FINAL REPORT
2005 addendum **DURATION: 03-04**
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-PIs: Steven W. Pechous (Research Associate, USDA-ARS, PQSL through 02/20/2004)
Nigel E. Gapper (Research Associate, USDA-ARS, PQSL beginning 10/18/2004)

Cooperators: Christopher B. Watkins
Cornell University, Department of Horticulture, Ithaca, NY
Susan Lurie, ARO Volcani Center, Department of Postharvest Science,
Bet Dagan, Israel
Jinhe Bai, Oregon State University, Mid-Columbia Agricultural Research and
Extension Center, Hood River, OR

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.

As stated last year, research priorities for the proposed objectives were amended. Work on the apple peel *HMG2* from 'Law Rome' was abandoned after finding that this gene is not up-regulated by ethylene and is unlikely to play a major role in production of α-farnesene during storage. In February 2004, postdoctoral Research Associate and Co-PI Steve Pechous left the PQSL after accepting a permanent position. Prior to his departure, in collaboration with Dr. Chris Watkins at Cornell, Steve completed the study proposed under objective 3. Using primers based on the 'Law Rome' *AFS1* cDNA, the *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 cvs. A report of this study was recently published (Postharvest Biol. Technol. 35:125-132, 2005). Efforts to clone the *AFS1* promoter as proposed under objective 1 were renewed with the hiring in October 2004 of plant molecular biologist Nigel Gapper, who filled an ARS Headquarters-funded postdoctoral position awarded in 2003. Genome walk experiments have yielded a 1.5-kb genomic DNA fragment upstream of the 'Law Rome' *AFS1* open reading frame. The first difficult and laborious step required for the transformation studies with RNAi constructs of *AFS1* proposed under objective 4 was taken this spring; shoots of 'Law Rome' and 'Delicious' were harvested from a commercial orchard and established in tissue culture. Seizing an opportunity, last fall we entered a collaboration with Dr. Jinhe Bai (Oregon State – MCAREC) to investigate scald control by 1-MCP in 'd'Anjou' pear in relation to *AFS* gene expression and α-farnesene synthesis and oxidation. Dr. Gapper has cloned the complete cDNA for *Pyrus communis AFS* and the study should be completed in several months.

Progress, significant findings, and future plans: Project accomplishments and findings during the past 12 months (06/04 – 06/05) are listed, and future plans and applications are described below:

- 5' and 3' primers were designed based on the cDNA sequences of 'Law Rome' and 'Idared' apple *AFS1* and used with reverse-transcribed peel tissue RNA from scald-susceptible 'd'Anjou' pear fruit to obtain a complete *Pyrus communis AFS* cDNA (Pc*AFS*) by 3'- and 5'-RACE. The open reading frame of Pc*AFS* is 97% identical to the corresponding apple sequences, and the deduced amino acid sequence of the encoded PcAFS protein is 95-96% identical to the sequences of AFS from 'Law Rome', 'Idared', and 'White Pearmain' apple (**Figure 1**).

- A collaborative study with Dr. Jinhe Bai at OSU-MCAREC compared expression of the pear α-farnesene synthase gene Pc*AFS*, accumulation of α-farnesene and its conjugated trienol oxidation products (CTols), and scald incidence in control and 1-MCP-treated (300 ppb) 'd'Anjou' pears stored up to 6 months at –1 °C. PC*AFS* expression, determined by quantitative RT-PCR using the 18S competimer system (Ambion, Inc.), showed a marked increase about one month earlier in control vs. 1-MCP-treated fruit, continued to increase up to 5 months, and remained about 1.5- to 2-fold higher in the controls from 2 to 5 months of storage (**Figure 2**). Both α-farnesene and CTols reached very high levels in control fruit (200 and 90 µg/g FW at 3 and 4 months, respectively) then declined after 4 months (**Figure 3**). Maximum levels of α-farnesene and CTols were 5 and 12-fold lower, respectively, in 1-MCP-treated vs. control fruit. Scald incidence was 100% in controls after 3 to 5 months of storage, whereas 1-MCP-treated fruit were scald-free until 6 months, when scald incidence was 13%.

