# Almond Board of California
# Project Report - 2003-04

**Project Title:** Survey of Sonora pellicle ink-staining in Central Valley almond varieties and production areas

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**Location:** Department of Pomology, University of California at Davis

**Objectives:**

1. Verify the basic types of pellicle staining characterized in 2002.
2. Survey regional trial samples from all major California almond-growing areas for the basic types of pellicle staining and provide samples to Dr. Jim Adaskaveg for fungal isolation studies.
3. Document the incidence of various pellicle stainings observed, including the variety, location, notes on orchard management practices, as well as findings from Dr. Adaskaveg's fungal isolation studies. Identify preliminary trends or associations of this affliction.

**Report 2003-04**:

Pellicle ink-staining on Sonora is an increasing concern for growers, processors and packers, particularly with the expanding acreage of this variety in the San Joaquin Valley. We have examined the incidence of pellicle ink-staining within *Sonora* as well as other varieties, as well as its distribution within the Central Valley. Nuts samples from 2002 almond trials have now been evaluated for the incidence and severity of the different types of pellicle staining. The location, variety, and any available notes on potentially pertinent orchard management practices have also been recorded. Over 3,300 affectedkernels have been selected in 2002-03 based on observed level of pellicle staining, ranging from distinct ink-staining to a more diffuse pellicle 'bluing', in 15 almond varieties including *Sonora, Ruby, Nonpareil, Mission, Butte, Padre, Monterey* and *Plateau,* as well as several breeding selections. Pellicle ink-staining has now been identified

from all major production regions in California. Pellicle stained samples have been classified into four basic groups based on a time of initial damage to the pellicle and final appearance. Pellicle damage resulted from trauma either early in seed development, as would occur from early insect (Lygus bug, etc.) damage or aberrant seed fill/development (Fig. 1), or from later damage to the seed coat, usually associated with pathogen (*Aspergillus, Alternaria, Rhizopus )* infection.The first two groupings resulted from early damage to kernel growth from a wide range of causes including insect damage and abnormal seed development. By the time of nut maturity, the damaged tissue was found by Dr. Adeskaveg's lab to be colonized by an assortment of secondary fungal pathogens including *Rhizopus, Alternaria, Penicillium, Aspergillus niger* and *Cladosporium*. The resultant dark staining could be limited to the point to damage (Figure 2) or cover extensive areas of the mature nut. Because of its early development, the underlying kernel tissue was often affected.

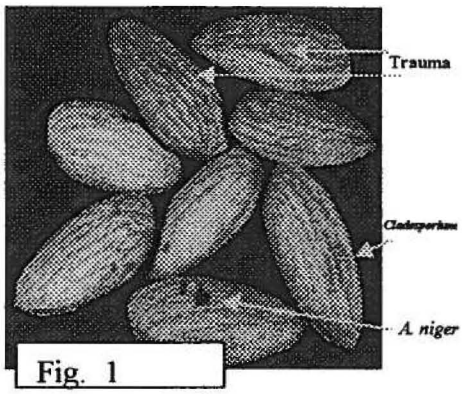


Fig. 1

A second, though rarer, developmentally based staining was found in certain varieties such as Sano and Savannah and less frequently in varieties such as Livingston and Fritz which can, under certain growing conditions, develop a more pronounced amaretto flavor. The pellicle is often rough in appearance and uniformly spotted with black to dark brown 'freckles' (Figure 3). Our experience indicates that this type of staining appears to be associated with the chemical breakdown of compounds leading to the amaretto flavor. In bitter almonds, and to much lesser extent, is some sweet almond cultivars including Mission, Fritz and Livingston, the amaretto flavor results from the breakdown of the compound amygdalin to benzaldehyde (which produces the amaretto flavor) and cyanide (which is highly toxic to living cells). This reaction only occurs when cells are damaged, such as by insect feeding, and appears to serve as a deterrent to feeding by insect and mammals. The dispersed flecking nature of this type of staining and its occurrence relatively early in seed maturity when the kernel is still largely protected by an intact shell, suggest that

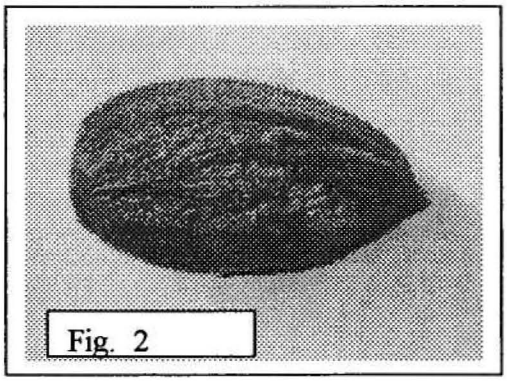


Fig. 2

some scattered cellular lysis is occurring at the pellicle at the time of kernel maturity (possibly associated with early cell desiccation and/or biochemical changes such as phenolic conversion) and that this isolated cell lysis is sufficient to invoke a broader cyanide induced damage (including tissue browning) to neighboring cells. Further evidence of the cyanide induced nature of this flecking comes from its association with bitter almonds and those sweet almonds (including Fritz, and Livingston) which are known to produce greater amounts of cyanide producing compounds in the kernel and pellicle. This type of pellicle staining, however, is rare in most varieties and does not appear to affect the underlying kernel.

