Almond Board of California

Project Report - 2002

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

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Objectives:

1. Characterize the basic types of pellicle staining, commonly observed on California almonds and develop descriptors for each such that they can be rapidly identified by field workers.
2. Survey regional variety and rootstock trial samples for the basic types of pellicle staining and provide samples 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. Attempt to identify preliminary trends or associations of this affliction for future studies.

Summary

Nuts samples from 2002 almond trials have 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 kernels have been selected for observed degrees 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. In affected orchards, it can be fairly widespread, though difficult to observe on nuts produced in the same orchards the following year. The pattern of almond kernel development also appears to play a role in predisposing the developing seed pellicle to ink stain damage. Kernels, selected as characteristic for each group of pellicle-type staining, have now been characterized into 6 classes, photographed and sent to Dr. Adaskaveg for fungal testing. The culturing and identification of fungal contaminates associated with stained pellicle sections will be completed by June, 2003. Final data will then be evaluated for relationships between the observed pellicle staining and variety, location and orchard practice.

Pellicle ink-staining on Sonora has become an increasing concern for processors and packers, particularly with the increase acreage of this variety in the southern San Joaquin Valley. Consequently, more information is needed concerning its incidence within *Sonora* as well as other varieties, and its distribution within the Central Valley. In addition, the occurrence of visual pellicle staining resulting from contamination by fairly innocuous fungi such as *Aspergillus niger* and *Rhizopus* may 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.

Over the past several years, sporadic cases of this type of staining of the pellicle or seedcoat of the variety *Sonora* have been reported. A dark, inky spotting or streaking on the outer pellicle surface often along the pellicle outer veins characterizes this affliction (figure 1).

Nuts samples from the 2002 Regional Almond Variety Trials as well as the Regional Rootstock Trials have 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 kernels have been selected for observed degrees of pellicle staining. We have identified similar pellicle staining, ranging from distinct ink-staining to a more diffuse pellicle 'bluing', in 15 almond varieties including Sonora, Ruby,

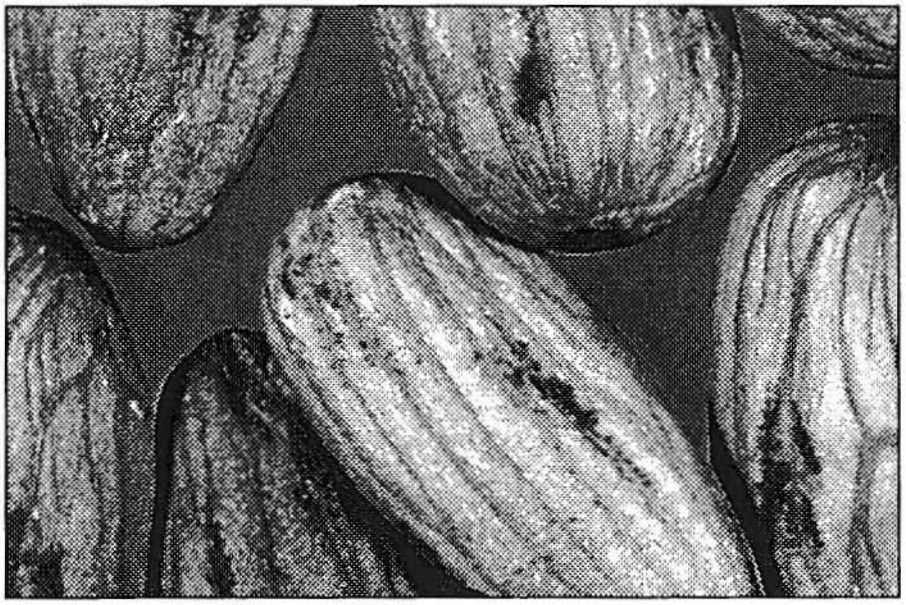

Figure 1.

Nonpareil, Mission, Butte, Padre, Monterey and Plateau as well as several breeding selections. In affected orchards, it can be fairly widespread, though 2001-2002 field observations indicate that it has often been difficult to observe on nuts produced in the same orchards the following year. The staining ranges from Sonora-type 'ink-spotting' to more diffuse pellicle staining. Kernels, selected as characteristic for each group of pellicle-type staining, have now been characterized, photographed and sent to Dr. Adaskaveg for fungal testing. The culturing and screening of fungal contaminates associated with stained pellicle sections will be completed by June, 2003. Final data will then be evaluated for relationships between the observed pellicle staining and variety, location and orchard practice.

The pattern of almond kernel development also appears to play a role in predisposing the developing seed pellicle to ink stain damage. Observations from 2002 indicate that processes involved in the development of vascular tissue connecting the maturing fruit to the developing seed typically may cause aberrant development in one of the two cotyledons. While pellicle staining can be found on both cotyledons, it appears to be more common and more pronounced on the cotyledons with suppressed development. This cotyledon is frequently associated with the site of attachment of the developing kernel with the fruit and ultimately the carbohydrate supplies from the photosynthesizing leaves. While this attachment can be at either of the 2 fruit carpel edges, it is almost always attached to the carpel opposite the smaller kernel half (seed cotyledon). This developmental relationship suggests that developmental differences in the kernel may predispose the tissue to pellicle staining and subsequent fungal colonization in inductive environments.

