FINAL REPORT
WTFRC Project #: PH-03-350 **USDA/ARS Project #:** 58-1275-0-004

Project title: Regulation of Farnesene Synthesis to Control Scald

PI: Bruce D. Whitaker
Organization: USDA-ARS, Produce Quality and Safety Laboratory
Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, MD

Co-PI: Steven W. Pechous (Research Associate, USDA-ARS, PQSL)

Cooperators: Christopher B. Watkins
Cornell University, Department of Horticulture
Susan Lurie Zaslavsky, ARO Volcani Center, Dept of Postharvest Science,
Bet Dagan, Israel

Objectives: (1) Obtain a genomic clone of the apple α-*FS* gene including the promoter and ethylene response element(s); (2) Isolate and characterize the promoter region from the genomic clone of the apple peel *HMG2* gene and determine whether expression of *HMG2* is regulated by ethylene; (3) Evaluate the correlation of ethylene-induced transcription of genes encoding enzymes in the α-farnesene synthetic pathway with accumulation of α-farnesene during storage, and with the incidence and severity of scald development; (4) Transform apple shoots with antisense or RNAi constructs of the *α-FS* gene (and the *HMG2* gene if time allows) driven by either the CaMV 35S or apple *α-FS* promoter and test transgenic apple plants for suppression of α-farnesene production.

Research priorities for the proposed objectives were amended for several reasons. Despite having finally obtained a full-length genomic clone of *HMG2* from 'Law Rome' roughly 12 months ago, we decided not to spend our efforts and limited resources on analysis of the *HMG2* promoter. This decision was based on the fact that both we, in studies with 'Law Rome', and our Israeli cooperators, in studies with 'Granny Smith', have not found evidence of ethylene-induced expression of *HMG2* in fruit peel tissue during the early weeks of 0 °C storage, as was reported for 'Delicious' fruit by Rupasinghe et al. (2001). We were successful in obtaining a full-length cDNA for the 'Law Rome' *HMG2*, which can be used in bacterial expression studies to biochemically characterize the HMG2 enzyme and eventually address the question of whether activity of the enzyme is regulated in vivo by post-translational modification. At the behest of the reviewers of our manuscript on the 'Law Rome' α-farnesene synthase gene *AFS1* (Pechous and Whitaker, 2004), about three months of work were devoted to biochemical characterization of the gene product (α-farnesene synthase; α-FS) expressed in *E. coli*. This work yielded valuable information about the enzymatic specificity of this unusual terpene synthase. Concerning efforts to clone the *AFS1* promoter as proposed under objective 1, a genomic fragment including about 500 bp of the open reading frame was obtained by PCR amplification using primers based on the cDNA sequence. We intend to use this fragment for a genome walk in the 5' direction to eventually obtain the promoter region. In February, postdoctoral Research Associate and Co-PI Steve Pechous left the PQSL rather abruptly after accepting a permanent position at NIH-NCBI. However, prior to his departure, Steve, in collaboration with Dr. Chris Watkins at Cornell, was able to complete the study proposed under objective 3. Using primers based on the 'Law Rome' *AFS1* cDNA, the corresponding *AFS1* cDNA from 'Idared' apple was obtained by PCR amplification after reverse transcription of peel tissue mRNA. This established a good experimental system for comparison of ethylene-induced *AFS1* gene expression and α-farnesene production and oxidation in fruit of the scald-susceptible Law Rome and scald-resistant Idared cultivars. A manuscript reporting this study has been submitted for publication. The

transformation studies with antisense or RNAi constructs of *AFS1* proposed under objective 4 await the hiring of another plant molecular biologist to replace Dr. Pechous. A suitable candidate has been identified and is eager to start the work, but he is a Canadian citizen and we have been waiting for nearly three months for clearance through Homeland Security. Funds from the WTFRC will fortunately not be forfeit since we were able to extend the existing ARS project into next year. Moreover, I was awarded an ARS Headquarters-funded postdoctoral position in 2003 to be hired in 2004 and continue the line of scald research proposed to the Commission last July.

