**PROJECT NO.**: 14105

**Project title:** Environmental Effects on Storage of Winter Pears

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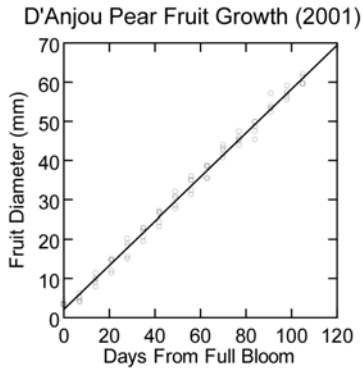
**Organization:** USDA, ARS, TFRL, Wenatchee, WA

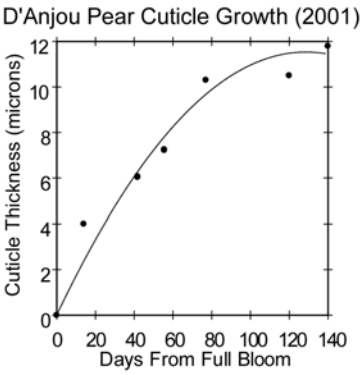
**Objectives:** There are complex interrelationships among climate, ripening, and the development of storage disorders, which, because they are not well understood, have resulted in incongruencies in reports of ripening behavior and scald development. This project is an attempt to better understand the influence of preharvest temperature on cuticle and wax development, fruit ripening potential, development of physiological disorders and their interrelationships by identifying effects of preharvest temperature on ripening behavior, fruit quality, storage disorders, cuticle development, wax accumulation, and antioxidant efficacy of 'd'Anjou' pears.

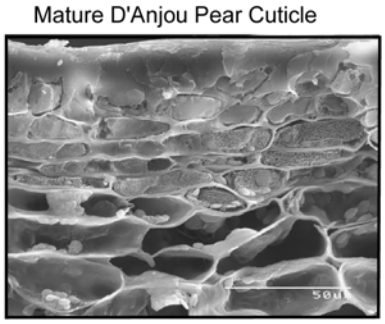
**Results and Discussion:**

Several studies were conducted this year. The first was to examine and measure development of the cuticle of pears growing at different elevations (microclimates). This was done both with light and electron microscopes.

As soon as the surface cells became exposed to dessication pressure, cuticle development began. This varied from location to location depending on weather conditions and loss of pubescence. It appeared early pubescence tended to delay cuticle development probably by increasing the relative humidity thereby reducing dessication pressure. As the fruit expanded and pubescence density decreased, exposure increased and wax development increased.



