Almond Bud-Failure

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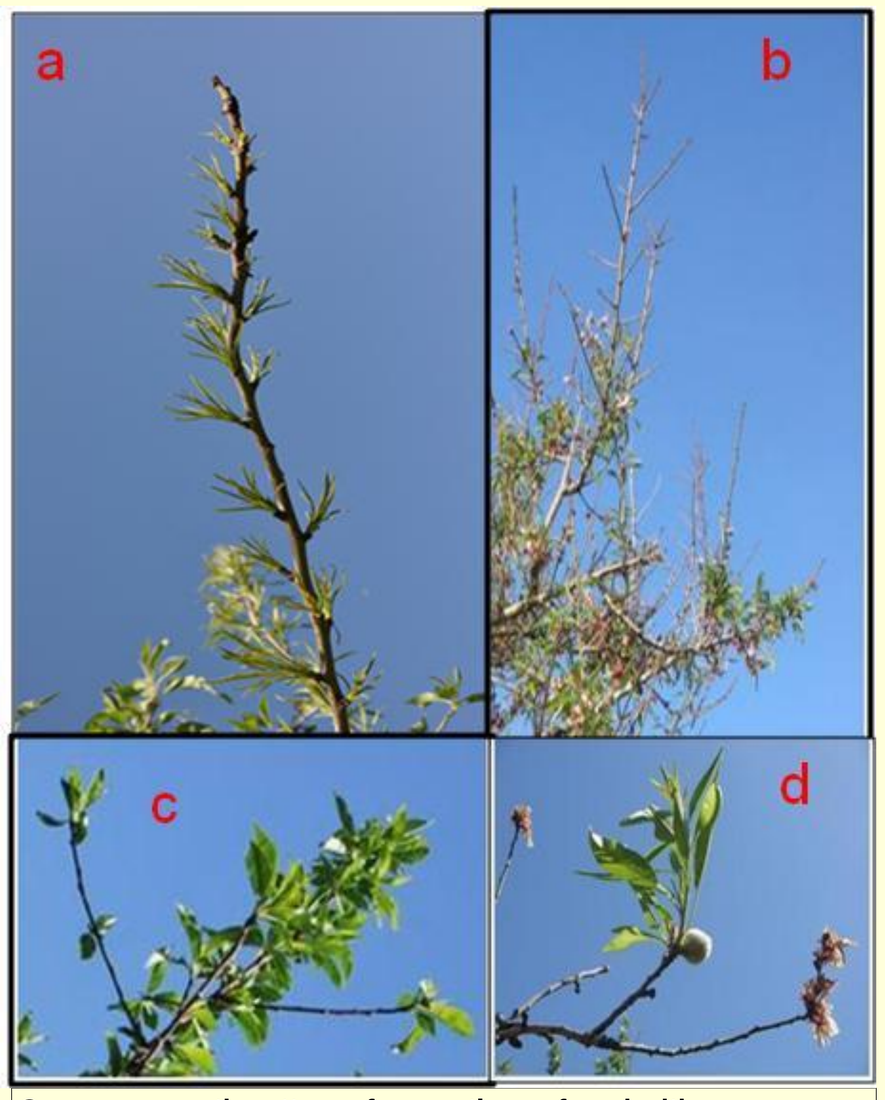
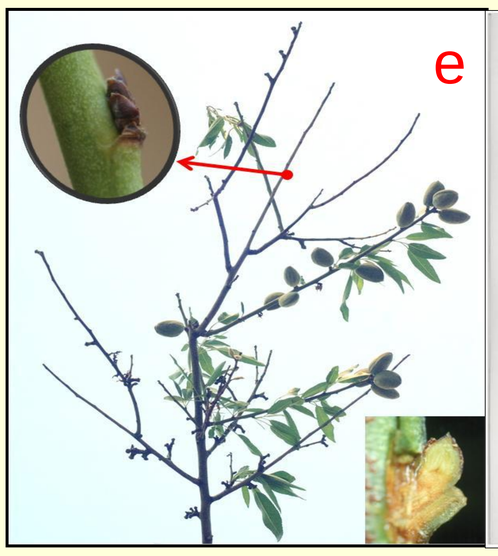
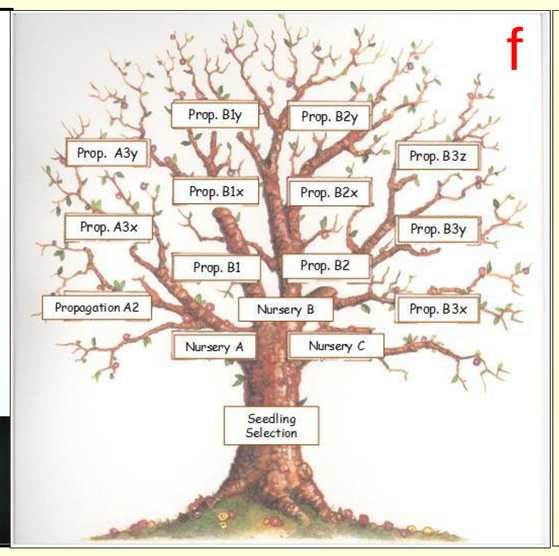


