## ALMOND BOARD OF CALIFORNIA ANNUAL REPORT

Project 88-K15 - Bud-failure

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## <u>Objectives</u>

- (1) Provide guidelines to the nursery industry and the Foundation Seed and Plant Materials Service (FSPMS) on use of the bud failure (BF) model to: (a) predict the future of BF-potentiality in 'Carmel' and other varieties by means of source pedigree analysis; and (b) apply the model to maintenance and distribution of source materials of different almond varieties.
- (2) Maintain shoot tip cultures of different Nonpareil source clones and rootstock material; use this material in current amino acid protein studies; develop culture as a means of maintaining, multiplying and distributing sources of low BF potential.
- (3) Extend the current amino acid and protein study to determine the significance of the differences being observed, and their potential as BF markers.
- (4) Complete analysis, summarization and publication of information on the project.

#### INTERPRETIVE SUMMARY

Objective (1): A cooperative program was begun in summer 1988 joining this project, the recently formed Nursery Fruit Tree and Grapevine Improvement Advisory Committee and the Foundation Seed and Plant Materials Service (FSPMS). New individual tree clonal source selections were made of Carmel, Butte, Price, Fritz and Ruby. These came from nominations of individual commercial nurseries with up to six individual selections of each. Approximately twenty-five trees of each source were propagated in a commercial nursery this summer to be planted into commercial orchard tests in southern San Joaquin valley. These trees will be evaluated for production of BF symptoms, freedom from nonproductive genetic disorders and trueness to type. Each tree source has also been started into a 2 year virus indexing program. Parallel trees have been propagated to be placed into the Foundation Orchard

of FSPMS, UCD. At the conclusion of these tests, the sources will be incorporated into the California Registration and Certification program.

This program puts into direct action principles learned from research sponsored by the Almond Board through this project.

<u>Objective (2)</u>: Aseptic culture. Shoot tip cultures of source-clones of "high" and "low" BF potential are being grown successfully. Several new potential rootstock clones are also being cultured.

Objective (3): Amino acid analysis. Data from this study carried out in 1986 and partly reported in the 1987 annual report has now been completely analyzed. The study involved the simultaneous analysis of the pool of free amino acids and related compounds of samples of leaves and buds of normal and BF affected Nonpareil plants collected bimonthly from April through November. In addition, comparisons of bud-forcing potential and incidence of necrotic bud symptoms were made to monitor the changing physiological condition of the buds and the pattern of symptom development.

Significant differences were found between the normal and BF shoots (buds and leaves) in both the bud physiology and the correlated amino acid patterns. The critical time period for symptom development during the study appeared to be a "window" occurring during August and extending into early September. During that period the buds of the normal plant began to lose their sprouting capacity, become "mature" and enter their resting period. This process accentuated through September and reached a maximum in early October. This period was marked by the simultaneous appearance of an amino acid arginine which increased through latter part of August to a maximum and then decreased to zero by the middle of September. The decrease in arginine was accompanied by the appearance and gradual increase of a second amino acid proline which reached a maximum in early October coinciding with the apparent development of "rest". The production of proline during periods of dormancy and after moisture stress is a well known biological phenomenon.

In contrast, the buds from the bud-failure plant continued to maintain their high bud forcing potential through August but then began to develop necrotic injury symptom in the buds. These began to appear in early September, continuing through the rest of the fall. The amino acid patterns in the bud failure plants were significantly different from that of the normal. No arginine appeared until after the first of September (10 days to 2 weeks after that of the normal plant). When arginine did appear it increased sharply, then decreased and then fluctuated periodically during the rest of the fall. Proline began to increase sharply in September following the appearance of arginine and increase to a level 2 or more x that of the normal continuing at this high level throughout the remainder of the fall.

It is hypothesized that the normal buds in late summer initiate the glutamic acid - arginine - proline sequence as a part of the maturation phase of development. In the BF affected buds, on the other hand, this reaction sequence is either inhibited during the high temperature period or is diverted into alternate pathways, possibly leading to the production of

toxic compounds. Later on during the cooler fall temperature periods, this same reaction sequence not only is triggered to develop but is sharply stimulated by the stress from the injured buds to give added impetus to the production of high levels of proline.

Differences between the amino acid levels of the bud-failure and the normal plants were also shown in the leaves. Further study needs to be made of this data to establish the significance of these differences.

