Diagnostic Markers for Bud-Failure

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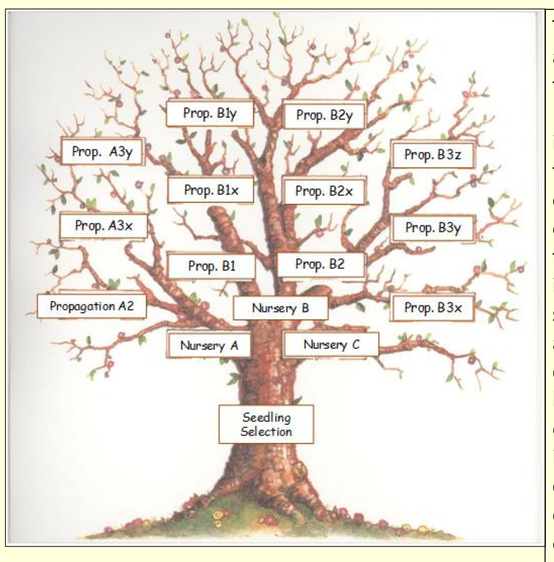
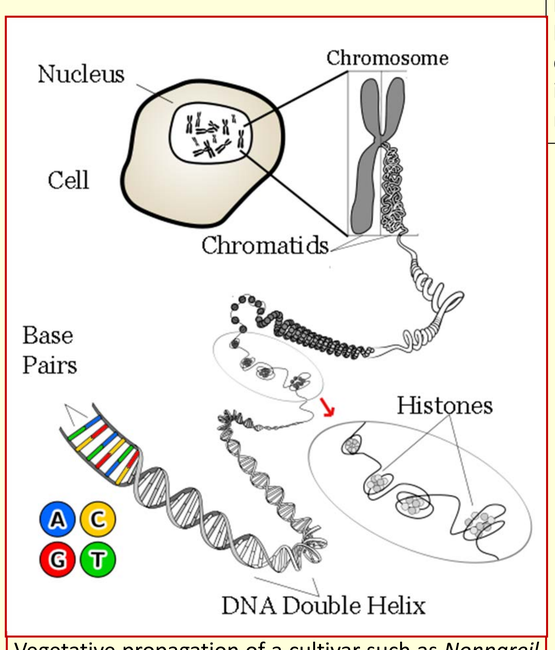
Introduction.
Non-infectious Bud-Failure (BF) remains a major threat to almond production in California. It is a particularly serious problem for the commercially important cultivars *Nonpareil* and *Carmel*, which together make up approximately 50% of total plantings. Clonal selection for low BF sources 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 -see below) 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 almost all currently commercially important cultivars).

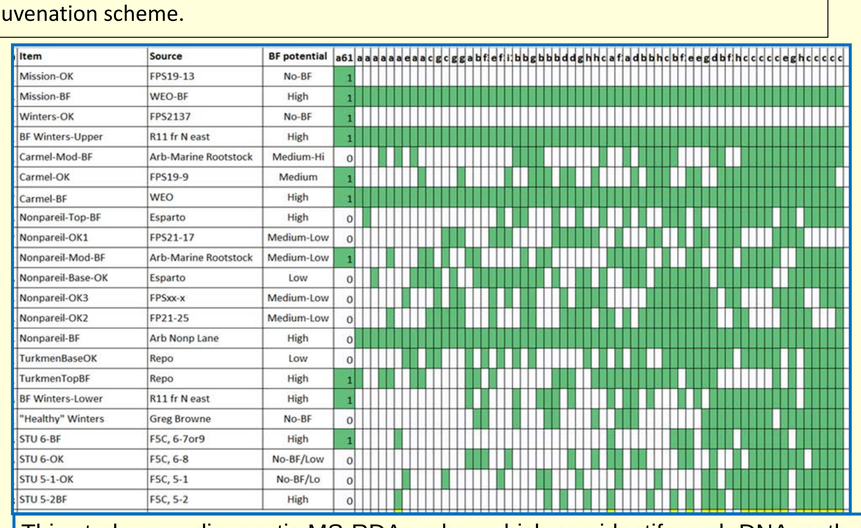
True Noninfectious-Bud-Failure (BF) is characterized by the death of terminal or sub-terminal shoot buds during the previous fall, which can be verified by a brown necrosis of the internal bud tissue at that time (lower right inset) as well as an arrest of all bud swelling and development during the 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. Flower buds are not affected and can often develop into fully formed nuts despite the absence of nearby leaves. A third distinct BF 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



True noninfectious Bud-failure (BF) is analogous to the graying of a person's hair as they age. The genetics of the individual is unchanged but the appearance or expression of those genes deteriorates with time (and stress). In the tree at left, the appearance of BF in the upper right branches of the tree will very likely be followed by BF expression in the upper branches of other scaffolds even though they are only attached at the trunk. This is because all terminal shoots have a common origin in the trunk and so are comparable in age. Because BF expression is strongly associated with age, expression in one section of the tree will likely be followed by expression in other parts of the tree as those shoots approach a similar age. The pattern or history of an individual tree growth is also analogous to the commercialization of a new cultivar. Here, the trunk is original seedling genotype of the new cultivar. The seedling cultivar will age as the tree grows, and also when buds and shoots are propagated in other orchards as clones of that cultivar. This branching out of different clonal propagations is analogous to the branching of the original tree as both inevitably progress with age. This model of BF development successfully predicts the progression of BF in individual trees and in individual cultivar propagation histories, and provides the only known method of control: clonal source selection. Thus, to minimize BF in commercially propagated trees of susceptible cultivars, nurseries use bud-wood sources as close as possible to the original seedling selection to minimize the deterioration with age. This approach, however, involves tedious and multiyear testing of vegetative progeny to identify the best propagation sources. Diagnostic markers for this aging process might allow the direct selection of low-BF propagation sources. Further, if these markers allowed the identification of the specific aging process, the process might be reversed through a rejuvenation scheme.

