaluate the involvement of antioxidant enzymes and genes in the resistance mechanism to postharvest superficial scald development using two primary systems:

1. Resistant and susceptible progenies of an apple cross between a scald resistant crab apple, 'White Angel' and a scald susceptible cultivar, 'Rome Beauty';
2. Heat-treatment of 'Granny Smith', which is known to reduce scald development in this cultivar.

In 2002 we asked for, and received (October 14), permission to revise our initial objectives. The US side decided to expand their results to include further work using commercial cultivars. Also, both sides wanted to include an emphasis on the interaction between these antioxidant enzymes and the α -farnesene pathway, with the cooperation of a third party, Dr. Bruce Whitaker, USDA-ARS, Beltsville.

Background: Superficial scald is a physiological storage disorder that causes damage to the skin of apple and pear fruit. It is currently controlled by use of an antioxidant, diphenylamine (DPA), applied postharvest by drenching or dips, but concern exists about such chemical usage especially as it also involves application of fungicides. As a result, there has been increased emphasis on understanding of the underlying mechanisms involved in disorder development. Our approach was to focus on the oxidative processes that occur during scald development, and specifically on using the two model systems described above to determine if the levels of specific antioxidants and/or antioxidant enzyme activities correlated with the presence/absence of scald. It was hoped that information about the role of antioxidant-defense mechanisms would lead to identification of candidate genes for future transgenic manipulation.

Major conclusions, solutions, achievements: Collectively, our results highlight the complexity of superficial scald developmental processes. Studies involving comparisons of antioxidant enzyme activities in different crab apple selection, commercial cultivars, and in response to postharvest heat and 1-methylcyclopropene (1-MCP) treatments, show no simple direct relationships with antioxidant contents and susceptibility of fruit to scald development. However, a correlative relationship was found between POX activity or isoenzyme number and scald resistance in most of the studies. This relationship, if confirmed, could be exploited in breeding for scald resistance. In addition, our investigations with key genes in the α -farnesene biosynthetic pathway, together with antioxidant processes, are being followed up by analysis of exposed and shaded sides of fruit of cultivars that show different degrees of scald control by 1-MCP. These data may further reveal productive areas for future research that will lead to long term control of the disorder. However, given the complexity of scald development, the greatest research need is the production of transgenic fruit with down-regulated genes involved in α -farnesene biosynthesis in order to test the currently popular hypothesis for scald development.

Achievements

We measured activities of the antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), peroxidase (POX), and superoxide dismutase (SOD) and their isoenzyme profiles using our respective apple systems. In Israel, the effects of heat and the ethylene perception inhibitor, 1-methylcyclopropene (1-MCP), treatments that can delay scald development on Granny Smith were investigated. Heat delayed scald development while 1-MCP prevented it, and α -farnesene accumulation and oxidation were slower in the skin of heated than in control fruit, and very low in 1-MCP treated fruit. Antioxidant enzyme activities either increased or remained stable during storage, except for SOD. APX activity was higher in heated than control apples and an additional POX isoenzyme was present. The activities of antioxidant enzymes were lower in 1-MCP treated fruit except for CAT during the first month of storage. Lipid soluble antioxidant activity, water soluble antioxidant activity, and free and total phenol contents in the peel were either affected by treatment or effects were inconsistent (Shaham et al., 2003). In the US, a study with the crab apple progeny (selections) and commercial cultivars showed that a hypothesis that POX activity is related to scald susceptibility was generally supported, but exceptions existed (Fernandez-Trujillo et al., 2003). In further work (Kochhar et al., 2003), no consistent relationships between total enzyme activities and scald incidence of the selections were identified, high activities of the enzymes being detected in selections with both high and low susceptibilities to scald. However, additional individual isoforms of some antioxidant enzymes, especially POX, were detected in the scald-resistant selections when compared with scald-susceptible selections. Differences in the presence of various isoenzymes for other enzymes were detected among the selections, but associations with scald susceptibility were also affected by fruit color or were inconsistent. From these studies, it was concluded that differences in enzyme activity of the peel of 1-MCP and heated apples, and the selections, may be involved in scald development, but that the presence or absence of individual isoenzymes, especially of POX, may be a better indication of scald resistance or susceptibility than the total enzyme activities.

Collectively, therefore, our results suggested that analysis of POX isoenzymes in a range of commercial cultivars with different scald susceptibilities was warranted,

especially if they could be used by breeders for the early detection of scald resistance in apples. We initiated a trial in Israel and the US where we assessed activity gel patterns of POX in cultivars from both countries. The results, which are being prepared for publication (Lurie et al.), demonstrated associations between presence of POX isoenzyme bands and scald resistance, although exceptions were found. In a related study, we found that calcium has a protective role for POX activity and specific isoenzymes during heat treatment (Kochhar and Watkins, in prep)