Progress and significant findings: Project accomplishments and findings during the past 12 months (06/03 – 06/04) were as follows:

- 5' and 3' primers were designed based on the genomic sequence of 'Law Rome *HMG2* and a complete cDNA clone was obtained by 3'- and 5'-RACE using template cDNA derived from reverse transcription of young leaf tissue mRNA. This full-length *HMG2* cDNA has been cloned into a bacterial expression vector for transformation of *E. coli*. Expression in *E. coli* will provide HMG2 protein for biochemical characterization of the enzyme.

- The 'Law Rome' *AFS1* cDNA was expressed in *E. coli* and the terpene synthase product was assayed with different substrates (farnesyl diphosphate, FDP or geranyl diphosphate, GDP), with different buffers and divalent cations (Mg^{2+} or Mn^{2+}), and over the pH range of 5.0 to 8.0. The soluble terpene synthase produced by the bacterial cells catalyzed conversion of FDP to (*E*,*E*)-α-farnesene (>99%) plus a small amount of (*Z*,*E*)-α-farnesene (<1%). Incubation with GDP and Mg^{2+} yielded trace amounts of (*E*)-β-ocimene, a monoterpene analog of α-farnesene. The pH optimum was between 7.0 and 8.0; activity was reduced by ≥ 50% at pH 5.0–5.5, but (*E*,*E*)-α-farnesene remained essentially the only product.

- 5' and 3' primers based on the full-length 'Law Rome' *AFS1* cDNA were used to amplify and clone the corresponding *AFS1* cDNA from scald-resistant 'Idared' apple after reverse transcription of total RNA extracted from 'Idared' peel tissue. Sequence analysis of the two *AFS1* genes showed that the 1728-bp open reading frames differ by only 7 nucleotides and the encoded protein sequences differ by only 5 amino acids (AAs), with two pairs of the non-identical AAs being chemically similar. The are no AA substitutions in either the RR(X8)W or the DDXXD motif, both of which are critical for enzymatic activity.

- Control and 1-MCP-treated 'Law Rome' (LR) and 'Idared' (IR) fruit were stored for up to 20 weeks at 0.5 °C and evaluated for scald after 20 weeks at 0.5 °C plus 1 week at 20 °C. In untreated control fruit, maximum levels of internal ethylene, *AFS1* mRNA, α-farnesene, and conjugated trienols were, respectively, 4.2-, 2.5-, 4.0-, and 33-fold higher in LR than in IR. Scald incidence was 86% in LR, whereas IR fruit had no scald. 1-MCP treatment strongly inhibited increases in ethylene production, *AFS1* gene expression, and α-farnesene synthesis over the first 4–12 weeks of storage, and reduced scald in LR to <1%. These data confirm that *AFS1* gene expression is stimulated by ethylene in cold-stored fruit and show that the level of *AFS1* mRNA is proportional to the amount of α-farnesene produced early in storage. They also show a good correlation between conjugated trienol accumulation and scald development, and indicate that factors involved in α-farnesene oxidation are a critical component of scald susceptibility.

Methods: Current methods in molecular biology and biochemistry (including RNA and DNA gel blot and protein immunoblot analyses, DNA cloning and sequencing, expression of cloned apple genes in bacteria using suitable expression vectors, and enzyme assays and characterization using appropriate substrates and cofactors) were employed to examine the mechanism whereby ethylene stimulates the α-farnesene biosynthetic pathway in peel tissue of apple fruit during the first few weeks of cold storage. An untagged construct of the 'Law Rome' peel tissue *AFS1* cDNA was

expressed in *E. coli*. Assays of the soluble terpene synthase from lysed bacteria were conducted over the pH range of 5.0–8.0 with farnesyl diphosphate (FDP) as the substrate for sesquiterpene synthesis, and at pH 7.5 with geranyl diphosphate as the substrate for monoterpene synthesis. The divalent cation cofactor in both assays was Mg^{2+}. Analysis of the terpene products was performed by GC-MS using a 30 m CycloSil B chiral capillary column that efficiently separates terpene enantiomers. Biochemical characterization of 'Law Rome' AFS1 determined pH and temperature optima, divalent cation requirements (Mg^{2+} versus Mn^{2+}), and major and minor terpene products. Collaborators at Cornell University provided peel tissue from scald-susceptible 'Law Rome' and scald-resistant 'Idared' apples that were untreated or treated with 1 μL/L 1-MCP and stored for 0 to 20 weeks at 0.5 °C in air. Primers based on the 'Law Rome' *AFS1* cDNA sequence and 5' and 3' RACE were used with cDNA reverse-transcribed from 'Idared' peel tissue RNA to obtain a complete 'Idared' *AFS1* cDNA (open reading frame plus 3'-untranslated region). Primers based on our full-length genomic clone of 'Law Rome' *HMG2* were used with cDNA reverse-transcribed from RNA extracted from young leaf tissue of 'Law Rome' to clone a full-length *HMG2* cDNA. A radiolabeled cDNA probe consisting of the 3'-untranslated region of the *HMG2* cDNA was used for RNA gel blot (Northern) analysis of *HMG2* transcript levels in peel tissue of control and 1-MCP-treated 'Law Rome' fruit after 4 and 8 weeks of air storage at 0.5 °C.

