ALMOND BOARD OF CALIFORNIA

Final Report
of the
NONINFECTIOUS BUD- FAILURE
Project
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Project Leader: Dale E. Kester, Department of Pomology, UCDavis
Cooperating Personnel: Tom Gradziel, Warren Micke, Ken Shackel, Mario Viveros, Mark Freeman

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History of the project

An Almond Board Project on Noninfectious Bud-Failure (BF) had been in operation for many years, first, as a part of the Almond Breeding Project and then as a separate project. Broad concepts of BF had been established as a result of the earlier work (Kester and Gradziel, 1996). Low- bud-failure potential single tree nursery sources of Nonpareil and a number of other cultivars (Peerless, Ne Plus Ultra, Price, Sonora, Padre, Mission) had been selected (Step I) and procedures were in place for their maintenance (Step II) in the Foundation Plant Materials Service (FPMS) at UCDavis and distribution (Step III) through the voluntary Registration and Certification program of the California Dept of Food and Agriculture (CDFA).

This 3-part program was expanded to extend the same goals to other varieties not represented in the early FPMS collection, including Carmel, Monterey, Ruby, Fritz and additional selections of Mission, Sonora, and Price. A joint meeting in April 1988 among representatives of the commercial nursery industry (AIB), Foundation Plant Materials Service (UCD) and UC pomologists identified new sources recommended from commercial nurseries. By 1994, new low-BF_{pot} Foundation Clones of all but Carmel of these cultivars had been progeny tested, approved and were available to the industry through FPMS. Carmel proved to be a bigger problem. In 1990, BF was appearing in commercial orchards at high rates and in very young trees from essentially all nurseries. The concern was so high that many nurseries and individual growers were considering discontinuing its propagation.

Two programs specifically dealing with Carmel were initiated:

(1) Search for individual tree sources with low BF potential (BF_{pot}) to be Foundation Clones

A. 1988. Six single trees of Carmel from a commercially originated source were identified (see previous paragraph) as candidate Foundation Clones. Progeny trees were propagated from each source tree in 1988, planted in a test plot as part of a Paramount Orchard commercial trial (Kern Co., near Wasco) in spring 1989. Yearly observations were begun in March 1990.

B. 1989. Six individual source trees from various origins (descriptions later) were tested. Progeny trees were propagated in 1989 and planted in the Paramount Wasco Orchard (I) in spring 1990. Yearly observations began in March 1991.

C. 1993. A third group of candidate Foundation Clones had been selected from the Manteca RVT plot and from a commercial orchard near Ripon. Progeny trees were propagated in 1993 and planted in three commercial orchard test plots in Fresno Co. Annual observations began in 1995 and have continued each year through 2000.

(2) Analysis of BF variability in commercial nursery sources of Carmel (BF HERITABILITY STUDY)

The idea for this study was developed in 1989-90; trees were propagated in 1990 and planted in February, 1991 in a commercial orchard of Paramount Farming

Corp (II) in northwest Kern Co. Observations were started in March 1992 and continued annually through March 1998.

The experiment consisted of approximately 3000 Carmel trees which were planted in a single 80 acre orchard in alternate rows with an equal number of Nonpareil trees to provide cross-pollination. The `Carmel' population trees consisted of (a). *vegetative progeny* trees of representative source trees made available for the project by eleven nurseries in which the pedigree of each tree was known for *nursery source, individual tree within source, individual branch* of the tree and *individual position of the bud* on the budstick) and (b). unpedigreed trees provided from specific commercial nurseries whose data has been used when appropriate. Some trees were omitted, including tree loss, replacement, incorrect identity, or lack of records.

'Nonpareil' nursery tree sources were planted as pollinizer rows. These included two source identified Foundation Clones (FPMS 3-8-2-70; FPMS 3-8-5-70) and five commercial nursery sources all planted in replicated rows across the orchard.

All individual trees were rated for BF annually (mid-March) for seven years (1992 through 1997) on a scale of 0 (none), 1 (slight), 2 (moderate), 3 (severe) and 4 (very severe) for seven years. (Note that the actual BF symptoms had developed the previous summer).

The objectives of the 1999-2000 project were:

1) To complete the analysis of the accumulated data from the Carmel heritability study, incorporate the findings into a comprehensive biological model on the nature of the BF phenomenon and prepare and publish the findings into scientific journals.

2) To prepare reports concerning the horticultural application of the findings and the education of almond producers and nursery persons to guide future management of the BF problem in the orchard and nursery.

Definitions of terms used in this report

1. *Source* - the trees from which budsticks are collected to propagate nursery trees. In this study, sources were identified as to (a) *block used by a specific nursery*, (b) *individual tree in that block*, (c) *individual branch of the source tree (up to five branches*), and (d) *relative position of bud on each budstick.* **None of the source trees showed BF symptoms at the time of collection.**

2. *Progeny* - the nursery trees that were propagated from specific source trees.

3. *BF potential* (BF_{pot}) - the inherent potential of the source to produce BF in its progeny trees as measured by the percentage of progeny showing BF within a specific interval of time.

4. *BF expression* (BF_{exp}) - The expression of BF symptoms in progeny trees rated on a scale of 0 (none), 1 (slight), 2 (medium), 3 (severe) and 4 (very severe). These differences primarily involve the distribution as well as the proportion of the canopy

affected at the time of observation. The proportion of buds failing on new shoots may also be included.

Section I. Pattern of Variability of BF_{exp} in the entire population of progeny trees

This section summarizes the distribution of BF symptoms (BF) within and among the 2,800 trees of the population of trees planted in the Paramount orchard in 1991. BF_{exp} is calculated either as (a) percentage of trees showing BF or (b) average rating (AvBF). Individual nursery sources were anonymous and identified by code letter based on the alphabetical sequence of the relative severity of BF from low to high.

A. Time Sequence of BF Incidence

The results (as of 1998) of the source comparisons for the seven-year test (Table 1 & 2) (figure 1) shows a continuously increasing trend. Significant differences occurred among all eleven sources and consistent patterns persisted among individual sources over time. All nursery sources produced some BF trees but large differences existed in the number, the severity of expression and the age at which these appeared.

Table 1. Percentage of trees showing bud-failure in different years for individual nursery sources from 1992-1998. Means followed by the same letter are not different at the 5% level of significance.

Nursery source (number of trees evaluated)	1992	1993	1994	1995	1996	1997	1998	Source mean
K (291)	49	61	84	94	95	98	99	83.0a
J (320)	22	27	45	74	69	86	93	59.4b
I (234)	20	23	38	68	64	81	85	54.0bc
H (97)	13	19	41	72	67	76	78	52.4bc
G (261)	3	10	36	69	67	80	86	50.1bc
F (407)	8	12	28	52	49	68	80	42.5cd
E (78)	9	12	28	50	47	68	83	42.5cd
D (235)	8	11	19	37	39	57	70	34.3d
C (297)	5	6	10	35	30	61	74	31.5d
B (234)	5	6	12	18	18	32	44	19.3e
A (217)	0	0	2	2	0	3	9	2.2f
Original Carmel tree (31)	0	0	0	0	0	3	10	1.8f
Year mean	11.8a	15.4a	28.5b	47.6c	45.5c	59.5d	67.6d	

Table 2. ANOVA for the effects of year and nursery source on the percent of trees showing bud-failure.