With the agreement of BARD, we focused to a greater extent on α -farnesene metabolism; expression of the genes encoding α -farnesene synthase (AFS1), and HMG2 and HMG3, which encode isozymes of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, were followed in the scald-resistant Idared and -susceptible Law Rome and Granny Smith cultivars (Lurie et al., 2005; Pechous et al., 2005). A sharp increase in AFS1 mRNA during the first weeks of storage preceded a proportional rise in α -farnesene and a subsequent increase in oxidation products (CTols). However, maximum levels of AFS1 transcript, α -farnesene, and CTols were greater in Law Rome than in Idared apples. 1-MCP suppressed the increases in AFS1 transcript and α -farnesene early in storage, although AFS1 expression, α -farnesene synthesis, and ethylene production recovered in treated Law Rome fruit. Heat treatment of Granny Smith apples reduced levels of α -farnesene and CTols, but less than for 1-MCP. AFS1 transcript was absent from 1-MCP-treated apples for the first 10 weeks of storage, and even at 16 weeks was lower than in heated and untreated control fruit. Levels of the HMG2 and HMG3 transcripts varied during storage and among treatments, and were not correlated with the incidence of scald. The effect of heat treatment is a temporary inhibition of both ethylene synthesis and α -farnesene accumulation, while 1-MCP has a much stronger and longer lasting effect, inhibiting ethylene perception and production and α -farnesene accumulation. The inhibition of α -farnesene biosynthesis by 1-MCP is at the level of transcript abundance of AFS1, whereas heat treatment does not affect transcript abundance. However, heat treatment retards the onset of both α -farnesene synthesis and oxidation, delaying the appearance of scald symptoms on the fruit.

The agricultural implications for the use of 1-MCP are clear; it can prevent or delay scald development on apple cultivars. From the studies of Idared and Law Rome, and

that of heat-treated Granny Smith apples, the critical step in the propensity of an apple to develop scald appears to be the level of accumulation of CTols; i.e. the oxidation of α -farnesene. The scald-susceptible Law Rome had more CTols than the scald free Idared, and in heated Granny Smith the CTols took much longer to accumulate than in control apples. Once they passed a threshold level the heated apples developed scald. There are indications from the research conducted in this project that POX may be involved in the lower level of CTols present in the apples that do not develop scald, and this enzyme could be the focus of breeding (traditional or molecular) studies for scald resistance.

Our cooperation has comprised of regular contact between the Israeli and US scientists during the course of this project by e-mail, discussions at scientific meetings and two visits to the US by Lurie. Interactions have included optimization of technical approaches, decisions about direction of the project, and a joint publication (in prep.).

Publications published, in press, or in preparation

- Arquiza, A., Whitaker, B.D., Conklin, P.L., Watkins, C.B. 1-Methylcyclopropene (1-MCP) effects on α -farnesene and conjugated trienols, α -farnesene synthase gene AFS1 expression, and antioxidant activities of exposed and shaded sides of apple fruit during storage. In prep.
- Fernández-Trujillo, J. P., Nock, J.F., Kupferman, E.M., Brown, S.K., Watkins, C.B. 2003. Peroxidase activity and superficial scald development in apple fruit. *J. Agric. Food Chem.* 51: 7182-7186.
- Kochhar, S., Conklin, P.L., Brown, S.K., Watkins, C.B. 2003. A quantitative and qualitative analysis of antioxidant enzymes in relation to susceptibility of apple fruit to superficial scald. *J. Amer. Soc. Hort. Sci.* 129: 910-916.
- Kochhar, S., Watkins, C.B. Ameliorating heat inactivation of peroxidase from *Arabidopsis* leaves and apple peel by Ca^{++} and calmodulin. In prep.
- Lurie, S., Lers, A., Shacham, Z., Sonogo, L., Burd, S., Whitaker, B.D. 2005. Expression of α -farnesene synthase AFS1 and 3-hydroxy-3-methylglutaryl-coenzyme A reductase HMG2 and HMG3 in relation to α -farnesene and conjugated trienols in 'Granny Smith' apples heat or 1-MCP treated to prevent superficial scald. *J. Amer. Soc. Hort. Sci.* 131: in press.
- Lurie, S., Arquiza, A., Sonogo, L., Lers, A., Kochhar, S., Conklin, P.L., Watkins, C.B. Peroxidase isoenzyme relationships with scald susceptibility of apple cultivars. In prep.
- Pechous, S.W., Watkins, C.B., Whitaker, B.D. 2005. Expression of α -farnesene synthase gene AFS1 in relation to levels of α -farnesene and conjugated trienols in peel tissue of scald-susceptible 'Law Rome' and scald-resistant 'Idared' apple fruit Postharvest Biol. Technol. - in press.
- Shaham, Z., Lers, A., Lurie, S. 2003. Effect of heat or 1-methylcyclopropene on antioxidative enzyme activity and antioxidants in apples in relation to superficial scald development. *J. Amer. Soc. Hort. Sci.* 129: 761-766.

Appendix

<i>Published papers where BARD is acknowledged.....</i>	<i>1</i>
<i>A copy of each 'in press', 'accepted' or 'submitted paper'.....</i>	<i>18</i>
<i>Unpublished data.....</i>	<i>32</i>