Our collaborators at the Volcani Center in Israel evaluated the correlation between inhibition of α-farnesene synthesis following prestorage heat or 1-MCP treatment and reduction or prevention of scald in 'Granny Smith' apples. Northern blot analysis employing probes based on our 'Law Rome' *AFS1*, *HMG2*, and *HMG3* cDNA clones was performed to examine the expression of genes in the α-farnesene pathway in relation to α-farnesene production and eventual development of scald in 'Granny Smith' fruit stored at 0 °C in air.

Results and discussion: Two very significant findings from our previous studies were that 1-MCP treatment of scald-susceptible 'Law Rome' apples at harvest (1) strongly inhibited α-farnesene synthesis and largely prevented scald (**Fig. 1**; Watkins et al., 2000), and (2) blocked the ethylene-induced upregulation of *AFS1* expression in the early weeks of cold storage and resulted in a decline in *AFS1* transcript to almost nil by 8 weeks (**Fig. 2**; Pechous and Whitaker, 2004). Recently, our Israeli collaborators have corroborated both of these findings with fruit of the highly scald-susceptible cultivar Granny Smith (Shaham et al., 2003; unpublished data), and a number of other studies have shown that 1-MCP treatment is usually very effective for scald control (e.g., Fan et al., 1999b; Rupasinghe et al., 2000b). On the basis of these observations and reports, we will continue to use the *AFS1* gene and the inhibition of α-farnesene synthesis by 1-MCP as our experimental system to determine, at the molecular level, how ethylene induces production of α-farnesene in apples after a short duration in cold storage.

Superficial scald has long been associated with synthesis and oxidation of α-farnesene (Huelin and Murray, 1966), and although there is some contradictory evidence (Rupasinghe et al., 2000a), it is still generally accepted that oxidation products of α-farnesene are directly linked with development of severe scald. The most compelling evidence in support of the hypothesis is the demonstration by Rowan et al. (2001) that treatment of 'Granny Smith' apples with synthetic conjugated triene alcohol and hydroperoxide oxidation products of α-farnesene induced symptoms indistinguishable from "natural" superficial scald. In addition, as stated above, studies comparing untreated control and 1-MCP-treated scald-susceptible apples have repeatedly shown that ethylene perception is critical for induction of both α-farnesene production and scald development (Fan et al., 1999b; Rupasinghe et al., 2000b; Watkins et al., 2000; Shaham et al., 2003).

An understanding of the genetic and physiological controls of α-farnesene synthesis in harvested apple fruit should enable molecular genetic manipulation to dramatically reduce production of the sesquiterpene. Alternatively, comparison of the *AFS1* gene and its regulation in highly scald-susceptible and scald-resistant apple cultivars (e.g., 'Law Rome' and 'Idared', respectively) should