- In April 2005, branchlets were cut from trees of 'Law Rome' and 'Red Delicious' growing in a commercial orchard, and young shoots were excised, sterilized, and placed on tissue culture medium. After initial severe problems with contaminant microorganisms from the orchard, it appears that modified procedures have enabled the establishment of viable shoots in sterile culture. These will be maintained and propagated, and ultimately used in transformation experiments with an RNAi construct of *AFS1* in *Agrobacterium*. On the basis of sequencing data, initial efforts to generate an RNAi construct using the 'Law Rome' *AFS1* cDNA appear to have been successful.

- Genome walk from the 5'-end of the 'Law Rome' *AFS1* open reading frame using genomic DNA prepared from young leaf tissue yielded about a 1.5-kb fragment of the *AFS1* promoter region. Experiments currently in progress will attempt to clone another 1.0 to 1.5 kb upstream of the existing promoter fragment prior to complete promoter sequence analysis and efforts to identify ethylene (and possibly low temperature) response elements. The same strategy will be used to clone the *AFS1* promoter from scald-resistant 'Idared' apple, with the aim of determining whether differences in regulatory elements of the *AFS1* promoters from the two cultivars can account for the very different levels of *AFS1* expression during air storage at 0.5 °C. It is also planned to use the 'Law Rome' *AFS1* promoter to drive the *AFS1* RNAi construct in transgenic apple plants, with the rationale that it should serve to silence the gene after fruit are placed in cold storage.

Publications in past 12 months:

Lurie, S., Lers, A., Shacham, Z., Sonego, L., Burd, S., Whitaker, B.D. 2005. Expression of α-farnesene synthase *AFS1* and 3-hydroxy-3-methylglutaryl-CoA *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.. **130**, 232-236.

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. **35**, 125–132.