Source	DF	MS	F	Pr > F
Year	6	5516	42.9	0.0001
Nursery source	11	3883	30.2	0.0001
error	66	128		

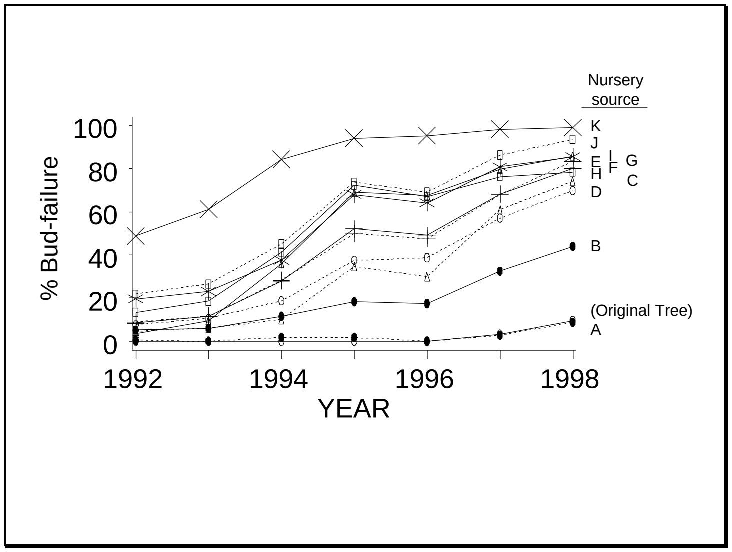

Figure 1 Increase in percentage of BF trees with age**.**

B. Tree Development

The accumulated numbers (percentage) of trees affected by BF increased regularly each year during the 7-year study. There were differences in the average severity of BF expression (AvBF), which reflected a change in individual ratings over time (see next paragraph). These resulted from an interaction between the change in tree morphology and the proportion of the tree affected at different ages. In general, the earlier in the life of the tree that BF occurred, the more severely the tree was affected at maturity.

- Much of the new growth was removed in the first dormant pruning and a complete range of bud-failure severity was observed on the primary branches in March of the following year. Rating were assigned to each tree from 0 (no bud failure) to 4 (very severe) based upon the proportion of buds that were dead (see photographs) (Fig. 2). A rating of 4 meant that about 75% or more of the buds on most shoots were dead, invariably in a gradient from base (alive) to tip (dead). A rating of 3 (severe) meant that the approximately 50 to 75% were dead again in a gradient from base (alive) to tip (dead). A rating of 2 (moderate) meant that only about 30% (range 25 -50) of the buds (upper end) were dead. A rating of 1 (slight) meant that only perhaps 10% (range 5 to 25%) were affected.

Figure 2 Different severity ratings were observed at the end of the first year.

- During the second year, secondary branching develops and the total volume of canopy increases. Individual shoots tend to be less vigorous. The bud failure pattern expressed in March (after the first year) was repeated and expanded on the new second year shoots that were expressed in March of the third year. Since more of the canopy was affected, our visual ratings of already affected trees tended to increase (Fig. 3). The result was that the earlier BF appeared in an individual tree, the more severe was the BF at the end of the seven- year study.

Figure 3

- As trees grew during this initial development period, vigor of individual shoots tended to decline; more secondary branching developed; flowers began to appear, first, laterally on shoots and then on spurs. Overall the total canopy increased in volume. Consequently, as new BF trees appeared in consecutive years, a spatial pattern of BF symptoms within the canopy developed with "new" BF symptoms affecting only the upper part of the tree. Consequently a second trend developed with age. The later that BF developed in the seven- year period, the less was the overall proportion of the affected part and so the tree rating decreased. The reason was that the symptoms were concentrated higher and higher in the tree and affected less and less of the canopy as a whole. (Fig. 4)

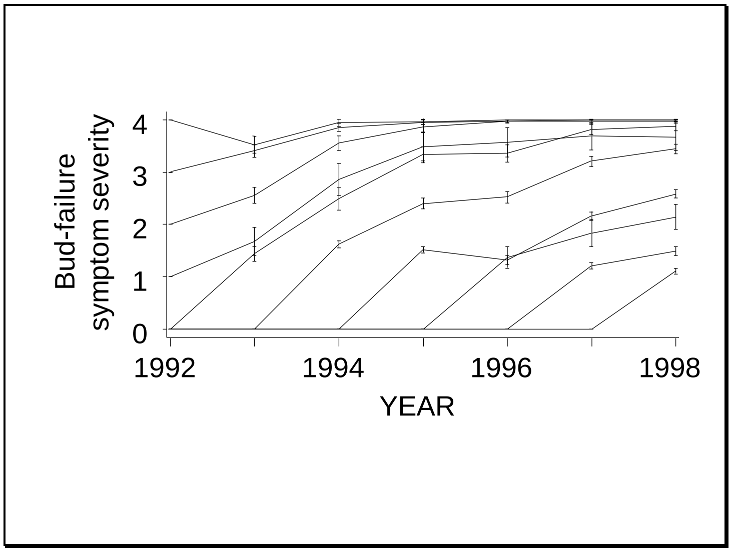

Figure 4 Time sequence of "new" BF trees in subsequent years from planting.

- Yield

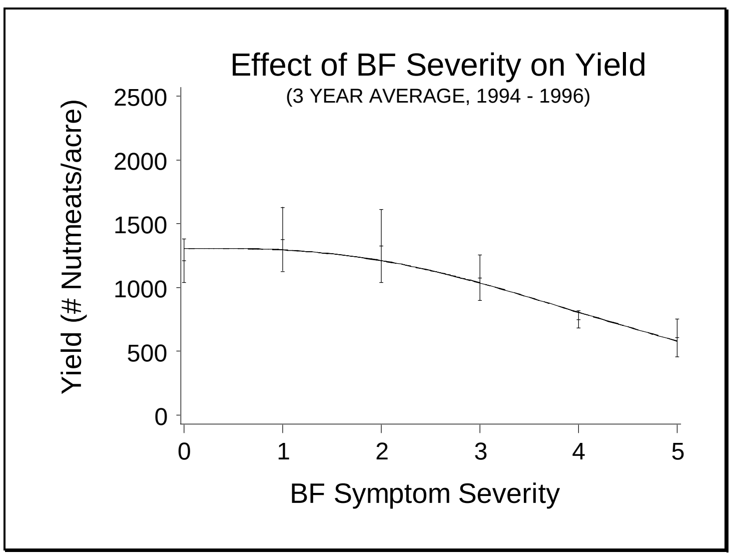

Figure 5 Relationship between symptom severity and yield.

Yield was reduced in direct proportion to the severity of BF that developed during the first five years. This occurred because the initiation of BF inhibited the formation of fruiting shoots and spurs and delayed the overall transition to spur bearing wood. The reduction in yield was in direct proportion to the extent of the canopy affected. There appeared to be an economic threshold at about five years beyond which new BF does not become economically harmful owing to the small proportion of the trees affected.