identify unique genetic markers that could be used in a conventional breeding program to select potentially scald-resistant lines at the seedling stage of development. With either approach, this is clearly a long-term endeavor, but the potential benefits to both the apple industry and the consumer are great. Specific elimination of α-farnesene synthesis in fruit of a highly scald-susceptible cultivar such as 'Law Rome' or 'Granny Smith' would provide proof of whether α-farnesene is required for scald induction. If it is, as many studies have indicated, it should be possible to develop new scald-resistant lines of popular commercial apple cultivars that no longer require a pre-storage drench with DPA plus a fungicide. Transgenic apple lines with antisense or RNAi suppression of *AFS1* would not include any foreign genes. Furthermore, specifically inhibiting α-farnesene synthesis by crippling a gene exclusively involved in the sesquiterpene pathway would not affect any other desirable trait of the fruit. The closest approximation to this is pre-storage treatment of apples with 1-MCP, which strongly inhibited α-farnesene synthesis, apparently by blocking ethylene-dependent expression of *AFS1*, and greatly reduced scald in 'Law Rome' fruit (**Figs. 1** and **2**; Watkins et al., 2000; Pechous and Whitaker, 2004). However, blocking ethylene action with 1-MCP affects many aspects of fruit physiology and biochemistry, not just α-farnesene production (Fan et al., 1999a,b). One additional, very useful outcome of this research will be a better understanding of how ethylene induces the expression of genes involved in production of volatiles and other ripening- and senescence-related processes. At present, there is very little known about the final steps of the ethylene signal transduction cascade, in which specific DNA-binding proteins recognize ethylene-response elements in the gene promoter and induce or suppress transcription of the gene (Deikman, 1997; Deikman et al., 1998).

Biochemical characterization of the 'Law Rome' AFS1 enzyme expressed in *E. coli* highlighted the advantages of this approach over previous efforts to assay the native α-farnesene synthase in extracts of apple peel tissue (Rupasinghe et al., 2000a). Assays with the crude enzyme from apple indicated an acidic pH optimum of about 5.6, whereas the bacterially expressed AFS1 clearly had a slightly alkaline pH optimum of about 7.5. This difference may have been due to the presence of potent pyrophosphase activity in the apple extracts, which cleaved much of the radiolabeled FDP substrate. Expression of AFS1 in bacteria also yielded a sufficient amount of active enzyme to clearly identify the terpene products using unlabeled FDP and GDP substrates (Pechous and Whitaker, 2004). A rather remarkable result was that AFS1 expressed from the cDNA and assayed in vitro yielded the 3*E*,6*E* and 3*Z*,6*E* isomers of α-farnesene in a ratio of about 200:1, which is close to the isomeric ratio of α-farnesene extracted from 'Granny Smith' apples.

Having proved that the 'Law Rome' terpene synthase gene that is upregulated in fruit peel tissue in response to ethylene during the early weeks of 0 °C storage encodes (*E*,*E*)-α-farnesene synthase, primers based on the 'Law Rome' *AFS1* cDNA were used to clone a corresponding *AFS1* cDNA from scald-resistant 'Idared' apple. Not surprisingly, the 'Law Rome' and 'Idared' *AFS1* cDNAs differ by only 7 nucleotides. As well, their encoded proteins differ by only 5 amino acids, with no substitutions in either the DDXXD or the RR(X8)W motif, which are both critical for enzymatic activity (**Fig. 3**; Pechous et al., submitted). An important next step toward understanding induction of α-farnesene synthesis in apple peel tissue by ethylene and low temperature will be to use primers based on the *AFS1* cDNA sequences to isolate genomic clones that include the entire promoter and ethylene-response element in the 5'-flanking region. This work has been initiated for 'Law Rome' *AFS1* and will resume as soon as another plant molecular biologist is hired. Because the open reading frames of the 'Law Rome' and 'Idared' *AFS1* genes are nearly identical and their bacterially expressed enzymes have the same activity, the markedly different rates of α-farnesene production in fruit of the two cultivars is likely the result of differences in the gene promoters and expression of the genes. In our recently completed study of levels of *AFS1* transcript, internal ethylene, α-farnesene, and conjugated trienols in relation to scald in 'Law Rome' and 'Idared' apples, we found that although fruit of both cultivars showed a rise in α-farnesene production over the first 8–12 weeks of storage, which was inhibited by 1-MCP, α-farnesene accumulation was much greater in 'Law Rome' than in 'Idared'

(**Fig. 4**). Moreover, maximum levels of *AFS1* transcript and internal ethylene were, respectively, about 2.5- and 4.2-fold higher in 'Law Rome' and roughly proportional to the amount of α-farnesene produced (**Fig. 5**; **Table 1**). The maximum level of conjugated trienols was about 33-fold higher in 'Law Rome' than in 'Idared' (**Fig. 4**), indicating that 'Idared' has more antioxidants and/or more active antioxidative enzymes, as reported by Fernández-Trujillo et al. (2003). We will explore this finding further in the coming year in our collaborative studies with Dr. Chris Watkins at Cornell. **Fig. 6** shows the temporal relationship of increases in *AFS1* mRNA, α-farnesene, and conjugated trienols during cold storage of untreated 'Law Rome' apples, clearly showing the correlation between *AFS1* expression and α-farnesene synthesis and oxidation. Future work to purify, characterize, and construct antibodies against the AFS1 protein should answer important questions about the levels of this critical enzyme in apple peel tissue at harvest and during storage.