Since the production of proline is also known to result from induced moisture stress, it will be important to learn if the reaction can be induced or prevented in moisture stressed BF plants. Likewise, it will be important to learn if the presumed inhibition of the reaction can is overcome by the lowering of the temperature. Studies are being proposed to obtain answers to these questions.

This study provides for the first time the framework for understanding the biochemical basis for the BF phenomenon.

### 1988 ANNUAL REPORT - PART I.

### Selection of Propagation Sources of Almond Varieties

by

### Dale E. Kester and Tom Gradziel

(Collaborating with Robert Wooley and Nursery FTNGI Board, Susan Nelson-Kluk (FSPMS) and UC Coop Extension (W. Micke, M. Viveros)

One of the long term objectives of this project has been to select improved nursery propagation sources of almond varieties which have low potential for noninfectious bud-failure (low BF- potential) either through the selection of specific sources or by providing guidelines to nurseries. Models to describe the variability of BF-potential with time and within orchards have been developed (see previous annual reports; publications listed at end of report). These guidelines point to the selection of specific individual tree sources to create new "source-clones". Selection is based upon "PEDIGREE ANALYSIS" and "VEGETATIVE PROGENY TESTING".

Other problems that involve variability among nursery trees include NONPRODUCTIVE GENETIC DISORDERS (Nonproductive syndrome or commonly known as "bull" trees) and other variants, and viruses. Research on each of these problems has been carried out over the past number of years and reported in this project, or summarized and presented as special reports (see publication list)

One solution to these problems is the selection of individual source trees to initiate SOURCE-CLONES which have been tested for known viruses, which are "true-to-type", and which are free of nonproductive genetic disorders. By combining source- clone selection for each of these problems with that of source clonal selection for low BF-potential it is possible to justify their incorporation into a REGISTRATION and CERTIFICATION program currently in place for the production of "clean stock" (i.e., virus-tested) nursery materials. Some almond selections with these attributes are currently maintained by the Foundation Seed and Plant Materials Service, UCD (see Appendix).

The possibility of extending this program to additional almond varieties was enhanced by the formation of the TREE FRUIT, NUT AND GRAPE NURSERY IMPROVEMENT BOARD in 1987 made up of members of the commercial nursery industry.

## Procedures

Selection for freedom from specific viruses is made by several standard indexing methods, as described elsewhere. Selection against nonproductive genetic disorders and selection for trueness-to-type is based on visual inspection of source trees and source pedigree analysis but must be verified by visual inspections of vegetative progeny trees. Selection for low BF-potential currently is based upon source pedigree analysis but most importantly on the visual inspection of vegetative progeny trees in areas of high BF potential.

### **Results**

1. Viruses.

A Special Report to the Almond Board has been prepared that summarizes past research on the distribution of specific varieties particularly as they relate to the RVT plots. It also describes some of the workings of the Foundation and Plant Materials Service and the Registration and Certification program. (See list of publications).

2. Nonproductive genetic disorders (Nonproductive syndrome)

A Special Report to the Almond Board summarizing the long series of research on the so-called "Bull Mission" problem was prepared in spring 1988. It also analyzes contrasting procedures for source selection carried out in the commercial nursery industry and their relation to the distribution of genetic disorders. (See publication list, end of report).

3. Status of current source-selections

Clonal source selection of particular varieties has been underway for a number of years and has been the basis of research in the project. These sources are maintained in the Foundation Orchard, UCD, of the FSPMS and some have been utilized in commercial nursery propagation. Only limited numbers of trees of each source are maintained in the Foundation Orchard such that commercial propagators need to establish their own source blocks. Essentially all of the sources have been utilized in the RVT plots for performance and yield evaluation. Information has been provided in annual reports. A detailed summary analysis has been completed but does not yet include 1988 data. When this is done, a report will be made to the Almond Board.

A Memorandum prepared in April 1988 summarizing the current status of almond varieties and sources within and without the FPMS program is in the Appendix. This report was utilized in compiling recommendations for new selections to be incorporated into the program.

4. Progress of source selection in 1988.

Meetings with the FTNGI Board, FSPMS, Paul Lavine (Almond Board) and investigators of this project resulted in the selection of a series of individual source trees to be used as "nuclear stock" for development of new clonal sources of the varieties specified. These were nominations made by individual nurserymen based on observations and performance.