Summary: Analysis of the seven years data of BF_{exp} development in the population of approximately 2800 progeny trees from eleven sources shows a pattern that duplicates what happens in commercial Carmel orchards in California. This data shows not only how the percentages of individual trees increase over time but also how the pattern of BF_{exp} within individual trees changes with the age of initiation. The data shows how BF affects yield as it begins at different ages and develops over time. It also demonstrates how the age of BF initiation interacts with the normal growth and development patterns in almond trees. It shows why it is important to examine young trees for BF during the first five years in the orchard which is the period at which future yield may be impacted.

Section II. Variability of BF_{pot} in the source trees

All budwood collections were made from trees which did not have BF symptoms (BF_{exp}). Nevertheless all source trees produced some progeny trees with BF_{exp} indicating that each source tree had some degree of BF_{pot}. Differences in BF_{pot} among the 70 commercial orchard source trees were shown by comparing the numbers of BF_{exp} trees and the AvBFat the end of four years (ratings made in March of fifth-year) (Figure 6).

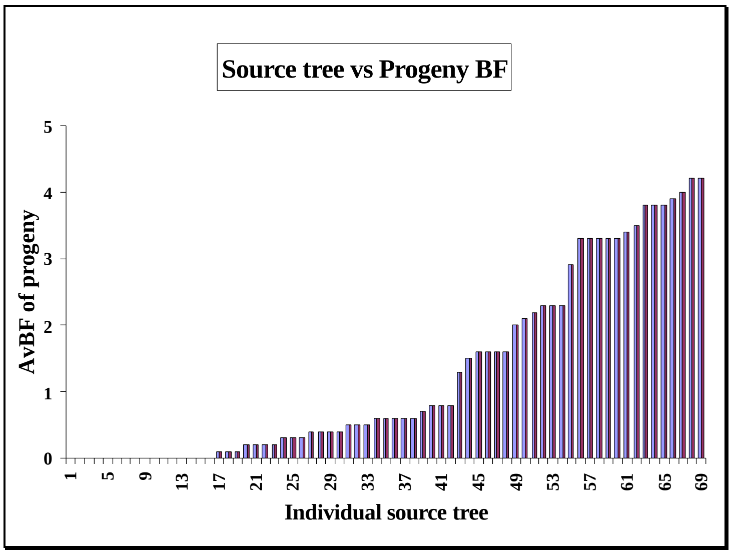

Figure 6 Relative BF_{pot} among the 70 source trees in the commercial orchard sources.

- This graph verifies the concept of *continuous variation* in source trees from very low to very high $BF_{pot.}$ Some source trees eventually showed BF_{exp}.

- BF_{pot} can be defined as "the number of years after a tree is planted that is required to produce BF_{exp} under specified environmental and site conditions".

- The array of results indicates that there is no such thing as a BF-free almond tree. Instead we must distinguish among different levels of BF_{pot} based on performance of progeny trees grown under a standard set of growing conditions.

- Table 3 attempts to establish criteria for selection for BF_{pot}. The origin of the data is the performance among the progeny trees of the individual tree sources utilized by the five commercial nurseries in this study who used commercial orchards as sources. The results suggests that a test should be conducted for at least four years (comparisons made in the fifth year) with follow up examination after 6 and 7 years. Both AvBF and % BF are used in the overall subjective rating.

Table 3. Distribution of BF_{pot} among 70 single tree sources from 6 commercial orchard sources. None showed BF at the time the buds were collected. AvBF at the end of four years progeny testing (1995) is shown with a followup of performance at the end of the 6th (1997) and 7th (1998) year.

Rating Class of BF_{pot} in source trees	No. of Source Trees	No. of Progeny Trees	AvBF Of single progeny 1995	AvBF Of single progeny 1997	AvBF Of single progeny 1998	% BF trees in progeny in 1998
Very low	9	12	0.0	0.0	0.0	0
Low	12	20	0.0	0.1-0.8	0.1 - 0.8	4 to 44
Medium	26	18	0.1 to 0.8	0.1 to 1.8	0.9 to 2.5	21 to 100
High	6	25	1.3 to 1.6	2.0 to 2.5	2.3 to 3.0	79 to 100
Very high	22	24	2.0 to 4.2	2.3 to 4.7	2.3 to 4.7	67 to 100

- Source: progeny relationships are shown in another way in Table 4. This analysis answers the question posed at the start of the experiment: what is the relative importance on the variation of BF in *source*, *trees*, *branch* and *bud location* on the budstick? This analysis utilizes a statistical procedure of calculating the percentage of the total variability of the BF data that is attributable to each parameter in the total population. The result is a correlation between *source* and *progeny* in a fashion analogous to that which plant breeders measure the *heritability* of individual traits in a parent - seedling progeny studies. In this case the *trait* is BF_{exp} which shows *variation* within the clone. The kind of variability described here has been previously defined in biological literature as *somaclonal variation.*

Table 4. Distribution of the total variance in AvBF among selection parameters of Carmel nursery sources as established from 1991 propagations.

Variance Parameter	1992 (%)	1993 (%)	1994 (%)	1995 (%)	1996 (%)	1997 (%)	1998 (%)
Nursery source	10**	14**	25**	32**	33**	37**	39**
Source tree	45**	47**	42**	41**	40**	36**	33**
Budstick within tree	24**	25**	17**	14**	12**	14**	14**
Position of bud	NS	NS	NS	NS	NS	NS	NS
Unaccounted	20	14	15	13	13	13	15

** = statistically significant at 1% level
NS = not significant

Previous breeding studies with seedling populations showed that variation in BF_{exp} within a clone is transmitted to progeny (i.e. inherited) (Kester, 1968). This means that BF_{exp} differences in consecutive annual growth cycles (referred to sometimes as "vertical" variation) and differences in separate branch sequences of the same plant or in branch sequences of different plants ("horizontal" variation) shown in Section I represent permanent changes in the BF genotype.

- The variance partitioning study (Table 4) shows that the most important selection parameter is the *individual tree* which, in combination with *individual branch,* accounts for 70% of the total variability in BF_{exp}. **This means that at the time of planting, each tree has an inherent BF_{pot} established by the combination of *source tree origin* and location of the *budstick within the tree.*** The differences among nursery sources became larger with age as more and more individual progeny trees began to express BF. This data indicates that not only trees but each part (i.e. branch, annual growth sequence) of the tree has its own inherent BF_{pot}.

- Although significant differences occurred in BF_{pot} among budsticks, no significant effect occurred due to relative position of the bud on the budstick. This relationship was hard to understand at first but makes sense if one recognize that the change occurs in BF_{pot} to all the buds on a stick some time during the annual cycle of growth. BF_{pot} of a budstick at the end of the season is greater than the BF_{pot} of the initiating bud at the beginning of the season. Such a change occurs in June after length growth has ceased (see Section I) and after individual buds have been formed. (See Section III) Vegetative buds of the currents years cycle from this point on have an increased BF_{pot}. This level is maintained into the next years cycle of growth or in new plants propagated from them. Continuous change in BF_{pot} is associated with increased age of individual plants and persists in consecutive propagation generations. Increasing BF_{exp} results from consecutive (and accumulative) changes in the specific gene(s) affecting BF.