A complete cDNA of 'Law Rome' *HMG2* was recently obtained using RNA isolated from young leaf tissue. We anticipate performing bacterial expression studies (as in Pechous and Whitaker, 2002) for biochemical characterization of the HMG2 enzyme. However, it is unlikely that we will attempt to transform apple with antisense or RNAi constructs of this gene in light of our finding (by Northern blot analysis) that *HMG2* expression appears to be stimulated rather than inhibited in 1-MCP-treated fruit.

The tools are now available for *Agrobacterium*-mediated transformation of apple tissue with antisense or RNAi constructs of the *AFS1* gene. Regeneration of stable transgenic plantlets will likely be the most difficult and time-consuming task in continuing this line of research. Because apple leaf tissue is capable of α-farnesene production, transgenic plants could be tested at the seedling stage for reduced levels of A*FS1* mRNA, as well as α-farnesene in the headspace volatiles (Mir and Beaudry, 1999). If we are able to generate stable transgenic plants of a highly scald-susceptible cultivar such as 'Law Rome' or 'Granny Smith' that exhibit a >90% reduction in α-farnesene synthesis, we will definitely follow through (undoubtedly as a collaborative effort) with the long-range processes of grafting to appropriate root stock and growing trees to the fruit-bearing stage.

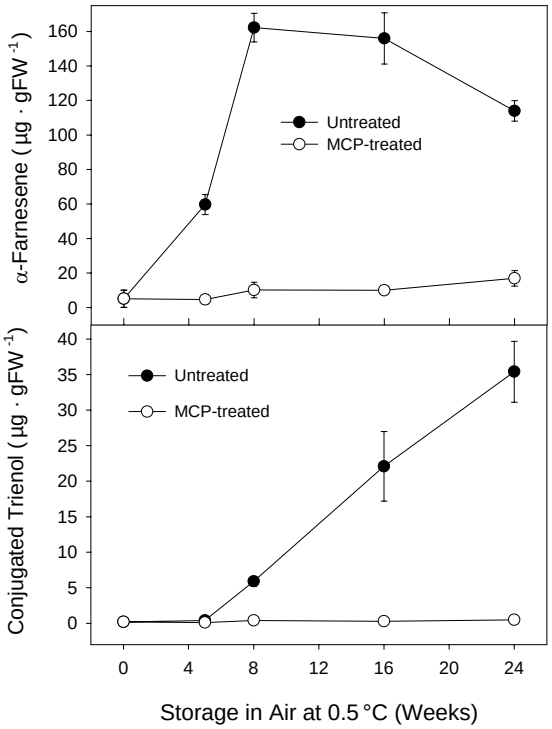

Fig. 1 Effect of 1-MCP on accumulation of α-farnesene and its conjugated trienol oxidation products during storage of 'Law Rome' apples. Scald incidence was 98% in control and 1% in 1-MCP-treated fruit.