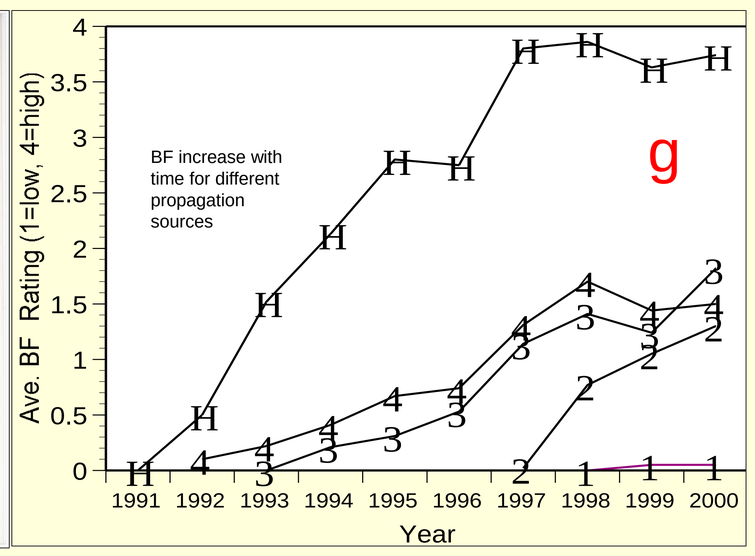
Common growth patterns frequently confused with noninfectious bud failure (a- nutrient deficiency, b- Monterey late-leafing-out, c- low winter chill, and, d- virus infection).

Introduction.
Non-infectious Bud-Failure (BF) remains a major threat to almond production in California. It is a particularly serious problem for the commercially important cultivars Nonpareil and Carmel, which together make up approximately 50% of total plantings. Clonal selection of low BF sources has allowed continued plantings of both Nonpareil and Carmel after BF first became a problem in these cultivars. However, BF-potential (which is related to the age and propagation history of the cultivar) in even the best clonal sources of Carmel may not be sufficiently low to ensure continued commercial use. Careful selection of low-BF Nonpareil clones in the 1970s, 80s and 90s has allowed continued plantings of this dominant variety, though recent BF expression in some Nonpareil sources caution that they may also be progressing towards a new round of BF expression. High BF expression was also a major contributor to the early abandonment of otherwise very promising cultivars such as Merced, and will likely be found in some of the recently released California varieties, particularly those which have the BF-susceptible cultivar Nonpareil as a parent (which includes virtually all currently commercially important cultivars).