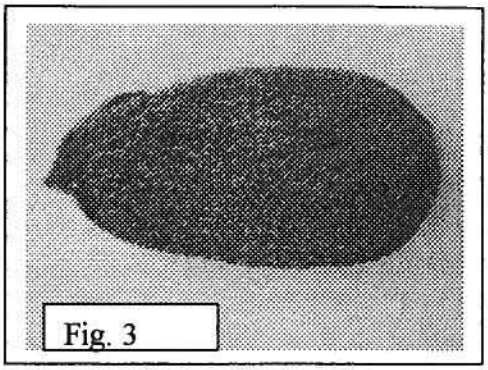


Fig. 3

The final two groups are characterized by damage to the pellicle late in nut development, probably during ripening. Staining is usually limited to the pellicle and does not affect the underlying kernel. However, if the damage is severe enough, infection by other secondary pathogens will occur, sometimes resulting in severe staining of the pellicle and a typical water-soaked damage to the underlying kernel.

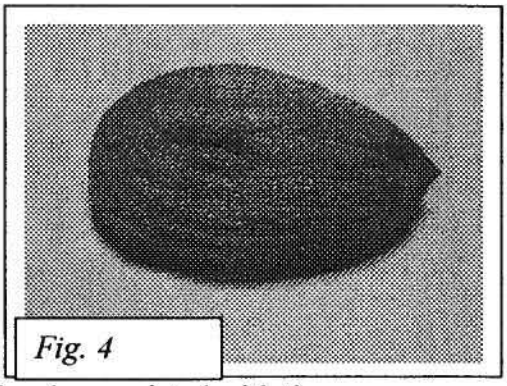


*Fig. 4*

The first of the ripening-associated stainings includes the typical 'Sonora ink-staining' which most often appears as narrow bands associated with the pellicle veins (Figure 4). Fungal isolation and identifications done in Spring, 2003 at the laboratory of Dr. Adeskaveg at UC Riverside, indicate that *Aspergillus niger* and *Penicillium* are the pathogens most often associated with this damage, though other pathogens such as *Alternaria* and *Rhizopus* are also commonly found. Our current observations suggest that this type of damage is associated with heat stress to the moist pellicle tissue prior to its drying at ripening.

The final ripening-associated staining is similar to "Sonora ink-staining" except that it initially occurs more as a pellicle 'flecking' than a banding pattern (Figure 5). This 'flecking' is often more pronounced in certain areas of the pellicle and occurs both at the pellicle veins as well as