Summary: Analysis of the BF_{pot} of the individual symptomless source trees used in this study provides evidence of a so-called "somaclonal" nature of the BF phenomenon. Variability of BF_{pot} among individual trees in commercial orchards is shown to be continuous whether or not BF symptoms are expressed or not. Variability in BF_{pot} occurs not only from tree to tree but progressively within individual trees as well. The greatest source of variability in BF_{pot} however is the individual tree and is the most important origin of low BF_{pot} sources. The shift in BF_{pot} that is shown to occur within an individual tree is within each annual season of growth.

Section III. Relationship Between Seasonal Temperature Patterns and Changes in BF_{pot} and BF_{exp}.

This phase of the project came about after all the data from the seven years was compiled and evaluated. When this analysis was done, it was observed that not only differences in the increase in BF_{exp} occurred in different years but also these differences were consistent among all sources for that year (Fig. 7). Differences among years were statistically significant indicating that something was occurring in specific years that affected this increase differently. Prior research had shown that BF was associated with the accumulated exposure of the trees to high summer temperatures.

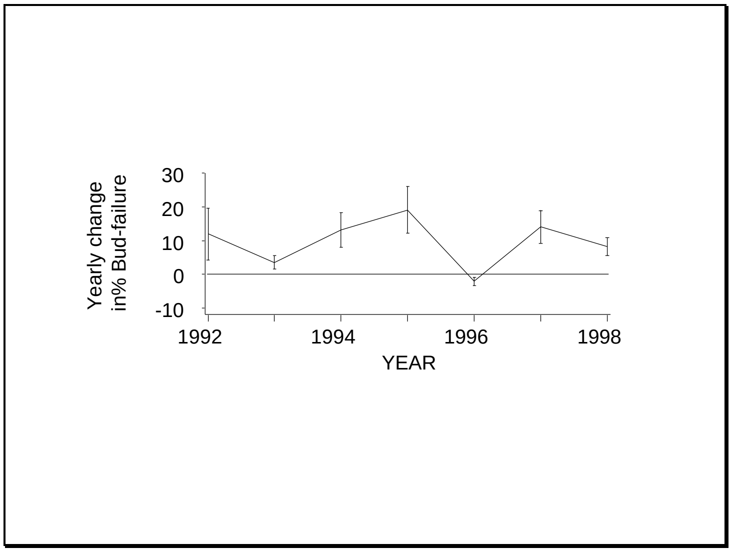

Figure 7 Variation in % BF change/year was chacteristic of year. Note the 1994 and 1995 had the greatest change but 1996 had the least.

The first step was to correlate total temperatures (Degreedays over 80°F) to yearly increases. The result was a positive relationship, which has been shown previously. One of the dogmas of BF distribution is that serverity is related to the hot summer temperatures that occur during midsummer of the previous year.

The second step was to correlate the average temperatures occurring during individual months of the year with annual changes in BF. The purpose was to pinpoint the specific time during the year that change occurred. The results from this analysis (Figure 8) show that June is the critical time for change in BF_{pot} extending into July. The critical temperatures are moderate and favor growth rather than in the range of 80°F or higher, that lead to heat damage.

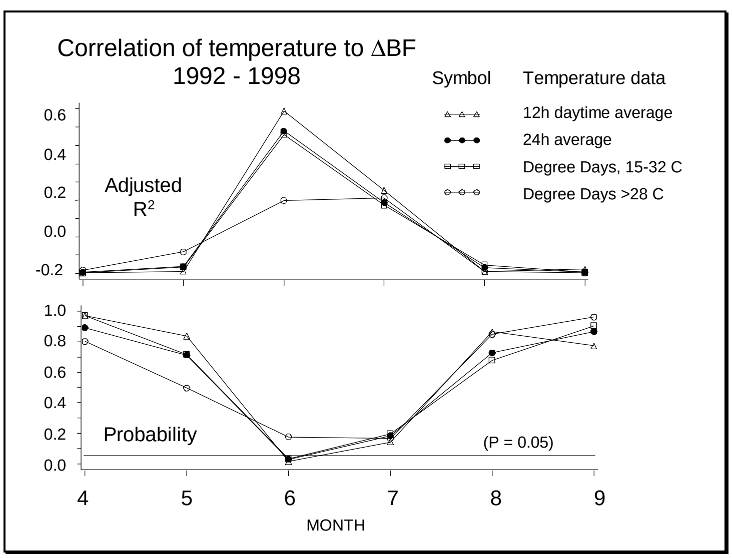

Figure 8 Above. Relationship between correlation valves (R_2) and month for four temperature data sets.
Below. Plot of Probability for each of the values above.

Fig 9 shows the regressions of temperature to yearly increase in BF_{pot}. When these temperature patterns were fitted to the BUD DEVELOPMENT MODEL (see Kester, et al., Chap. 9 in Almond Orchard Manual 1996) a picture of bud development, BF development and temperature appears. Table 5 describes seven stages of annual bud development and the changing pattern in the "normal" plant, one with BF_{pot} and one showing BF_{exp}.

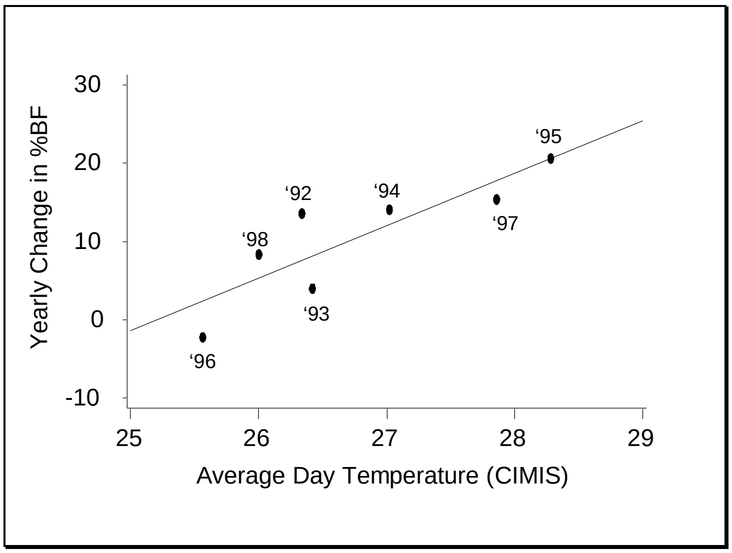

Figure 9 Regression of yearly change on BF % to average day temperature in June. Shows a range falling between 77°F (25°C) to 86°F (30°C).

Table 5. Seasonal pattern of shoot and bud development in almond trees in an orchard. HTD is high temperature dormancy.