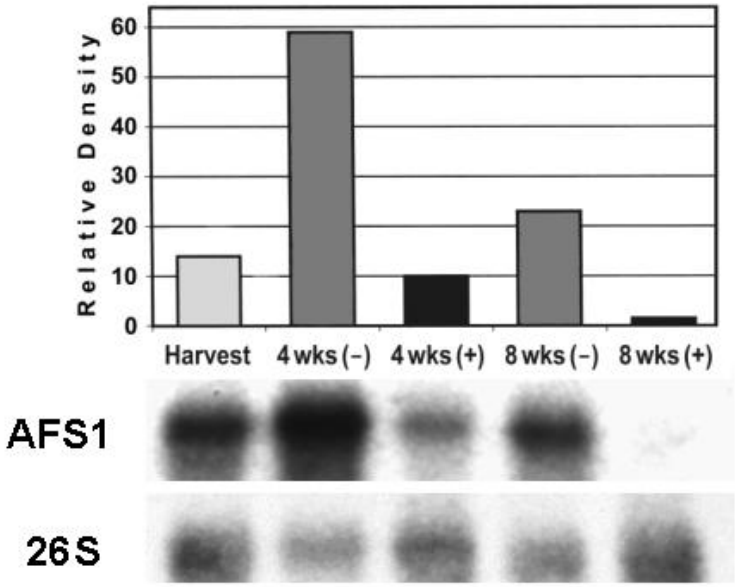

Fig. 2 Effect of 1-MCP treatment (**+**; black bars) on changes in the level of α-Farnesene Synthase (**AFS1**) mRNA in peel tissue of 'Law Rome' apples over 4 to 8 weeks of storage in air at 0.5 °C.

Fig. 3 Alignment of deduced amino acid sequences of 'Law Rome' and 'Idared' *AFS1* genes

(*E*,*E*)-α-farnesene synthase [*Malus domestica*] Length = 576 amino acids
Law Rome – accession no. AAO22848; Idared – accession no. AAS01424
LR vs IR: Identities = 571/576 (99%), Positives = 573/576 (99%)

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AAO22848   1
MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
AAS01424   1
MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60

AAO22848   61
YRKLSEKLIEEVKIYISAETMDLVAKLELIDSVRKLGLANLFEKEIKEALDSIAAIESDN 120
AAS01424   61
YRKLSEKLIEEVKIYISAETMDLVAKLELIDSVRKLGLANLFEKKIKEALDSIAAIESDN 120

AAO22848   121
LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180
AAS01424   121
LGTRDDLYGAALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180

AAO22848   181
FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVVHSLELPSHRRVQWFDVKWQINAYE 240
AAS01424   181
FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVVHSLELPSHRRVQWFDVKWQINAYE 240

AAO22848   241
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AAS01424   241
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AAS01424   301
GVAFEPEHSSFRICLTKVINLVLIIDDVYDIYGSEEELKHFTNAVDRWDSRETEQLPECM 360

AAO22848   361
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LRNGCISSSVSVLLVHSFFSITHEGTKEMADFLHKNEDLLYNISLIVRLNNDLGTSAAEQ 480

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AAS01424   481
ERGDSPSSIVCYMREVNASEETARKNIKGMIDNAWKKVNGKCFTTNQVPFLSSFMNNATN 540

AAO22848   541 MARVAHSLYKDGDGFGDQEKGPRTHILSLLFQPLVN 576
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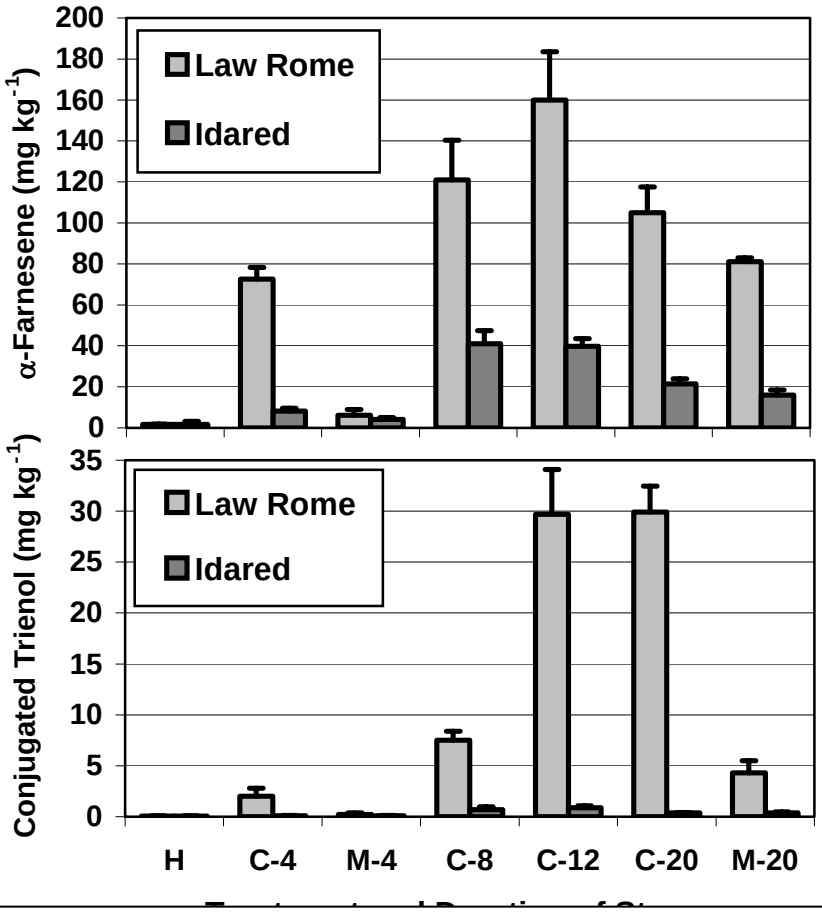