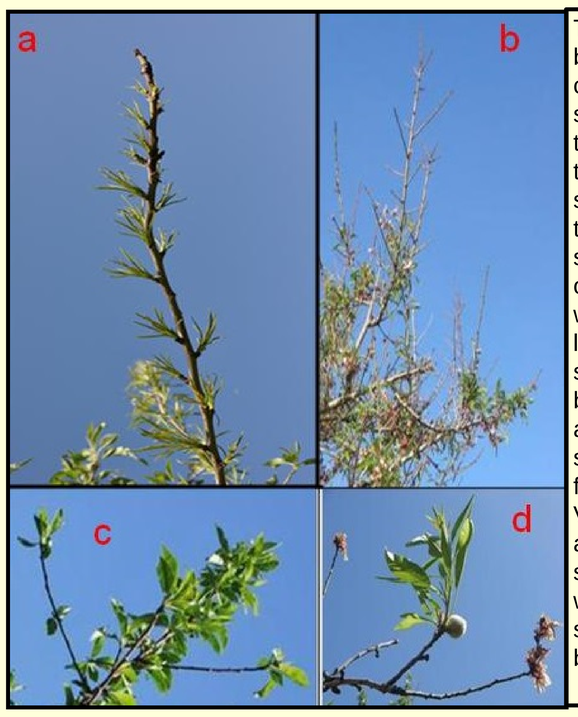


Vegetative propagation of a cultivar such as *Nonpareil* or *Carmel* results in identical trees or clones because the genotype remains unchanged. The genotype contains the instructions for plant growth and development within the DNA sequence contained in each cell's chromosomes. (In much the same way as the text of a book or the code in computer software has very specific meaning). Although this DNA text remains the same in all chromosomes of all cells and in all propagations of the cultivar, changes in the chromosome structure or properties can cause the misreading of the DNA text and even the failure of the DNA text to be read. In human genetics, this has often been found to be the result of a process called DNA methylation where the DNA text remains the same but becomes unreadable.



Item	Source	BF potential
Mission-OK	FPS19-13	No-BF
Mission-BF	WEO-BF	High
Winters-OK	FPS2137	No-BF
BF Winters-Upper	R11 fr N east	High
Carmel-Mod-BF	Arb-Marine Rootstock	Medium-Hi
Carmel-OK	FPS19-9	Medium
Carmel-BF	WEO	High
Nonpareil-Top-BF	Esparto	High
Nonpareil-OK1	FPS21-17	Medium-Low
Nonpareil-Mod-BF	Arb-Marine Rootstock	Medium-Low
Nonpareil-Base-OK	Esparto	Low
Nonpareil-OK3	FPSxx-x	Medium-Low
Nonpareil-OK2	FP21-25	Medium-Low
Nonpareil-BF	Arb Nonp Lane	High
TurkmenBaseOK	Repo	Low
TurkmenTopBF	Repo	High
BF Winters-Lower	R11 fr N east	High
"Healthy" Winters	Greg Browne	No-BF
STU 6-BF	F5C, 6-7or9	High
STU 6-OK	F5C, 6-8	No-BF/Low
STU 5-1-OK	F5C, 5-1	No-BF/Lo
STU 5-2BF	F5C, 5-2	High

This study uses diagnostic MS-RDA probes which can identify such DNA methylation events to test whether they are associated with BF and/or clonal aging. 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 identifies DNA fragments differentially methylated between two genomes (for example, BF *Nonpareil* and normal *Nonpareil*). MS-RDA analysis reveals a large number of potentially useful methylation-sensitive markers since many show differences among clones with differing BF potential and/or different clonal ages (green chart above, left). These markers may be an indicator of clone age (which previous research has shown to be correlated with BF 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 DNA reading 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 and possibly varieties showing differing levels of BF (chart above at right). In addition, some markers (blue arrows) show a high correlation with high BF expression. Many discrepancies are also apparent, however, as would be expected because of the pooled nature of this preliminary analysis and 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. However, while 'age' relationships within a clone of the same variety can often be worked out, determining age-relationships among different varieties is much more difficult since the initial expression of BF differs among varieties (for example, very early in *Carmel* but relatively late in *Nonpareil* and very late (rare) in *Mission*). Similarly within an individual tree, while basal (trunk epicormic) and terminal shoot buds represent the youngest and oldest 'age' respectively, it is very difficult to accurately quantify the age of intermediate growth. We are pursuing a parallel analysis where BF-independent MS-RDA markers can be used to quantify relative clonal age, which will then facilitate the possible identification of BF-specific markers. (This process may have to be pursued independently for each variety evaluated, depending on how closely related they are.) The ideal marker would be able to differentiate between non-symptomatic clonal sources which have low BF potential (i.e. no BF expression in clonal source and 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). Statistical methods of analysis are currently being developed to identify the most promising markers for BF when compensating for these interactions with our most current, though still imperfect, iteration plotted below.



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 should 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 some propagation sources of the Spanish variety Marcona (**d**). Diagnosis of virus or infectious bud failure is by graft or bud transmission to a susceptible indicator host, or by molecular screening.