Budwood was obtained for budding in June onto certified Nemaguard seedling rootstocks by the Burchell Nursery. Virus indexing were started at the same time by the FSPMS, UCD and trees propagated for inclusion in the FSPMS Orchard. Nursery trees are to be dug in 1988-89 digging season and planted into test orchards in the southern San Joaquin valley area as a vegetative progeny test for evaluation for BF, nonproductive disorders and trueness to type.

Table 1 summarizes the varieties, sources and numbers of trees propagated for this test. A pedigree analysis of these materials is underway but is still incomplete.

Variety		Numbers of sources	Numbers of trees	Initial Virus status*
Carmel		6	109	negative
Monterey		5	79	unknown
Price		11	243	negative
Butte		6	96	negative
Mission	(1)	6	123	positive
	(2)	3**	67	negative
Padre	. ,	1**	28	negative
Sonora		1**	26	negative
Fritz		6	26	positive
Ruby		1	13	positive

\* Tested on (short-term Shirofugen Index; those that are positive are undergoing 2 year index program on 6 indicators \*\* previously tested in RVT plots

## 1988 ANNUAL REPORT. PART II

## Amino Acid Analysis of BF and Nonaffected Plants

by

#### Dale E. Kester and Don Durzan

## (Collaboration of Frank Ventimiglia and Linda Liu)

The distribution of BF within and between orchards is, on the one hand, related to differences in <u>F-potential</u> of the trees growing in the orchard. However, it is also recognized that <u>BF-expression</u>, i.e., the characteristic of the disorder to produce the specific symptoms of "bud-failure" and "roughbark", is also strongly dependent upon environmental conditions and (possibly) management factors. Environmental conditions include high summer temperatures but high moisture stress during this same period can apparently be a factor. Although a major emphasis for long range control of the problem has been placed upon selection of source materials which have a greater "resistance" to the conditions inducing bud-failure symptoms, another emphasis has been to understand the physiological basis under which the symptoms of BF develop. This knowledge could lead to earlier, objective methods of identifying or measuring BF-potential and possibly to management operations that could depress the development of symptoms.

Past research has determined the basic morphological pattern that occurs during the season and has shown that internal injury to the vegetative buds can be identified visually in late summer and early fall. Orchard, growth chamber and laboratory experiments have shown that symptom development can be induced by exposure to high temperatures which is proportional to the accumulated exposure to temperatures over  $80^{\circ}$ F. In the orchard, the high summer temperature period of August and (sometimes) early September has been implicated as the critical time of symptom induction. Orchard experience and some controlled experiments have also implicated severe moisture stress during this period as also inciting severe BF symptoms at least under conditions of high temperature. Some laboratory experiments have shown that the stomates on leaves of BF affected plants do not respond normally to increased temperature and thus result in higher internal temperatures.

Studies on bud development show that in the nonaffected plant, active shoot growth essentially stops from May and later. Vegetative buds become dormant (quiescent) and develop budscales in June. Buds subsequently mature, remain dormant and gradually go into a rest period from August through September and later. During this time they are apparently highly resistant to high temperature and moisture stress. In contrast, studies now carried out over a number of seasons show that the vegetative buds of the BFaffected plant do not follow the same pattern from mid-summer on and evidently fail to develop the maturity and resting condition characteristic of nonaffected plants. Earlier studies showed that these differences could be associated with hormonal levels in abscisic acid and gibberellin, i.e., nonaffected = high ABA/low GA; BF = lower ABA/higher GA.

Preliminary analyses of amino acid patterns were started in 1985 and a full scale analysis carried out in 1986. Part of these results were presented in 1987 Annual Report, particularly those relating to the pattern of symptom development and shoot forcing response. The pattern was similar to that previously found.

- (a) During August 1986 the vegetative buds of the nonaffected plant began to decline in forcing potential as the buds became mature and a "rest period" gradually began to develop. This decline increased rapidly during September to a minimum in early part of October.
- (b) The pattern of the BF-affected buds was somewhat similar, except that the forcing potential remained high through August, declined during late September but was always higher than the comparable nonaffected shoots.
- (c) Necrotic buds began to appear in early September with the number of affected buds increasing during the remainder of the fall.