Fig. 4 Accumulation of α-farnesene and its conjugated trienol oxidation products in peel tissue of control and 1-MCP-treated fruit of scald-susceptible 'Law Rome' and scald-resistant 'Idared' over 20 weeks of storage in air at 0.5 °C. H = harvest, C = control, M = 1-MCP-treated

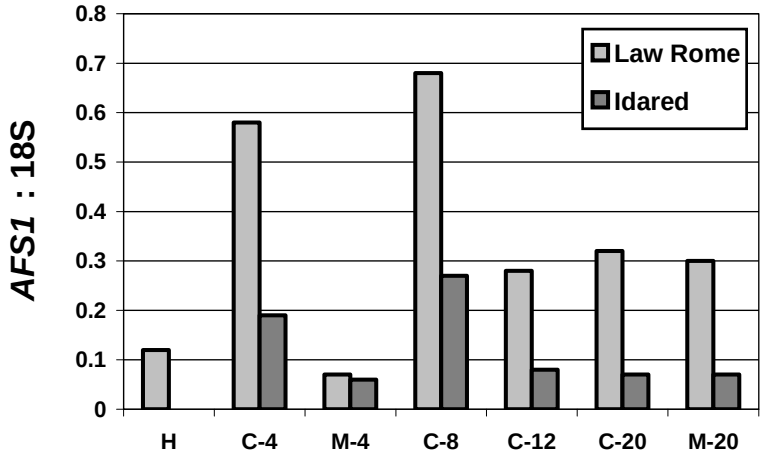

Fig. 5 Changes in *AFS1* transcript levels in peel tissue of control and 1-MCP-treated 'Law Rome' and 'Idared' apples over 20 weeks in air at 0.5 °C. H = harvest, C = control, M = 1-MCP-treated

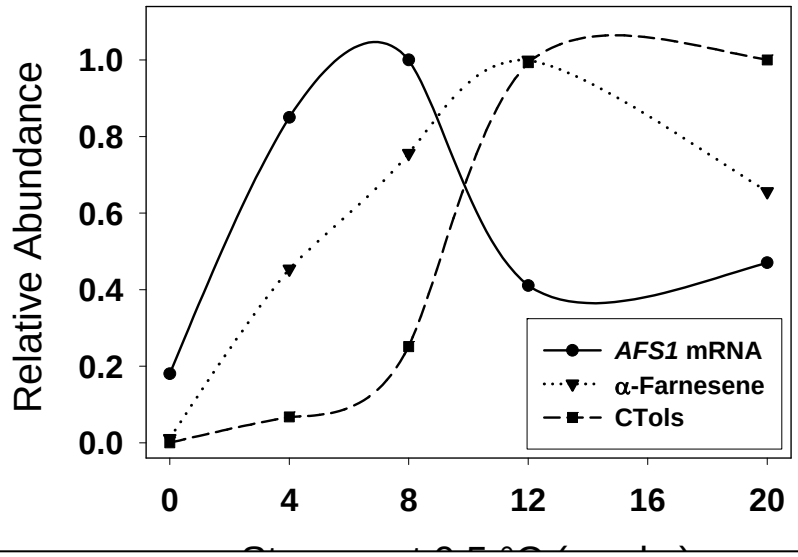

Fig. 6 Changes in the relative levels of *AFS1* mRNA, α-farnesene, and conjugated trienols (CTols) in peel tissue of untreated 'Law Rome' apples over 20 weeks of storage at 0.5 °C in air. Data points were normalized relative to the highest value determined for each constituent, which was assigned a value of 1.0.