Stage	Date	Description: **Normal**	**BF_{pot}**	**BF_{exp}**
I	Jan, Feb	Emergence of buds and flowers	Emergence of buds and flowers	Emergence of buds and flowers
II	Mar, Apr	Active growth of new shoots	Active growth of new shoots	Active growth of new shoots
III	May	Growth cessation and bud maturation	Growth cessation and bud maturation	Growth cessation and bud maturation
IV	June	Budscale formation; induction of HTD	Budscale formation; "Somaclonal" increase in BF_{pot}	Budscale formation; "Somaclonal" increase in BF_{pot}
V	July, Aug	Veg buds develop HTD; Flower buds are initiated	Veg buds have new level of $BF_{pot;}$ Flower buds are initiated from cells with higher BF_{pot}	Veg buds have new level of BF_{pot} and initiate necrosis; Flower buds are initiated with high level of BF_{pot}
VI	Sept, Oct	Veg. Buds initiate rest period Flower buds continue to differentiate	Veg buds have new level of $BF_{pot;}$ Flower buds continue to differentiate	Veg buds develop n**ecrosis** Flower buds continue to differentiate with high level of BF_{pot}
VII	Nov, Dec	Veg. Buds in Rest period; Flower buds continue to differentiate; chilling occurs	Veg buds have new level of BF_{pot} ; Flower buds continue to differentiate; rest period; chilling	Veg buds show **necrosis** Flower buds continue to differentiate; high level of BF_{pot} ;rest period; chilling
VIII; I for next year	Jan, Febr	Emergence of buds and flowers	Emergence of buds and flowers: increased BF_{pot} in new shoots and gametes	Veg buds**: bud -failure** Flower buds emerge with high level BF_{pot} in gametes.

Three components of the BF syndrome have been identified as follows:

Component I. BF_{exp} - This symbol designates the active expression of noninfectious bud-failure (BF) as shown by *necrosis of the growing points* in summer and fall followed by *bud- failure* in the spring. The seasonal pattern of BF_{exp} was characterized in earlier studies (1978-1990) by using a **petri dish bioassay** of single node cuttings which measured *per cent of buds sprouting* (Kester, paper in preparation).

Buds emerge in January or February (Stage 1), grow actively through March and April (Stage II) , stop growth and mature in May (Stage III) and develop budscales in June (Stage III). For the rest of the season, the vegetative buds appear to be dormant but begin to develop *necrosis* at various times during the summer and fall depending upon temperature, BF_{pot} and moisture stress. Necrosis was not observed in irrigated plants until September and later. Moisture stressed BF plants developed severe necrosis as early as July. It appears that necrosis is the result of interactions among high temperature (July, August), moisture stress and BF susceptibility. Flower buds differentiating in July and August (Stage V), continuing through the remainder of the season (Stage VI, Stage VII) are not affected by the toxic effect of BF_{exp}. This disorder is a genetic trait which we showed in earlier studies (Kester et.al. 1968ab) to be inherited and whose severity increases continuously with time in growth cycles of vegetative propagation.

Component II. High Temperature Dormancy - This specific trait of the almond species was discovered and characterized utilizing the same **petri dish bioassay** described above but measured as *rate of bud sprouting* (Kester, et al., 1990). In the "normal" i.e., low BF_{pot} , plant, Stages 1, 2, 3 and 4 of the normal and BF_{exp} are the same. However, in the normal plant, vegetative buds in Stage 4 begin to develop *dormancy* which is enhanced by high temperatures occurring in late June, July, Aug, and early September (Stage 5). During September and October, the buds remain dormant but now develop the "rest period" (Stage 6) which is subsequently overcome by chilling temperatures during November, December and January (Stage 7). This trait dubbed HTD (High Temperature Dormancy) apparently evolved as an adaptation to the deserts of central Asia where the almond originated. The same trait adapted the almond to the cultural conditions in the Mediterranean climates of Europe and Asia where the almond was nonirrigated. On the other hand, under the environmental and management conditions which utilize summer irrigation, the bud lose resistance to stress and increase susceptibility to BF. The result is that in California, BF evolved over time as an aberrant form of the HTD gene. Instead of going dormant the growing points lose their resistance to stress and become necrotic apparently due to the production of a toxin from the surrounding tissue or leaves.

Component III. BF_{pot} - this symbol is used to designate buds which are in the transition from HTD to BF_{exp} It is measured by the length of time (years) required from planting to produce BF_{exp} . Transition occurs in June and the rate (amount) is proportional to the favorability of (lower) temperatures to promote growth. Although BF has been characterized by the *increase* in BF_{pot}, in actuality the process is a *decline* and represents the deterioration of a clone due to a loss (change) of a climatic adaptive gene (HTD). We have described this unique kind of change as **somaclonal variation** and suggest that noninfectious bud-failure be described as **somaclonal decline**.

Summary: The most important new discovery obtained within this study was the relationship between temperatures in June and the annual increase in BFpot. This phenomenon is different from the relationship between hot temperatures in July and August on the severity of BFexp. Prior experiments (1978 - 1990) are used to show that three distinct components of BF occur. BF is explained as a gradual decline in the ability of a specific almond gene(s) to induce high temperature summer dormancy at a specific stage of the annual bud development cycle. The apparent continuous gradient in both BFpot and BFexp results from many small consecutive changes in what is called somaclonal decline.

Section IV. Patterns of BF from separate commercial nursery sources

This section describes the results of source-progeny tests in which the heritability concept is applied to the evaluation of specific nursery sources of Carmel. This analysis includes the original Carmel seedling tree and subsequently, its propagation history from the time of discovery in 1949 planted in a commercial Nonpareil orchard near LeGrand. Subsequent history included introduction in 1966 and first commercial planting into the industry in 1972. The commercial history of Carmel includes five consecutive orchard generations designated as S_o (original seedling tree), S_1 (first scion generation), S_2 (second scion generation) and so on. Within this sequence we have identified six VegLines which trace propagation sequences from the original seedling tree to each nursery source used in this study.

The eleven nursery sources in this study could be separated into four groups (Table 6):

Table 6. Characteristics of source and progeny trees of individual nursery sources at the end of the 7 year trial at Paramount West orchard in Kern Co.

Source ID	No. of Source trees	No. of Progeny trees	BF %	Significant differences among:			Remarks
				Sources	Trees in source	Within trees	
I. Original seedling tree							
NS-A	1	31	1.8	A	**	*	Severely pruned for budwood
II. Commercial Orchards							
NS-E	25	266	34.3	C	**	Ns	S_4 ; VegLine 1B
NS-F	8	79	42.5	CD	**	**	S_4 : VegLine 1B
NS-G	10	307	42.5	CD	**	**	S_3 ; VegLine 1B
NS-I	Unk	100	52.4	DE	Unk	unk	Commercial nursery trees
NS-J	7	215	54.1	DE	**	**	S_5 ; VegLine 1B
NS-L	10	295	83.0	F	**	**	S_5 ; VegLine 1B
III. Pre-selection							
NS-C	10	234	19.3	B	**	**	S4 ; VegLine 2
IV. Managed Source blocks							
NS-B	25	210	2.2	A	Ns	na	Nursery increase block; VegLine 3
NS-D	21	297	31.5	C	**	**	Nursery scion block; Vegline 4
NS-H	unk	315	50.1	DE	Unk	unk	Nursery scion block; VegLine 5
NS-G (cl)	3	98	55.0	DE	Ns	**	Orchard scion block; clonal selection of NS-G
NS-K	16	420	59.4	E	**	**	Orchard scion block; VegLine 5
NS-M	19	72	81.0	F	Ns	ns	Nursery scion block; VegLine 5

** = statistically significant at 1% level; ns = non-significant; na = not applicable

Group l. original seedling tree (NS-A) (NS = Nursery source)

This source tree originated in a commercial orchard near LeGrand, CA in 1949 apparently as a rootstock tree whose bud failed in the nursery. In recent years, the tree has been annually pruned to main scaffolds to generate bud wood for nursery production. The tree tests positive for Prunus *ringspot virus* but this characteristic does not seem to adversely affect orchard production. The progeny showed the lowest percentage of BF (1.8) of any source. "Slight" symptoms only appeared after the sixth year in upper parts of the tree.