Figure 1. Alignment of deduced amino acid (AA) sequences encoded by α-farnesene synthase genes cloned from fruit peel tissue of three apple cultivars and 'd'Anjou' pear. AA substitutions are in bold type (chemically similar) or white bold type with gray highlighting (chemically dissimilar).

```
White Pearmain  MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
Idared          MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
Law Rome        MEFRVHLQADNEQKIFQNQMKPEPEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
d'Anjou         MEFRVHLHADHEQKILQNQMKPEHEASYLINQRRSANYKPNIWKNDFLDQSLISKYDGDE 60
                *******:**:****:******* ************************************

White Pearmain  YRKLSEKLIEEVKIYISAETMDLVAKLELIDSVRKLGLANLFEKEIKEALDSIAAIESDN 120
Idared          YRKLSEKLIEEVKIYISAETMDLVAKLELIDSVRKLGLANLFEKKIKEALDSIAAIESDN 120
Law Rome        YRKLSEKLIEEVKIYISAETMDLVAKLELIDSVRKLGLANLFEKEIKEALDSIAAIESDN 120
d'Anjou         YRKLSEKLIEEVKIYISAETKDLVAKLELIDSVRKLGLANHFEKEIKEALDGIAAIESDN 120
                ****************** ****************** ***:******.******** 

White Pearmain  LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180
Idared          LGTRDDLYGAALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180
Law Rome        LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKGTLENHHFAHLKGMLELFEASNLG 180
d'Anjou         LGTRDDLYGTALHFKILRQHGYKVSQDIFGRFMDEKDTLENHHFAHLKGMLELFEASNLG 180
                *********:************************.********************** 

White Pearmain  FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVVHSLELPSHRRVQWFDVKWQINAYE 240
Idared          FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVVHSLELPSHRRVQWFDVKWQINAYE 240
Law Rome        FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVVHSLELPSHRRVQWFDVKWQINAYE 240
d'Anjou         FEGEDILDEAKASLTLALRDSGHICYPDSNLSRDVVHSLELPSHRRVQWFDVKWQINAYE 240
                ************************************************************ 

White Pearmain  KDICRVNATLLELAKLNFNVVQAQLQKNLREASRWWANLGFADNLKFARDRLVECFSCAV 300
Idared          KDICRVNATLLELAKLNFNVVQAQLQKNLREASRWWANLGFADNLKFARDGLVECFSCAV 300
Law Rome        KDICRVNATLLELAKLNFNVVQAQLQKNLREASRWWANLGIADNLKFARDRLVECFACAV 300
d'Anjou         KDICRVNATLLGLAKLNFNMVQAQLQKDLREASKWWANLGIADNLKFARDRLVECFACAV 300
                *********** *******:*******:*****:******:********* *****:*** 

White Pearmain  GVAFEPEHSSFRICLTKVINLVLIIDDVYDIYGSEEELKHFTNAVDRWDSRETEQLPECM 360
Idared          GVAFEPEHSSFRICLTKVINLVLIIDDVYDIYGSEEELKHFTNAVDRWDSRETEQLPECM 360
Law Rome        GVAFEPEHSSFRICLTKVINLVLIIDDVYDIYGSEEELKHFTNAVDRWDSRETEQLPECM 360
d'Anjou         GVAFEPEYSSFRICLTKVINLVLIIDDVYDIYGSEEELKHFTNAVDRWDSRETEQLPECM 360
                *******:****************************************************** 

White Pearmain  KMCFQVLYNTTCEIAREIEEENGWNQVLPQLTKVWADFCKALLVEAEWYNKSHIPTLEEY 420
Idared          KMCFQVLYNTTCEIAREIEEENGWNQVLPQLTKVWADFCKALLVEAEWYNKSHIPTLEEY 420
Law Rome        KMCFQVLYNTTCEIAREIEEENGWNQVLPQLTKVWADFCKALLVEAEWYNKSHIPTLEEY 420
d'Anjou         KMCFQVLYNTTCEIAHEIEKDNGWNQVLPQLTKVWADFCKALLVEAEWYNKSHIPTLEEY 420
                ***************:***::************************************** 

White Pearmain  LRNGCISSSVSVLLVHSFFSITHEGTKEMAGFLHKNEDLLYNISLIVRLNNDLGTSAAEQ 480
Idared          LRNGCISSSVSVLLVHSFFSITHEGTKEMADFLHKNEDLLYNISLIVRLNNDLGTSAAEQ 480
Law Rome        LRNGCISSSVSVLLVHSFFSITHEGTKEMADFLHKNEDLLYNISLIVRLNNDLGTSAAEQ 480
d'Anjou         LRNGCDSSSVSILLVHSFFSITHEGTKEMADFLHKNEDLLYNLSLIVRLNNDLGTSAAEQ 480
                ***** *****:******************.***********:****************** 

White Pearmain  ERGDSPSSIVCYMREVNASEETARKNIKGMIDNAWKKVNGKCFTTNQVPFLSSFMNNATN 540
Idared          ERGDSPSSIVCYMREVNASEETARKNIKGMIDNAWKKVNGKCFTTNQVPFLSSFMNNATN 540
Law Rome        ERGDSPSSIVCYMREVNASEETARKNIKGMIDNAWKKVNGKCFTTNQVPFLSSFMNNATN 540
d'Anjou         ERGDSPSSIVCYMREVNASEETARKNIKGMIDNAWKKVNGKCFTTNQVPFLSSFMNNATN 540
                ******************** ************************************** 

White Pearmain  MARVAHSLYKDGDGFGDQEKGPRTHILSLLFQPLVN 576
Idared          MARVAHSLYKDGDGFGDQEKGPRTHILSLLFQPLVN 576
Law Rome        MARVAHSLYKDGDGFGDQEKGPRTHILSLLFQPLVN 576
d'Anjou         MARVAHSLYKDGDGFGDQEKGPRTHILSLLFQPLVN 576
                ********************************** 
```

Figure 2. Relative quantitative RT-PCR of Pc*AFS1* in control and 1-MCP-treated fruit using universal 18S competimer oligos and 18S primer pairs at a 9:1 ratio.