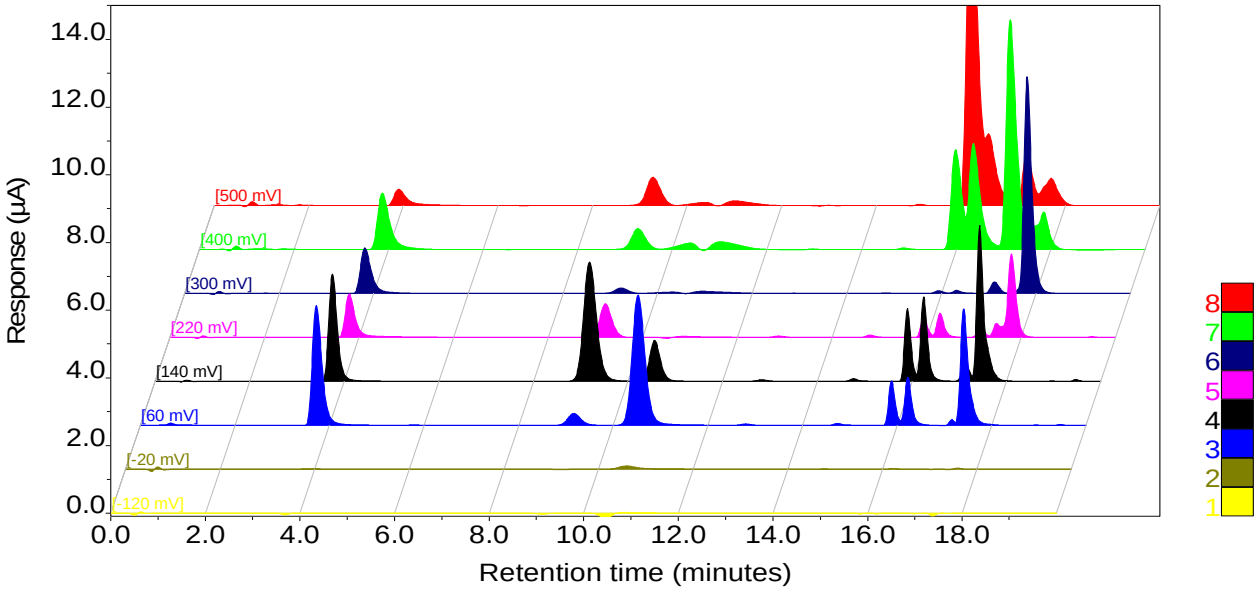




Mature d'Anjou pear fruit peel showing cuticle ranging in thickness from about 12 - 22 microns.

Wax ‘grows’ in the form of microtubules about 100-120 nm in diameter. These are composed of a number of compounds with a predominance of nonane and associated long chain carbon derivatives. These tubules, being ‘sticky’ to each other, associate into rows of tubules having the appearance of walls or platelets (similar to the walls of a log cabin). Cuticle development occurs by layered wax platelets. That is, the first wax plates to develop on the cell surface generally remain closest to the cell throughout fruit growth. As subsequent platelets develop, they meld and polymerize becoming an insoluble matrix of polyphenolic hydroxy esters. Platelets continue to develop on the surface of the previous layer which then polymerizes to the previous platelet. My theory is the microtubules are ‘attached’ to the epidermal cells throughout development of the wax and cuticle. Therefore, changes in environmental conditions such as temperature and light affect metabolism of the epidermal cells and therefore the composition of the wax and the subsequent cuticle. The antioxidants which are most likely to have an impact on scald development are those which are closest to the cell, i.e., those formed early. However, epidermal cells both increase in size and number as long as fruit expansion continues. As seen in the figure above, the actively dividing cells are within the top 3-4 layers of cells, whereas beneath this, cells are mainly for

storage of water, and soluble and insoluble carbohydrates. As cells divide and new cells form, triggered by expansion pressure from the internal cells, new microtubules form creating new areas of wax deposition and cuticle formation. As long as fruit growth occurs, new platelets will form, therefore, it seems the rate of expansion prior to harvest (and, therefore, the temperature, sunlight, and other factors contributing to fruit growth) is also an important key toward understanding the role of antioxidants in scald development in storage. For example, when the temperature prior to harvest is warm, the wax composition may be composed of longer chain hydrocarbons than when the temperature is cool. This will affect the composition of the cuticle developed during this particular period. In a similar fashion, the phenolic composition within this hydrocarbon matrix will also change depending on the temperature and the amount of light. Recall, that scald development both on pears and apples occurs predominantly on the shaded partion of the fruit. Solar radiation incident on the fruit surface triggers the cell to increase phenolics which can act as antioxidants against radicals generated during the photosynthetic energy conversions. Thus, high temperatures and low light levels before harvest due either to canopy shading or clouding would be the worse conditions for scald development. Understanding development of the cuticle is important in these respects.

Samples were collected from the same 5 orchards used in the past, beginning at full bloom. Fruitlet samples were collected weekly for the first 6 weeks, bi-weekly for the next 6 weeks, and every three weeks thereafter. Hexane-soluble wax was extracted and examined qualitatively using UV absorbance at selected wavelengths. The samples were frozen at -80C and kept for further analysis. Because of delays in signing the federal budget, I was unable to acquire the equipment necessary to continue until recently. A photochemiluminescent detector is to be installed within the next two weeks which will enable measurement of total water soluble and lipd soluble antioxidants (in the form of free radical quenching). These will be compared with HPLC analysis of water-soluble phenolic antioxidants in samples throughout the growing period and during storage. The objective is to identify patterns in antioxidant extracts during fruit development that correspond to scald development in storage. This work continues.