Group 2. Commercial Carmel orchards (NS-E, NS-F, NS-G, NS-I, NS-J, NS- L)

One of the key concepts of BF selection has been that the probability of BF_{exp} tends to increase with each scion generation of propagation. Five of the nursery sources utilized commercial orchards which were determined to be of the 3^{rd}, 4^{th} and 5^{th} generation. Plotting % BF against scion generation of progeny of these and other sources confirmed this increasing trend (Figure 10). This relationship cannot be attributed to a so-called "juvenility" effect but can now be explained as the progressive accumulation of individual "somaclonal mutations" during consecutive annual cycles.

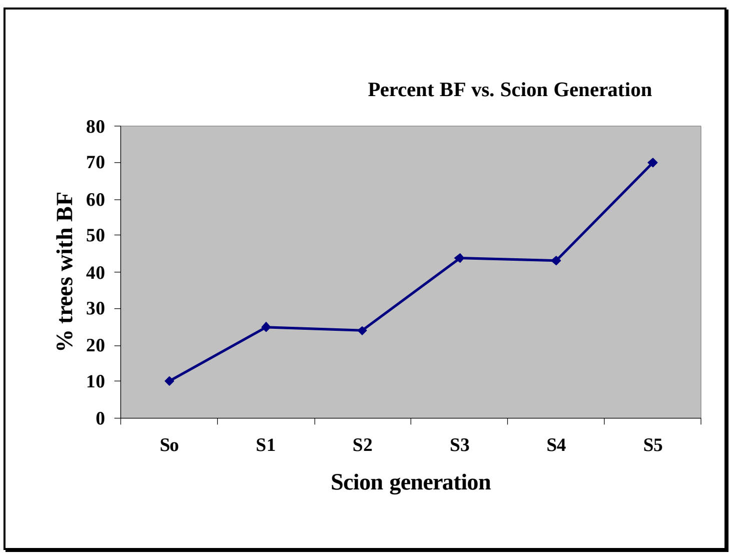

Figure 10 Average percent BF of orchards representing consecutive scion generation.

Scion generation is apparently not the only factor that determines the BF pattern among orchards; much variability was associated with location, growing conditions, vigor and perhaps even rootstock of the individual orchard.

All of the commercial source orchards used showed great variability in BF_{pot} among individual source trees and even within trees, i.e., branch to branch. This characteristic underscores the point that using commercial orchards as budwood sources is not a good practice to control BF and is probably the primary reason BF has not only continued to be a problem in the industry over the past 75 years but has increased its occurrence.

Group 3. Pre-selected sources (NS-C)

One nursery source (NS-C) utilized a unique procedure which turns out to have promise in identifying individual low BF_{pot} tree sources within commercial orchards. The orchard utilized was 18 years old, S_4 generation and a different VegLine from those described previously. BF_{exp} was present in the top of 20 percent of the trees in the orchard but not in the ten trees selected as sources. Budsticks were cut from watersprouts (suckers) emerging from the base of the trees above the graft union. Progeny from individual trees were previously propagated and compared for BF in progeny tests over several years to identify the best individual tree sources. In our tests from 10 of these previously selected trees, the average level of BF was significantly lower than progeny from other commercial orchards of comparable age, the main source of variability being among branches of the same tree.

This study demonstrates that significant variation in BF_{pot} exists in both horizontal (separate branches) and vertical (base vs. top) locations within individual trees and can be exploited in selection procedures for BF (see Section IV)

Group 4. Managed budwood sources (NS-B, NS-D, NS-H, NS-G [cl] , NS-K, NS-M)

Five nursery sources were managed under conditions to produce budwood rather than nuts and included severe annual pruning. Maintenance of these source blocks utilized some variation of "hedge-row" pruning system either in the nursery or in the orchard or utilized "increase nursery blocks". Results were as follows:

- NS-B utilized unsold nursery trees to provide budwood for the next generation of nursery plants. This procedure involved recycling the budwood source every few years which theoretically increased the scion generations considerably more than the commercial orchards listed in Group II. The nursery was located in a cool summer site and the source of the original budwood was unknown. However, under this combination of conditions BF_{pot} was very low with very little BF_{exp} in the progeny (2.2 percent at seven years with very mild expression).

- NS-D source trees were planted in a closely-planted hedge row. This source originated from a commercial orchard which represented a completely separate origin from all others (VegLine 4). The percentage of progeny trees with BF (31.5 percent) was significantly less than many of the other sources. The BF trees that did appear came from specific source trees. Increased variability also came from separate sides of the source trees.

- NS-H (commercial) and NS-M (test) appear to have been propagated from the same nursery source block, a row of nursery trees maintained in a hedgerow over consecutive years. The original source was not known for certain but apparently was a commercial orchard of perhaps an S_4 generation. This source was designated as VegLine 5. Many of the progeny trees of NS-M died during the first year in the orchard which we believe was related to their high BF_{pot}. The percentage of the individual source trees producing BF progeny was very high (81%). Furthermore, the % of trees with BF of NS-M (commercially supplied trees) was also high.

- NS-K was maintained as a typical orchard scion block and produced very vigorous budwood material. There was a wide range of BF_{pot} both among and within the individual trees of the source. The budwood used to establish this scion block apparently came from VegLine 5 and had a high $BF_{pot,}$ comparable to that of NS-H and NS-M.

- NS-G (cl) is interesting in that it originated as a single tree selection from the same source orchard as NS-G but was maintained as a scion tree row and pruned as a hedge. The significance was that the average BF of the progeny of the three individual source trees was the same as the entire NS-G orchard source but the uniformity in BF % was much higher. This source is also represented in the clonal selections shown in Table 6 (row 114). This material is further discussed in Section IV.

Summary: Controlling BF through nursery procedures follows two basic principles: a) select sources with low BF_{pot} at the outset and b) stabilize, maintain and manage the source blocks to continue distribution of the material over time without increasing the BF_{pot} of the source.

Of the sources used, NS-A and NS-C followed both principles. NS-B also appeared to have followed those principles but we cannot be sure why, whether this method is superior to other techniques or if the cooler summer location provided a favorable environment not present with the other nursery sites.