What appears to be significant is that there was a "window" of time (or development) during August when the BF-susceptible buds failed to "mature" and/or develop resistance. Immediately following this time, injury occurred inside the buds that could be visually observed by the early part of September.

#### Procedures

The procedures for the experiment was described in the 1987 report. What has been carried out in the meantime is completion of the statistical analysis and the graphics for the entire pattern of amino acids of the leaves and the buds. This involved a computerized 3-dimensional graphic display of the extensive data that required special programming carried out by Frank Ventimiglia.

### <u>Results</u>

Earlier analyses concentrated on the conspicuous difference in proline content of the BF-affected plant as compared to that of the normal. Proline is a special amino acid that is commonly observed to develop to high levels when plants are subjected to stress.

A more precise pattern of sequential flow of different amino acids and related compounds began to emerge when enough samples were analyzed close together in time. For example, one can compare the actual concentration, the per cent of total amino acids and the percentage of nitrogen represented in specific amino acids (nitrogen flux) in both normal and BF leaves and buds as they change continuously throughout the entire summer and fall. Certain compounds predominated throughout the entire period which represent pools of soluble amino nitrogen. For instance, asparagine is an important translocation form of nitrogen. Alanine is a first nitrogen containing

product of photosynthesis and also serves as a nitrogen source to other amino acids.

However, most interest centered on the glutamic acid family which involves a series of reactions that include citrulline and ornithine leading to arginine and then proline as the final product. In buds of the normal plant, arginine began to accumulate in mid to late August, increasing to a maximum and then decreasing to non-detectable amounts for the remainder of the fall. Proline began to appear, increasing steadily to a high level through October where it remained through the rest of the fall. This pattern was correlated to the decrease in bud activity and appeared to mark the maturation and the induction of the resting condition in the bud.

During the "window" in August, just prior to the development of necrotic bud symptoms in the bud, no arginine and little proline appeared in the buds of the affected plant. Instead, arginine began to appear ten days to two weeks later (early September) and then began to appear in pulses throughout the fall. Following the appearance of arginine, proline concentration increased to a very high level 2 to 4 times that occurring in the normal plant. These high proline concentrations appeared during or after the induction of the symptoms and evidently represent the effect of injury stress.

Differences also occurred in the leaves between the normal and budfailure plants particularly in the arginine, proline and other compounds of the glutamic acid family. These differences in timing could be shown (as well as in the buds) by plotting the concentration of the BF against the concentration of normal on the same sampling date. Many of the compounds showed a high correlation with a direct line relationship. Others showed wide fluctuating patterns which identify variation between the normal and the BF tissues in time sequences.

### Discussion

The important difference between the normal and BF tissue appeared to occur during the "window" in August prior to the production of symptoms. At that time it appears that in the BF plant, the induction of the glutamic acid -- (ornithine) -- (citrulline) -- arginine -- proline sequence which accompanies the normal physiological response of "maturation" and "shutting down" of metabolic activity that should occur at this time of the year is either (a) prevented from occurring or (b) is diverted to other compounds which may be toxic in high concentrations.

The accumulation of proline is also associated with the plant reaction to stress and to the seasonal development of growth cessation and maturation. It is hypothesized that the abnormality of the BF tissues involves the enzymes of this specific biochemical reaction which are sensitive to temperatures. Thus, it is hypothesized that during the high temperatures of late summer this reaction is inhibited whereas during the milder temperatures in mid September and later, this reaction can not only take place but occurs at a very high level due to the effect of injury stress.

The hypothesis poses several questions that are researchable and need to be the subject of future work.

- 1. Since moisture stress also leads to the production of proline, would imposition of severe moisture stress earlier in the summer period also induce an arginine proline reaction sequence in normal plants (assuming the same high temperature exposure)?
- Exposure of BF plants to severe moisture stress early in the season (high temperature exposure) produced immediate production of symptoms (1979 experiments). Would such moisture stressed BF plants fail to develop the arginine-proline reaction.
- 3. What would be the pattern of the arginine -- proline sequence in normal and BF plants at cooler summer temperatures with and without moisture stress? And how would this relate to more direct glutamate -- proline sequence?

These questions are the basis for a new study proposed to the Almond Board for 1989. This study would not only confirm the findings of this experiment but also extend the concept to the interaction of moisture stress as a contributor to the BF symptom expression.