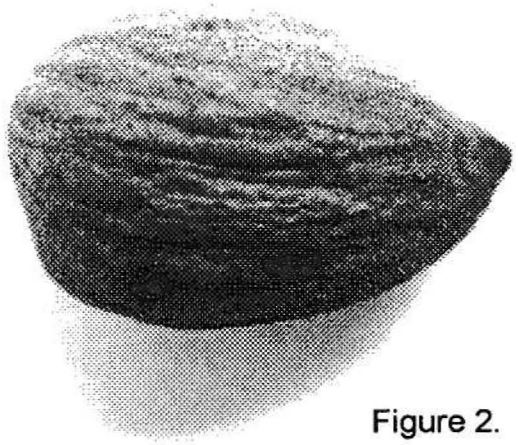

Figure 2.

Six general classes of pellicle discoloration have been characterized in this work. A brief description of each class follows. Studies in 2003 will verify, and possibly expand these classes, and will examine the correlation of general class grouping with associated fungal contaminants (as identified by Adeskaveg's lab). Additional studies in 2003 will examine the relationship between classes of pellicle discoloration in preharvest/harvest field practices (in cooperation with Mario Viveros and other Farm Advisors). Kernel development in those almonds identified as having a higher risk of pellicle discoloration will also be examined, particularly in regards to the nature of vascular attachment to the maternal tissue and associated vascular development within the seed pellicle.

General classes of pellicle staining.

Pellicle vein staining. This type of staining was most commonly identified in the variety Sonora and to a lesser extent Ruby. The staining is typically inky-black and located along the longitudinal pellicle veins covering the cotyledons (figure 1). In Ruby, Butte, Padre, and similar Mission-type kernels, the ink staining often appears as small splotches associated with pellicle veins (figures 2 and 3). At maturity, the cotyledon tissue underlying the ink staining does not appear to be damaged, however, after extended periods of storage these areas often appear to be softer and more water-soaked than adjacent tissue.

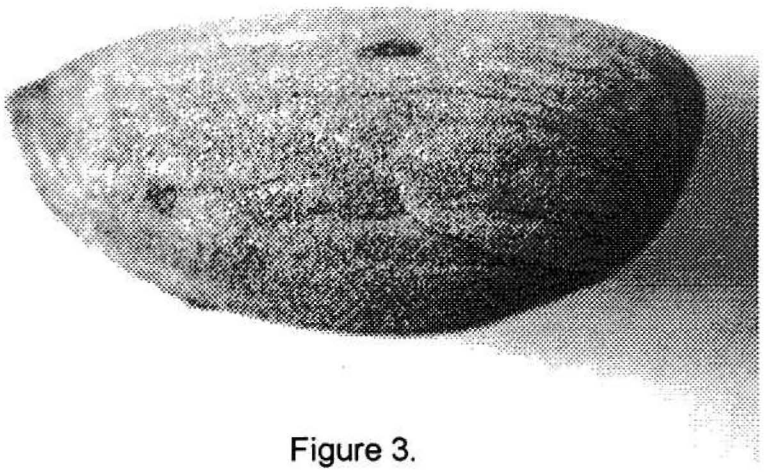

Figure 3.

Damage from probing insects. This common type of pellicle staining results from damage to the pellicle and sometimes underlying cotyledon tissue by probing insect pests such as Lygus bug and False-Footed Leaf bug. The damaged area is usually very distinct, with a small spot of dark to brownish, corky tissue which may or may not be associated with pellicle veins (figure 4). Since this damage occurred early in fruit development before shell hardening, wounded tissue or callus is often associated with the damage. This type of damage was more common in paper shell varieties such as Nonpareil, and because of its known and unique origin was not considered a typical pellicle ink stain in this study, and samples were not forwarded to Adeskaveg for testing.

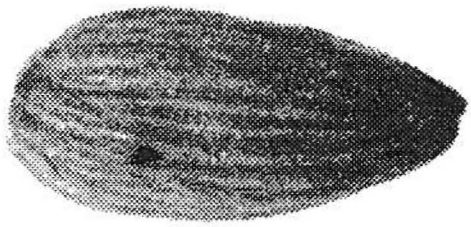

Figure 4.

Band streaking. This class of pellicle staining was characterized as a broad band of dark colored streaks running longitudinally along one of the two pellicle halves (figure 5). Banding was sometimes associated with abnormal cotyledon development (such as multiple embryos within a seedcoat). In most observations, some distortion of the underlying cotyledons tissue was observed. The pellicle staining did not appear to be associated with any external damage to the developing seed and was strongly dependent on genotype (more frequently occurring in certain genotype and virtually absent in others).

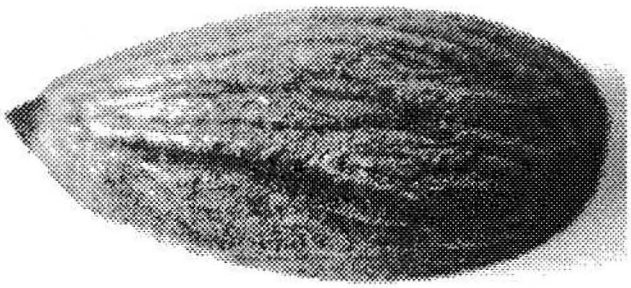

Figure 5.