True noninfectious bud failure is 'noninfectious' i.e. it cannot be transmitted to other trees by budding or grafting and is not the consequence of nutrient deficiencies or chemical toxicities. In contrast, bud-failure from nutrient deficiencies/toxicities (including some herbicide toxicities) often show some bud development during the winter chilling period and subsequent spring growth, as is the case with zinc-deficiency in **(a)**. Leaf and shoot appearance is often characteristic of the specific toxicity/deficiency. Normal growth can also be restored with the proper nutrient treatment. Similarly, some late-blooming varieties and more recently some Monterey (**b**) show a late leafing-out on terminal shoots that give an early impression of BF. Close examination of shoots, however, typically show buds are developing although at a delayed rate. This can also be confirmed by revisiting the orchard 1 to 2 weeks later when normal shoot development should be observed. In years with low winter-chill, some varieties, including Carmel, may also show a delay in terminal or sub-terminal lateral bud development **(c).** Again, a close examination of the buds will show some degree of swelling or development from the previous fall, ruling out noninfectious bud failure. As with late blooming varieties, buds may continue development at a later date, though in some cases they appeared to become dormant or even desiccated. A similar appearance is sometimes caused when shoots or branches rub together in the wind causing the sloughing of buds. Finally, a form of bud failure often observed on old trees is caused by virus infection (typically Prunus Necrotic Ringspot Virus). Where noninfectious bud failure will typically first appear in the more rapidly growing shoots at the tops of trees, virus or infectious bud failure tends to be more prevalent at the slower growing shoots on the lower branches. New shoot growth tends to show shortened internodes and be willowy, giving a 'mules-tail' appearance as seen for PNRV infection common in commercial propagation sources for the variety Marcona (**d**). Diagnosis of virus or infectious bud failure is by graft or bud transmission to a susceptible host, or by molecular analysis.







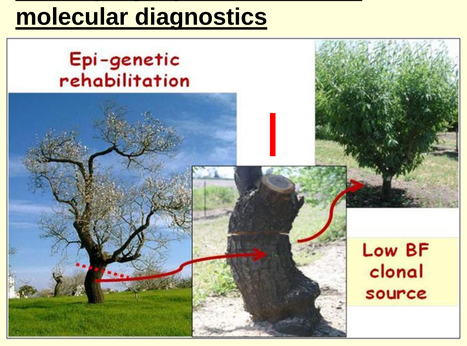
Expression. True Noninfectious-Bud-Failure (NBF) is characterized by the death of terminal or sub-terminal shoot buds during the previous fall, which can be verified by a brown necrosis of the internal bud tissue at that time - see insets in (**e**) as well as an arrest of bud swelling and development during the subsequent winter and spring. The disorder becomes evident with the failure of buds to grow the following spring resulting in sections of blind or bare shoot-wood with the subsequent pushing of the still-viable basal vegetative buds (**e**). Flower buds are not affected and can often develop into fully formed nuts despite the absence of nearby leaves. A third distinct NBF characteristic is that once bud-failure symptoms develop, normal growth is not restored in subsequent seasons but rather the disorder gets worse with each following season (though the extent and rate of failure may vary depending upon growth rate, heat stress, etc. from the previous summer). This recurring sequence of terminal shoot failure followed by pushing of viable basal buds, results in a punctuated and erratic shoot development pattern commonly termed "crazy top" (**e**). In severe cases of NBF, the bark on young shoots develops a characteristic cracking called 'rough bark' (**h**). NBF results from a genetic aging process and so will be expressed first at the oldest, terminal portions of the tree. This tree model (**f**) for NBF increase is also analogous to the variety's commercial propagation. The trunk would represent the original breeder developed tree and each branch would represent different nursery propagation sources (clonal sources). As the clonal source (branch) becomes more distant from the trunk through multiple propagation cycles, the NBF potential for expression increases. The NBF-potential of different clonal-sources can be determined by growing trees propagated from these different sources-clones over many years under conducive climates and observing the rate of NBF development (**g**) though this is laborious and time-consuming. Since expression of BF during the initial 5 years of tree growth will compromise later productivity, source clones expressing BF 7-10 years or more after planting are required for commercial use.

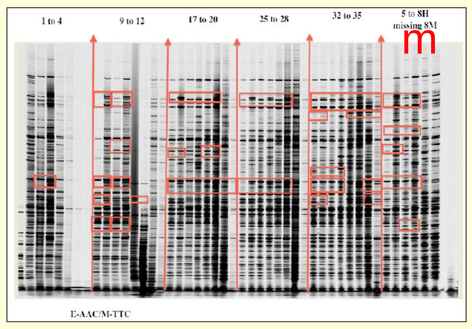
BF Monitoring

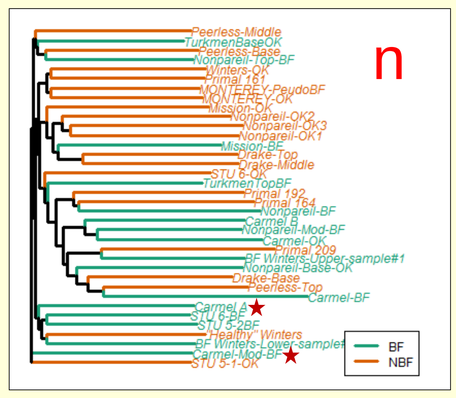
Because True NBF is irreversible, control is primarily through the identification and utilization of low-BF clonal sources for each susceptible variety followed by careful field monitoring of BF-stability as well as investigation and diagnosis of new potential outbreaks.