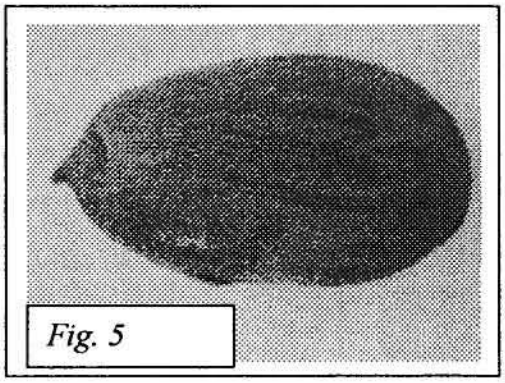
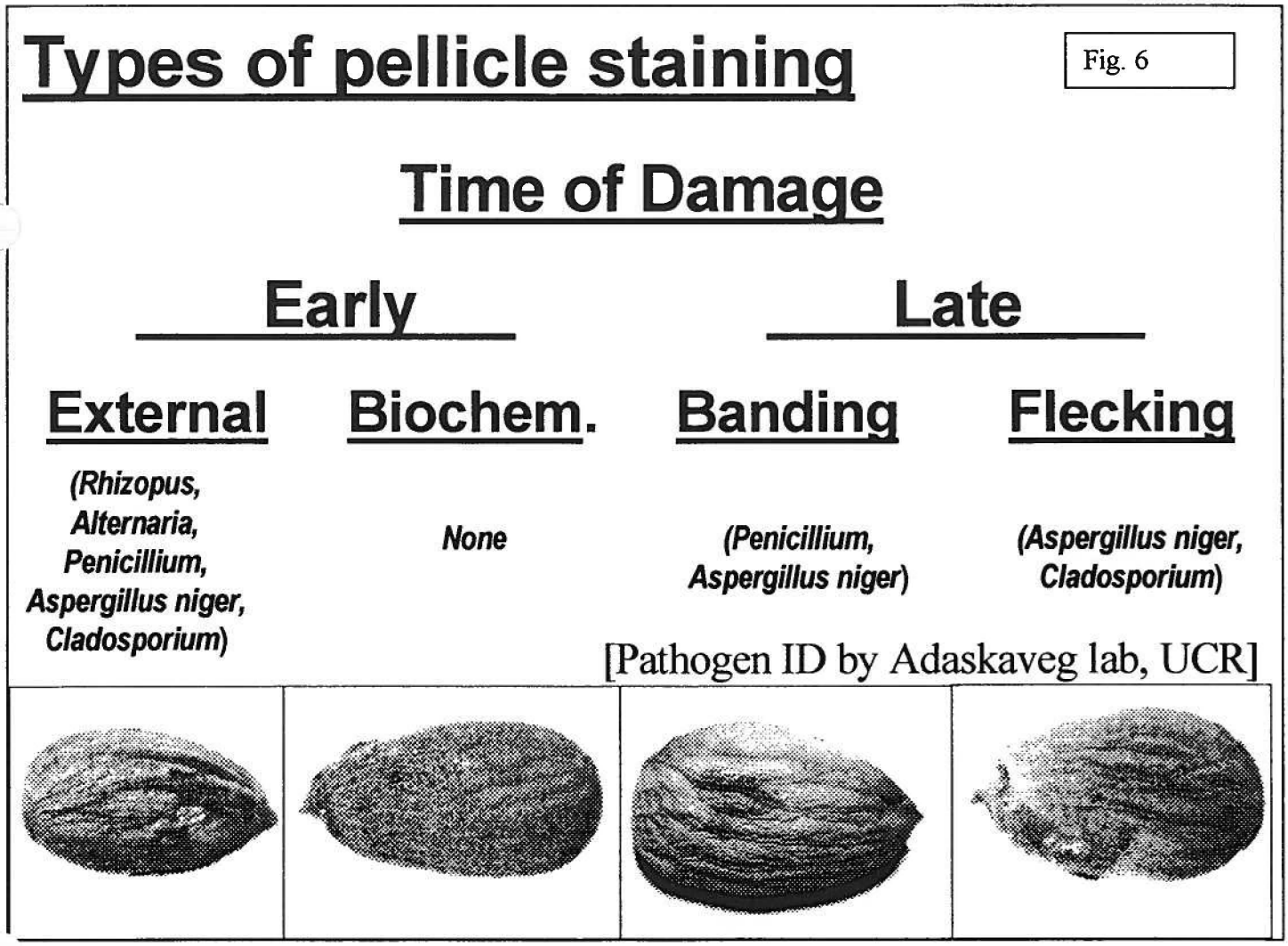


*Fig. 5*

between the veins. If extensive, flecks can coalesce into bands giving a typical Sonora staining appearance or covering larger sections of the kernel (particularly along the edges). Unless damage is extensive the underlying kernel tissue does not appear to be affected (although this is based on field observation only and not on blanching studies). The pathogens isolated by Dr. Adeskaveg's lab were primarily *Cladosporium* but some *Aspergillus niger* and other fungi also detected.

Based on 2002 findings, a template for characterizing basic pellicle stain type has been developed (Fig. 6). This template has been used to catalog samples of stained almond kernel pellicles collected in 2003 from central and southern Sacramento Valley, and Northern, Central, and southern San Joaquin Valley production areas. Approximately 4000 affected kernels have been collected, with representatives from each cultivar/site grouping classified for pellicle



staining type and sent to Dr. Jim Adeskaveg's lab at UC Riverside for pathogen isolation and identification. More extensive pellicle damage was observed in 2003 relative to 2002, both within and among cultivars and locations sampled. This apparent increase in the incidence of pellicle staining may be a consequence, however, of the high summer temperatures and the unusual late summer rains, both of which are thought to be factors in promoting pellicle staining latency development, and not necessarily an indication that the problem is becoming more severe.