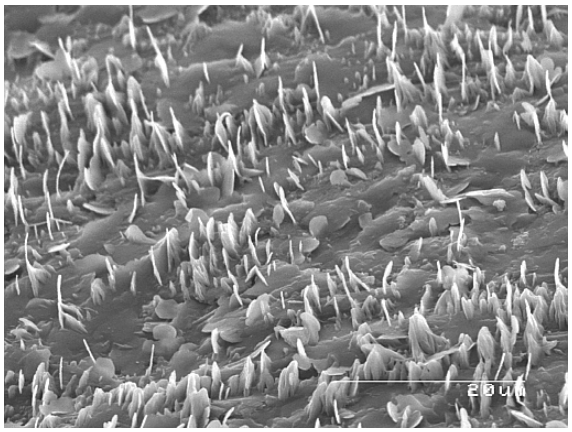



This Figure shows a typical chromatogram using an coulometric array of water soluble phenolics. Patterns from pear peel extracts from fruitlets through mature fruit will be examined using principal component analysis to determine correlations with scald development.

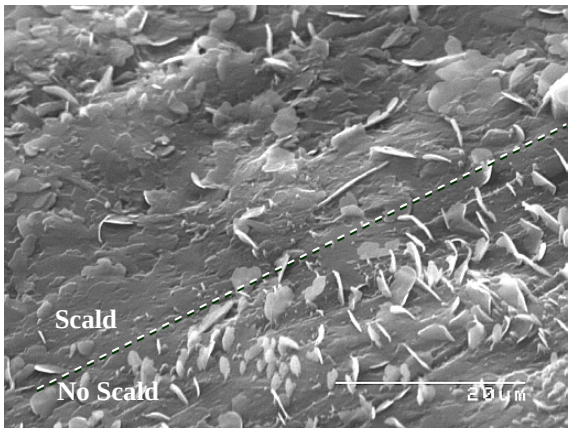
The second experiment was a continutation of the previous years work. The objective was to determine if quantity and quality of wax was related to the amount or retention of antioxidant applied to the fruit for scald control. We harvested samples from the 5 orchards used in the study, treated with either ethoxyquin or DPA for scald control, and placed fruit in regular and CA storage. At harvest and at 1, 2, 4,

and 6 months in storage we extracted peel for wax analysis and for analysis of DPA and ethoxyquin. We initially ran into some snags related to co-elution. This could be resolved using different more selective detectors, however we hadn't the resources to acquire the necessary equipment or to outsource the samples for analysis. Thus, we had to use more extensive procedures to separate components. Analysis is close to completion.

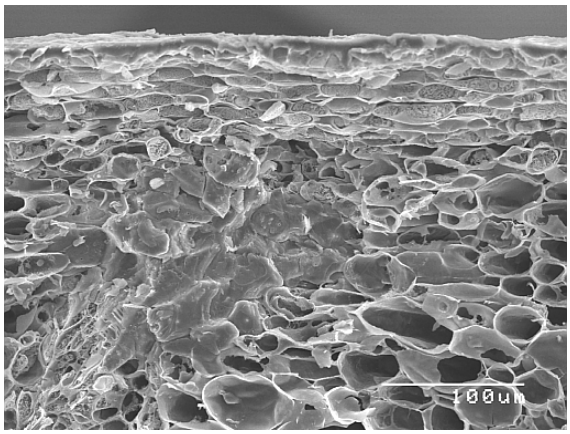
The third set of studies was to examine the nature of scald on the underlying tissue. That is, when scald appears, is the oxidation due to the cuticle itself, the layer of cells embedded in the cuticle, or subsequent underlying tissue. Samples of stored Anjou pears having areas of scald were used. Tissue with or without scald were excised and snap-frozen in liquid nitrogen. While frozen the tissue is shattered and freeze dried. This maintains the structural integrity of the fruit cells.