	Hvst	Untreated controls				1-MCP treated	
		4 wks	8 wks	12 wks	20 wks	4 wks	12 wks
Law	3.0	27.7	34.8	26.9	11.2	1.1	9.8

Table 1 Internal ethylene concentrations (μL/L) in untreated and 1-MCP-treated 'Law Rome' and 'Idared' apples at harvest and from 4 to 20 weeks of storage at 0.5 °C in air.

Budget: One year of funding to pay ~86% of the GS-11 salary for postdoctoral Research Associate Steve Pechous was awarded in September 2003 and received in May 2004.

Project duration: 2003-2004

Current year: 2004

Budget request

Yearly breakdown over project duration

Item	Year 1 (2003)	Year 2 (2004)
Salary (GS-11-02)	45,000*	0
Total	45,000	0

* Represents ~86% of GS-11-02 salary with locality pay = $52,279

Other funding sources: Base funding for our research is provided by ARS through CRIS Project # 1275-43000-007-00D, "Quality Maintenance and Food Safety of Fresh and Fresh-cut Fruits and Vegetables," which includes 3 staff scientists, 4 technicians, and several other support personnel. The total allocation for FY-2003 was $1,013,079. Of this amount, $921,374 was expended on salaries and indirect research costs, leaving $91,705 for all other expenses.

References:

Deikman, J. 1997. Molecular mechanisms of ethylene regulation of gene transcription. Physiol. Plant. **100**, 561–566.

Deikman, J., Xu, R., Kneissl, M.L., Ciardi, J.A., Kim, K.-N., Pelah, D. 1998. Separation of *cis* elements responsive to ethylene, fruit development, and ripening in the 5'-flanking region of the ripening-related E8 gene. Plant. Molec. Biol. **37**, 1001–1011.

Fan, X., Blankenship, S.M., Mattheis, J.P. 1999a. 1-Methylcyclopropene inhibits apple ripening. J. Amer. Soc. Hort. Sci. **124**, 690–695.

Fan, X., Mattheis, J.P., Blankenship, S.M. 1999b. Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. J. Agric. Food Chem. **47**, 3063–3068.

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.

Huelin, F.E., Murray, K.E. 1966. α-Farnesene in the natural coating of apples. Nature (Lond.) **210**, 1260–1261.

Mir, N.A., Beaudry, R. 1999. Effect of superficial scald suppression by diphenylamine application on volatile evolution by stored Cortland apple fruit. J. Agric. Food Chem. **47**,7–11.

Pechous, S., Whitaker, B.D. 2002. Cloning and bacterial expression of a 3-hydroxy-3-methylglutaryl-CoA reductase cDNA (*HMG1*) from peel tissue of apple fruit. J. Plant Physiol. **159**, 907–916.

Pechous, S.W., Whitaker, B.D. 2004. Cloning and functional expression of an (*E*,*E*)-α-farnesene synthase cDNA from peel tissue of apple fruit. Planta **219**, 84–94.

Rowan, D.D., Hunt M.B., Fielder S., Norris J., Sherburn M.S. 2001. Conjugated triene oxidation products of α-farnesene induce symptoms of superficial scald on stored apples. J. Agric. Food Chem. **49**, 2780–2787.

Rupasinghe, H.P.V., Paliyath, G., Murr, D.P. 2000a. Sesquiterpene farnesene synthase: partial purification, characterization, and activity in relation to superficial scald. J. Amer. Soc. Hort. Sci. **125**, 111–119.

Rupasinghe, H.P.V., Murr, D.P., Paliyath, G., Skog, L. 2000b. Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. J. Hort. Sci. Biotechnol .**75**, 271–276.

Rupasinghe H.P.V., Almquist, K.C., Paliyath, G., Murr, D.P. 2001. Cloning of *hmg1* and *hmg2* cDNAs encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase and their expression and activity in relation to α-farnesene synthesis in apple. Plant Physiol Biochem **39**, 933–947.

Shaham, Z., Lers, A., Lurie, S. 2003. Effect of heat or 1-methylcyclopropene on antioxidative enzyme activities and antioxidants in apples in relation to superficial scald development. J. Amer. Soc. Hort. Sci. **128**, 761–766.

Watkins, C.B., Nock, J.F., Whitaker, B.D. 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. **19**, 17–32.