In the spring of 2003, isolated though widespread occurrences of BF-like symptoms in the variety Monterey were reported in both the San Joaquin and Sacramento valleys. (Similar symptoms were reported in other varieties such as Fritz, Aldrich and Nonpareil but at much lower frequencies). While many trees showed extensive shoot bud-failure symptoms characteristic of the later stages of NBF (i), most trees had not shown symptoms in previous years. The sudden appearance of BF throughout the tree rather than progressing with relative limb age indicated a cause other than NBF. Similarly, while the pattern of failed buds and viable flowers on terminal shoots was similar to NBF, the absence of a crazy-top growth patterns (j) again indicated another cause. Finally, an examination of individual buds showed that most buds were still viable but had failed to push. With 1 to 2 weeks of additional time, many shoot buds did push (k) resulting in normal tree appearance by midsummer. [Many buds failed to push, however, resulting in a concern that a frequent recurrence of this Monterey-BF-syndrome could eventually impact tree productivity]. While the diagnosis of Monterey-BF is still very preliminary, it appears to be associated with environmental conditions such as late summer and winter heat stress and irrigation water quality and quantity. [This problem may also be associated with Monterey's greater sensitivity to high salts in the irrigation water as observed in several west-side San Joaquin Orchards this last fall].

Developing rapid and accurate molecular diagnostics







Current evidence indicates that there is no genetic difference in high-NBF and low-NBF selections of the same clone, but rather the epigenetic state (i.e. turned on or off, etc.) of the controlling gene is affected. Based on our current tree-model of NBF (**f**), clonal sources closest to the original tree origin would have the lowest NBF potential, as has been verified in vegetative progeny tests. [Clone #1 in (**g**) is the original seedling Carmel tree]. Similarly, rehabilitation of high-BF almond varieties to a lowered NBF status can sometimes be achieved by 'pushing' basal epicormic buds from older trees of that variety as the genetic aging appears suppressed in these basal epicormic meristems [fig.(**l**) as has been applied to 2012-13 FPS clone sources (see annual report). Molecular strategies may represent more effective tools for diagnosing (and possibly correcting) such epigenetic differences (including ageing effects) as they may allow diagnosis of the underlying mechanism directly, without environmental interference.

In attempts to molecularly distinguish differing clonal sources for BF-potential, we have used Methylation-Sensitive Representational Difference Analysis (MS-RDA), which utilizes the methylation-sensitive restriction endonuclease *HpaII* to recognize the 5'-CCGG-3' 4-bp motif and thereby isolates DNA fragments differentially methylated between two genomes. Preliminary results show a large number of potentially useful methylation-sensitive markers (**m**) as many show differences among clones with differing BF potential and/or different clonal ages. Statistical methods of analysis are currently being developed to identify the most promising markers for NBF. These markers may primarily be an indicator of clone age (which previous research has shown to be correlated with NBF expression); or the marker may be physically linked to the BF-gene and so a good indicator of its presence; or the methylated-marker may target the BF-gene directly (since methylation is one of the known mechanisms for epigenetic gene suppression). Initial results, comparing a pooled analysis of all the methylation-sensitive markers with BF expression for different clonal sources shows good promise for differentiating between clones of different clonal-age as well as clones showing differing levels of BF (**n**). Many discrepancies are apparent, however, as would be expected because of the pooled nature of this preliminary analysis. Continuing work will analyzed the specific relationship of each individual marker with known differences in clonal age as well as known differences in BF expression. The ideal marker would be able to differentiate between non-symptomatic clonal sources which have low BF potential (i.e. no BF expression in vegetative progeny) versus medium BF potential (no BF expression in clonal source but BF expression in some of the vegetative progeny) [such as Carmel-A★ and Carmel-Mod-BF★ in Fig. **n** at left].