Abnormal kernel development. Abnormal or distorted kernel development was often associated with localized staining of the outer pellicle. Staining was typically more extensive than in other classes, being similar to band streaking but localized to sections or quadrants of the developing kernel (figure 6). The seed or cotyledon tissue underlying the stained areas were often distorted, though it could not be determined whether this distortion was a cause or consequence of pellicle staining. Often both sides of the kernel would show staining, but the amount of staining and the amount of underlying kernel distortion were typically greater on the kernel half showing suppressed development . The pellicle staining was also more frequently located at the base of the kernel where the seed radical emerges. The seed tissue in this area, where the embryo attaches to the two cotyledons, a is often more irregular or lumpy, leading to more frequent cavities or air spaces between the mature pellicle and underlying seed tissue. This discontinuity between the pellicle and underlying seed tissue is most pronounced once the kernels have been dried and is sometimes associated with flaking of the dry pellicle tissue exposing the underlying embryo radical. This region also appears to be an important entry point for post-harvest fungal contamination, undoubtedly due to the breakdown of the pellicle barrier but perhaps also a result of inherent weaknesses in the inner lipid membrane located between the pellicle and embryo.

Figure 6.

Fungal contamination. Several varieties showed a a general type of pellicle staining ranging from sooty-black to a more bluish-black (figure 7). The staining was more diffuse and not strongly associated with pellicle veins. The smaller of the two cotyledon halves was more frequently affected, often leading to a further reduction (by desiccation) in size of the pellicle and underlying tissue in this area. In some instances, fungal filaments, and occasionally, spores are were observed (figure 8). As with abnormal kernel development, a more frequent separation between the dried pellicle and underlying kernel tissue was observed in this class of pellicle staining.

Figure 7.

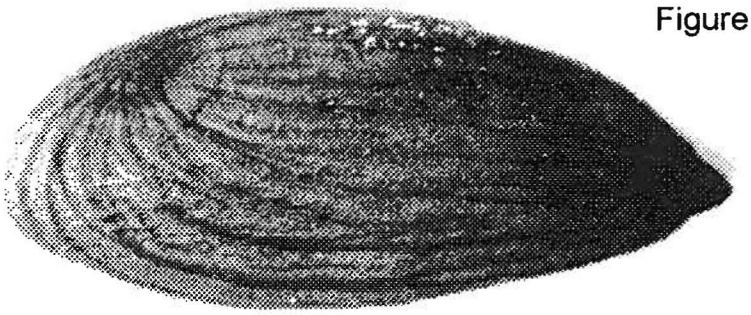

Figure 8.

Cyanide associated pellicle speckling. This unique class of pellicle staining was characterized by an extensive spotting or speckling of the pellicle tissue and was associated with genotypes possessing higher levels of cyanide and benzaldehyde forming cyanoglucosides. The dark necrotic spots forming the speckles were observed on both sides of the kernel though usually more pronounced towards the kernel base

where the seed radical emerges (figure 9). While this type of seed speckling was most commonly observed on breeding selections having a higher almond (benzaldehyde) flavor, it was also observed on varieties such as Fritz and Livingston which are also associated with a more pronounced benzaldehyde flavor and are known to carry the cyanide gene (but which is commonly masked by the 'sweet' or non-bitter gene in these varieties).

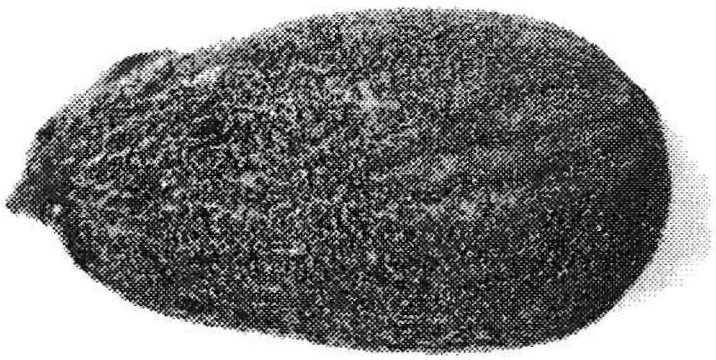

Figure 9.

Plans for 2003.

Isolation and identification of fungal contaminants associated with 2002 pellicle staining will be finalized by June 2003. A major objective of this study is to determine whether specific classes of pellicle stains are associated with specific fungal types or whether a group of unrelated but opportunistic fungi are involved. The type of fungi identified (pathogenic or saprophytic) will also be determined as an indicator of whether the staining is pathogen induced or whether both staining and fungal contamination are consequences of environment-related damages to the developing seed. Field evaluations will be repeated in 2003 to verify and advance findings from 2002 isolations. If present, specific associations between class of pellicle staining and either fungal contaminants or inducing environments, could then be useful to growers, processors and researchers to more accurately characterize and perhaps control this problem.