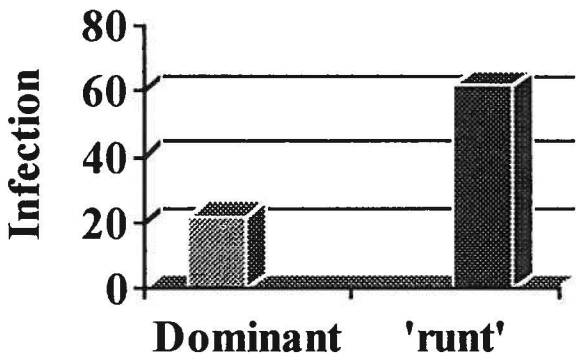



Fig. 7. Different incidence of 'ink-staining' type infection between the larger (dominant) and less developed (runt) cotyledon within individual affected seed.

The occurrence of visual pellicle staining resulting from contamination by fairly innocuous fungi such as *Aspergillus niger* and *Rhizopus* may further serve as an indicator of potentially problematic harvest factors, such as excessive pellicle moisture, which could predispose the crop to more serious problems such as contamination of aflatoxin forming *Aspergillus flavus* fungi or *Salmonella* bacteria. While both pellicle-staining and Aspergillus and Salmonella contamination appear more frequently in certain cultivars, their incidence is very sporadic under field conditions which confounds their study. In addition, these afflictions have been very difficult to reproduce under controlled, laboratory conditions. These findings suggest that their occurrence results not only from the combination of susceptible cultivars with the appropriate, triggering pathogens, but that the environment both in terms of external (climate) and internal (biochemical state) also must be conducive. Clarifying the internal and external environmental conditions conducive to pellicle staining may thus lead to better understanding of the more serious problems of aflatoxin and Salmonella contamination. Studies in 2003 identified a greater incidence of pellicle staining when one of the two cotyledons (which comprise the majority of the almond kernel) was distinctly less-developed and the other (Fig. 7). Furthermore, it is the less developed of the two cotyledons (i.e. the 'runt') which is more prone to pellicle staining which is consistent with the hypothesis that this affliction is more common in stressed or otherwise abnormally developing

tissue. This association is presently being tested using previously stored almond kernel samples known to be affected by pellicle staining, and in a separate study, known to be infected with aflatoxin producing *Aspergillus* strains. If a clear difference in disease vulnerability between the two ontogenetically paired but developmentally dissimilar cotyledons is found to exist, it would represent a very useful model system for studying the internal/biochemical/developmental events which predispose otherwise healthy tissue to susceptibility.

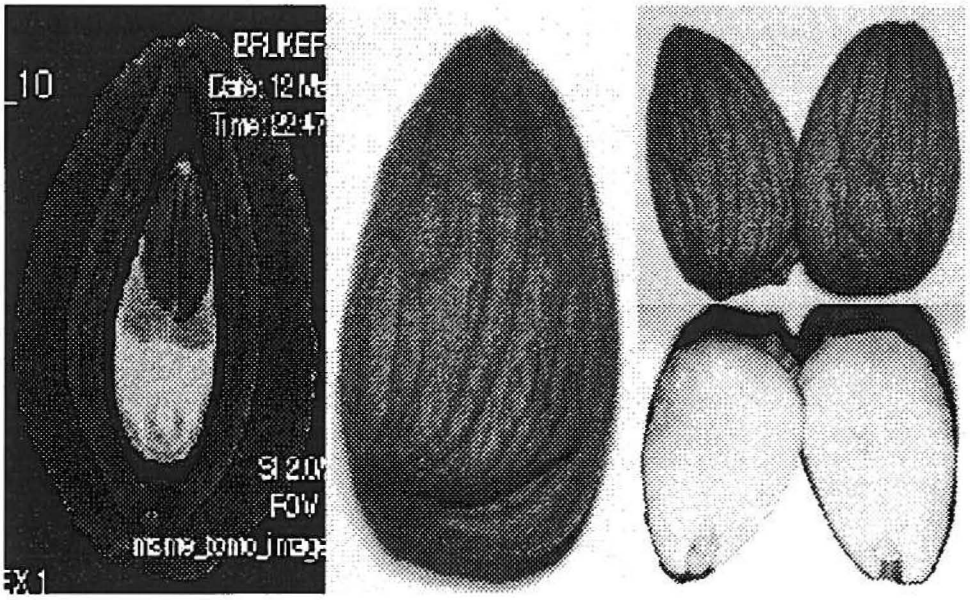


Fig. 8. Examples of ontogenetically paired but developmentally disimilar cotyledons within intact seed. NMR image of early seed development within young almond fruit showing that developmental disimilarities are already present at this early stage [far left]; Mature almond kernel showing differences in the size of the 2 seed cotyledons [center]; split sections of the previous seed showing differences in cotyledon tissue maturity between the 2 halves.