The primary conclusion from this section is that use of commercial orchards as budwood sources violates both principles and over time their use can be expected to increase the BF problem for a specific nursery.

Section V. Selection of low BF_{pot} Foundation Clones of Carmel

At the beginning of this report, three different tests of single tree sources were described. The results of the progeny tests of the three groups of candidate clones are given in Table 6. The first group came from a commercial nursery (NS-G) whose bud-source was a S_3 commercial orchard. Certain rows (e.g. R116) were planted with progeny from a single tree in the prior generation, designated as "clone source" and pruned back each year for budwood. Progeny from trees R114 -1 through 6 were utilized in the test. Three trees of the group were retested in the nursery trial (section III) as NS-G (cl), B114-3 was included in the Group 2 and others were in Group III. The average BF_{pot} of the "clone trees" was not significantly different but the range in BF_{pot} was much more restricted making for high uniformity among progeny trees. The pattern of BF_{exp} was slower to develop than most other nursery sources (3^{rd} to 5^{th} year) and most of the trees were only slightly or moderately affected. Although these sources were an improvement over many if not most of the nursery sources tested, somewhat more BF was produced in the long run than other sources, particularly in Group II and III and none have been retained.

Group II (established 1990) included three sources (D13-2, D13-13, and D13-7) from the Delta RVT plot, Manteca. Two selections (W1-4 and W-9) were from individual trees (S_1) in a nursery test plot propagated from the original seedling tree. A few trees propagated directly from the original seedling source (S_0) were included. BH is the source used by the commercial orchard in which the test was established. D13-2 and D13-13 produced no BF until the 8th year and their expression was very slight. D13-7 produced some BF in the 7th year and the expression was also very slight but was found in a relatively high percentage. W1-4 had low BF_{pot} but W1-9 had very high BF_{pot}. B114-3 duplicated Group I.

Group III selections were obtained by using the procedure followed in NS-C and described in Section IV. Of the seventeen selections tested in commercial orchard plots in Fresno County, eight had produced no BF expression by 1999 (5th year).

From these tests, low BF_{pot} sources have now been established as FOUNDATION CLONES in the FPMS Orchard at UCD. These are identified by specific FPMS numbers and are listed below. These source trees have been *registered* as virus-tested clones under regulations of Registration and Certification of the CDFA, Sacramento. Their maintenance is supervised by FPMS staff and its Advisory Committee, which includes UC and USDA researchers, commercial nursery persons (AIB) and regulatory personnel from CDFA.

FPMS 3-56-1-90. This selection originated as D13-2 from the Delta RVT plot and results of its progeny test is shown in Table 6, Group II. Some very slight BF has appeared at the 8th and 9th year of the progeny test. Material was released to the nursery industry in 1994 and some commercial orchards were planted in winter spring 1995. No BF has been reported to date in any commercial orchard propagated from this source. Nor has any BF appeared in the RVT plots where this source was planted in 1993.

FPMS 3-56-2-90. This selection originated as D13-7 from the Delta RVT plot (see Table 6, group 2). Although originally released with D13-2, its use has been discontinued because of its slightly earlier initiation of BF in the progeny test.

FPMS 3-56-6-93. This selection originated with NS-B (Table 5). Originally found to be PRSV positive, it was since heat-treated to eliminate the virus. No commercial information of subsequent performance is available.

FPMS 3-56-7-92. This selection has the same origin and history as 3-56-6-93.

FPMS 3-56-8-92. This selection is D4 from the Delta plot. No BF has appeared in the progeny test through 1999.

FPMS 3-56-9-92. This selection is from D8 from the Delta plot. No BF has appeared in the progeny through 1999.

Table 7. Progeny tests of single tree candidate selections for low BFpot Foundation Clones in Carmel

Group I. Commercial Nursery Carmel selections

Source ID	No. trees	1990	1991	1992	1993	1994	1995	1996
A. BF percentage								
114-6	14	0	0	0	0	21	29	29
114-2	19	0	0	5	11	21	26	42
114-5	11	0	0	0	18	55	64	64
114-4	16	0	6	21	50	62.5	69	69
114-1	13	0	0	3	31	62	92	77
114-3	13	0	0	38	62	77	92	100
B. AvBF								
114-6	14	0	0	0	0	0.29	0.29	0.29
114-2	19	0	0	0.11	0.11	0.32	0.42	0.69
114-5	11	0	0	0	0.27	0.73	1.18	1.00
114-1	13	0	0	0.03	0.38	1.15	1.38	1.08
114-4	16	0	0.06	0.50	0.81	1.12	1.25	1.19
114-3	13	0	0	0.23	0.62	1.54	1.54	1.77

Group II. Miscellaneous Carmel selections made in 1989.

Source ID	No. trees	1991	1992	1993	1994	1995	1996	1997	1998	1999
A. Per cent BF										
S_o	8	0	0	0	0	0	0	0	0	0
D13-2	39	0	0	0	0	0	0	0	5	5
D13-13	39	0	0	0	0	0	0	0	11	14
W1-4	27	0	0	0	15	7	0	0	41	22
D13-7	39	0	0	0	0	0	0	51	74	41
B114-3	40	0	0	0	22	28	43	75	92.5	70
BH	57	0	10.5	12	35	39		68	81	70
W1-9	38	0	36	78	97	100	100	100	100	100
B. AvBF										
S_o	8	0	0	0	0	0	0	0	0	0
D13-2	39	0	0	0	0	0	0	0	0.03	0.08
D13-13	39	0	0	0	0	0	0	0	0.11	0.09
W1-4	27	0	0	0	0.15	0.1	0	0	0.4	0.5
D13-7	39	0	0	0	0	0	0	0.8	0.9	1.0
B114-3	40	0	0	0	0.3	0.4	0.5	1.3	1.4	3.9
BH	57	0	0.11	0.14	0.5	0.8	1.0	1.3	1.7	2.0
W1-9	38	0	0.5	0.6	2.1	2.8	3.6	4.4	4.4	2.9

Group III Carmel source selections made from UC selections in 1992 and 1993 plus commercial selections. Trees planted in January 1994.