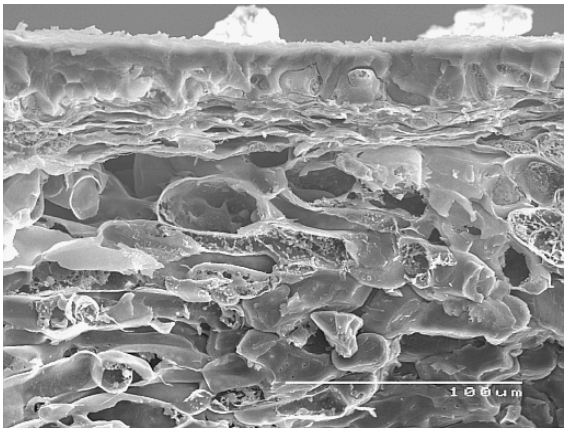

Anjou Peel Surface – 180 days RA – NO SCALD



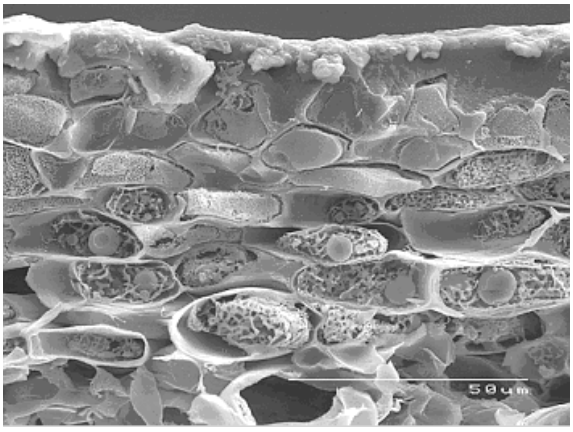

Anjou Peel Surface – 180 days RA – SCALD

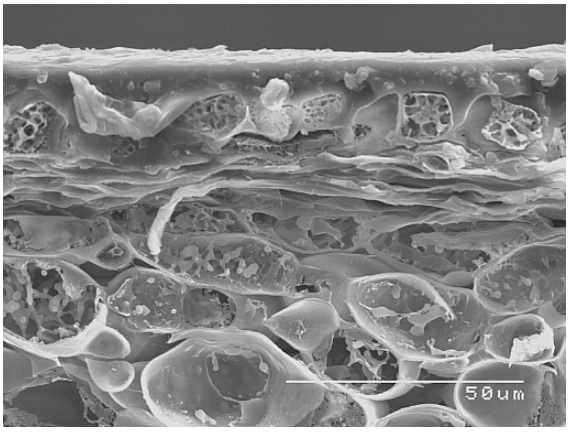



Anjou Peel Section – 180 days RA – NO SCALD




Anjou Peel Section – 180 days RA – SCALD



In the images above, several points are evident. The section of peel exhibiting scald (top right) shows a lack of actively growing platelets. This is reflected in the lack of integrity or collapse of the epidermal layers 2-4 (right middle and left bottom). Contrast this with those images on the left of scald-free peel where platelets are actively growing and the sub-surface epidermal cells are expanded and intact. The appearance of scald apparently comes not from the cuticle which, when isolated and removed from the cellular tissue beneath, is clear and indistinct from that isolated from scalded peel, but rather from the collapse of tissue in layers 2-4 where phenolic compounds in the cell walls is oxidized and intensified.

This work is also continuing. We are in the process of examining the make up of cuticle isolates from scalded and normal fruit peel to determine if indeed the nature of the cuticle itself is changing (or changed). That is, is the cuticle part of the problem or an innocent bystander in scald development.

**Budget:**

**Project duration:** 1998 - 2001

**Current year:** 2001

**Original budget request:**

| **Year** | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|
| **Total** | 38,000 | 44,500 | 42,000 | 27,000 |