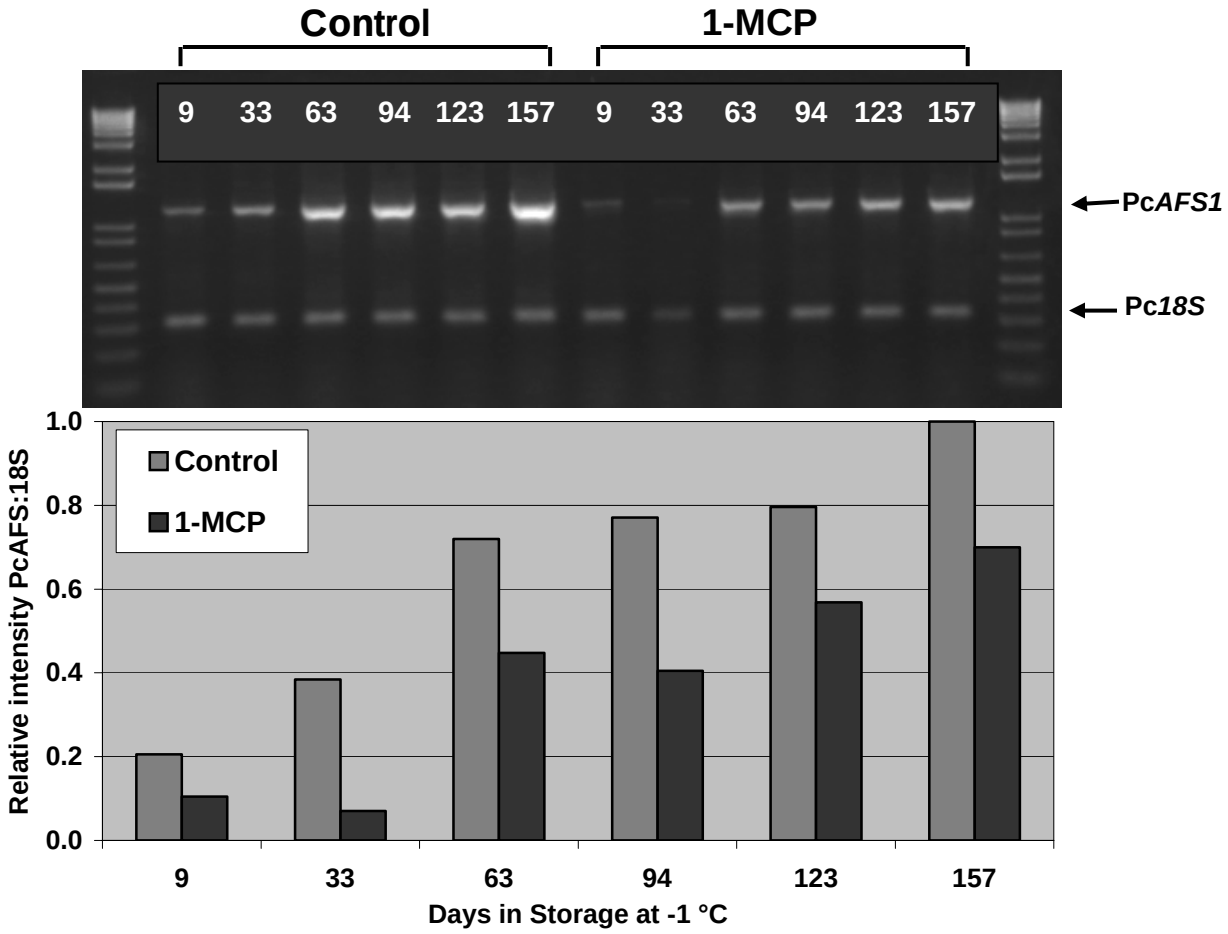

Figure. 3 Accumulation of α-farnesene and its conjugated trienol oxidation products in peel tissue of control and 1-MCP-treated (300 ppb) fruit of scald-susceptible 'd'Anjou' pear over 5 months of storage in air at -1 °C.