Source ID	Test plot	No. progeny trees	March1998		March 1999	
			% BF trees	AvBF	% BF trees	AvBF
Sources from commercial orchard near Ripon						
VG 8-8	Fresno 1	2	0	0.0	0	0.0
	Fresno 2	10	0	0.0	0	0.0
VG 8-14	Fresno 1	2	0	0.0	0	0.0
	Fresno 2	10	0	0.0	1/10	0.1
VG 8-18	Fresno 1	2	0	0.0	0	0.0
	Fresno 1	10	0	0.0	0	0.0
VG 8-23	Fresno 1	2	0	0.0	0	0.0
	Fresno 2	10	0	0.0	0	0.0
VG 11-3	Fresno 1	2	0	0.0	0	0.0
	Fresno 2	10	0	0.0	0	0.0
VG 11-6	Fresno 1	2	0	0.0	0.0	0.0
	Fresno 3	10	0	0.0	0.0	0.0
VG 8-7	Fresno 1	2	100	3.0	100	3.0
	Fresno 2	10	60	1.2	100	3.0
VG 8-10	Fresno 1	2	0	0	0	0.0
	Fresno 2	10	10	0.1	20	2.0
VG 8-12	Fresno 1	2	50	1.0	50	3.0
	Fresno 3	10	70	2.1	80	1.7
VG 8-13	Fresno 1	2	0	0.0	0	0.0
	Fresno 2	10	10	0.1	-	-
VG 8- 15	Fresno 2	10	30	0.5	40	0.5
VG 11-1	Fresno 1	1	50	-	100	1.5
	Fresno 2	9	67	50	50	0.4
VG 11-5	Fresno 1	2	0	0.0	0	0
	Fresno 2	10	20	0.3	70	1.3
Sources trees tested from Delta RVT plot, Manteca						
D2	Fresno 1	2	0	0.0	0	0.0
	Fresno 3	10	0	0.0	0	0.0
D4	Fresno 1	2	0	0.0	0	0.0
	Fresno 2	10	0	0.0	0	0.0
D8	Fresno 1	2	0	0.0	0	0.0
	Fresno 3	10	0	0.0	0	0.0
D20	Fresno 1	2	0	0.0	0	0.0
	Fresno 2	10	20	0.2	60	0.9
Trees from commercial selections						
Mach	Fresno 1	4	0	0.0	25	0.25
	Fresno 2	7	30	0.3	14	0.14

	Fresno 3	8	37.5	0.5	60	0.9
72-2E	Fresno 1	3	0	0.0	67	1.3
112-40	Fresno 1	3	0	0	67	1.0
	Fresno 3	4	25	0.5	67	1.0
112W-4	Fresno2	7	40	0.9	41	0.9
27-448	Fresno 1	16	75	2.1	95	2.1
Background commercial source within test orchard						
Unknown	Fresno 1	59	44	0.7	70	0.8
	Fresno 2	66	61	1.5	64	1.2
		68	54	1.1	-	-
	Fresno 3	73	55	1.1	57	1.0

Summary: Progeny results of 35 separate single tree sources are detailed in three major tests. Out of these results, individual selections have been established as Foundation Clones at the FPMS Repository at UC Davis. Of these, FPMS 3-56-1-90 has the lowest BFpot and was released to the nursery industry in 1994. Four other Foundation Clones are also maintained in the FPMS repository as low BF_{pot}.

Section VI. Relationship between propagation method and the control of noninfectious bud-failure

Biologically the relationship between the seasonal changes in BF_{pot} and the propagation process (Fig. 8) are very different from that of the seasonal changes during orchard development as described in Table 5. Table 8 shows the pattern during June bud propagation utilizing scion orchard and/or hedgerow propagation blocks. In the "normal" and/or low BF_{pot} source, shoots emerge (Stage I), grow rapidly during March, April (Stage II), cease growth and mature in May (Stage III). Propagation takes place at that time and needs to be completed before June when the budscales appear (Stage IV). Shoots may remain in place for the rest of the year and are pruned away at the next dormant season. Next years shoots start over from latent buds or from those at the very base. Under these conditions, the BF_{pot} is stabilized at an initial level and the process is repeated next year.

Table 8. Seasonal pattern of shoot and bud developmental stages during June bud propagation in almond using hedge row management.

Stage	Date	Description		
		Normal	**BF$_{pot}$**	**BF$_{exp}$**
I	January, Febr	Emergence of buds	Emergence of buds	Emergence of buds
II	March, April	Active growth of new shoots	Active growth of new shoots	Active growth of new shoots
III	May	Growth cessation and bud Maturation **Propagation**	Growth cessation and bud Maturation **No change**	Growth cessation and bud Maturation **No expression**
IV	June	Budscale formation; induction of HTD	Budscale formation; **Possible slight increase in BF$_{pot}$**	Budscale formation; Increase in BF$_{pot}$ **No expression**
V	July, Aug	Veg: HTD	Veg: **Possible slight new level of BF$_{pot}$**	**No expression**
VI	Sept October	Veg. Buds: initiation of rest period	Veg: **Possible slight new level of BF$_{pot}$**	Veg: **No expression**
VII	November, December	Veg: Rest period; chilling. Dormancy **Severe pruning**	Veg: **possible slight new level of BF$_{pot}$**	Veg**: No expression**
VIII, I for next year	January, February	Emergence of shoots	Veg:**emergence**	Veg**: emergence**

Operational control of noninfectious bud-failure in orchards thus is at the level of nursery propagation. A program for control of BF has three steps:

I. Select single tree sources with low BF-potential to become FOUNDATION CLONES

The most important step in a program to control BF is the selection of specific low BF$_{pot}$ single tree sources (literally single bud). The present analysis of BF distribution in the industry shows that essentially every branch of every tree has its own unique BF$_{pot}$ whose potential must be performance tested through the propagation of their progeny.

Because of the possibility of variation among separate branches of the tree, several branches must be included for each tree (we suggest five). In contrast, there is little variation within a single shoot so that only one bud per shoot theoretically needs be tested, although three to five are suggested to provide a margin for error. The test should be conducted in a region of higher than average temperature and which is known to favor BF expression. The length of the test should be enough to bring the tree into bearing. We recommend that the progeny trees should be examined at the 5th year from planting (4 years of exposure with an annual followup each of the next 2 or 3 years. Both percentage progeny affected and age of expression should be considered. At present there is no molecular fingerprinting system to identify BF_{pot}.

II. Maintain stabilized FOUNDATION CLONES in a FOUNDATION BLOCK.

Stabilization means that the BF_{pot} does not increase during the natural annual cycles of growth which we have shown to occur in commercial orchards (where the objective is to grow a nut crop). In a budwood source block the trees need to handled for budwood production by a system of hedge row and scion orchard establishment in which shoots are annually pruned back to the main or secondary scaffolds. Our studies indicate that change in BF_{pot} occurs in June and is associated with higher than average temperatures at that time. This period occurs after budwood collection and propagation in normal "June budding" operations.

We may need to look further into the process of establishing such stock trees during their first years in the orchard. Nevertheless, if the source is inherently low in BF_{pot} the method may not be critical.

The procedures used to maintain Foundation Clones in virus-control programs have been successful for maintaining Nonpareil and other varieties in "clean stock" programs for many years now.

III. Multiplication and Distribution for commercial propagation.

This step is carried out by commercial nurseries who develop their own *scion orchards, hedge row blocks* or *nursery increase* rows. The same comments that we made in step II apply here. Note that the time of collection and budding is again earlier in the year than when the change in BF_{pot} occurs. Also fall budding and spring budding with stored scions should be satisfactory as long as low BF_{pot} sources are used. Results from this experiment indicates that it takes a number of years of consecutive exposure to increase the BF_{pot} from a low level to one that produces BF_{exp}. This conclusion fits the general experience and results of other experiments.

Summary: A summary of steps for controlling BF in the nursery industry is described which involves a combination of (a) selection for low BF_{pot} Foundation Clones combined with (b) management of source blocks.