4. Assuming that defective reactions controlling the glutamic acid -arginine -- proline pathways are directly involved in bud-failure expression, if the necrosis symptom due to direct lack of resistance of the non-dormant BF tissue? Or is it due to the production of aberrant unknown toxic substances? The answer to this question would determine how effective this reaction could be utilized as a biochemical marker.

Studies to identify potentially toxic compounds related to arginine and glutamate metabolism are under way in Durzan's laboratory using a new specially designed, automated analytical method.

## 1988 ANNUAL REPORT. PART III

Shoot Tip and Callus Culture of Normal and BF Sources of Nonpareil

by

## Dale E. Kester, Linda Liu and Karen Pelletreau

Evaluation and identification of sources and varieties for BF-potential has required the identification of specific symptoms that occur on a mature trees following a distinct season al pattern of development, as described in part II of this report. Considerable effort has been expended in recent years to develop alternate systems that could allow expression of the BF syndrome under controlled environmental conditions, as in the laboratory or/and greenhouse. For example, a number of experiments have shown that differences in BF expression can be produced in plants growing in containers. Furthermore, we have found that almond plant material can be maintained in aseptic culture systems, as shoot tips, callus, and cells. Under these conditions, differences between normal and BF tissue can be documented.

<u>Procedure</u>: Procedures for growing almond plant material in culture has been previously described.

<u>Results</u>:

1. Almond cells originating from normal and BF source trees had been maintained in suspension culture for a number of years and was the basis of part of the thesis work of Dr. Lou Fenton. Information from this work has been published (see bibliography). These culture lines had been maintained both as cell suspensions and as callus. A number of additional experiments have been performed, including the effect of nitrogen nutrition, artificial moisture stress produced by polyethylene glycol (PEG) and short term heat exposures. Comparisons have been made between normal and BF source tissue to determine the effect on growth performance, response to tetrazolium chloride (for viability) and proline production. Results of these experiments are being summarized to provide background for further research which may be reinitiated in the future.

Because of the extended age and relative poor health of these cell cultures, as well as the time required for their maintenance, these cultures have now been discontinued.

Future work with such material will require the development of new culture lines which could be developed.

 Emphasis has been placed in the past two years on developing the shoot tip culture procedure. New material was started in 1987 and also in 1988 from source material of Nonpareil with low BF- potential and sources with high BF-potential. In addition, six additional potential rootstock clones are being grown.

These procedures are now highly successful, culture lines appear to be stabilized and extensive numbers of new unrooted microshoots can now be produced regularly. Transfers at 3 to 4 weeks are needed to maintain the plants in a healthy condition. No rooting studies have been carried out. However, several experiments were carried out to measure the proline content of shoots of normal and BF tissue with time of culture and after differential temperature exposure. Although differences have been produced, more comparisons need to be made.

### Discussion

The experimental systems involved in growing almond plants in culture have a great deal of potential not only for studying the BF problem but also as bioassays for BF-potential. There value is based on the fact that the differences in the inherent BF potential is maintained in the cells (buds or tissue) used for propagation. The future application of these systems is likely to be most useful when used in conjunction with the other aspects of this project.

Culture "lines" could be established that parallel the "vegetative progeny tests" of part I. In fact, maintaining and multiplying the original source plants under temperature controlled conditions could have uses in preserving the BF-potential of the source plant, as well as being a significant testing procedure to measure the changes in BF-potential with time under controlled environmental conditions.

To be used as a bioassay, specific biochemical methods of measuring BF-potential are needed. These culture procedures could have significant use in establishing such biochemical interactions of moisture and temperature stress of normal and BF plants in conjunction with the work being carried out on the amino acid reactions described in Part II.

Nevertheless, maintaining the cultures and carrying out the needed experiments is time-consuming and expensive. These disadvantages needed to be balanced against their potential experimental value in shortening the time required to get answers when dealing with a perennial crop like almond with long seasonal and developmental cycles.

## **Publications**

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Kester, Dale. February 1988. Report of Research on Nonproductive Syndrome (NPS) ("Bull" Mission) in almond. Special Report to the Almond Board of California, Sacramento, CA. 59 pp.

Kester, Dale E. and G.Nyland. 1988. Research on the Distribution of viruses of the ringspot complex in almonds. Special Report to the Almond Board. Mimeograph. March 1988, revised December 1988. pp.

## REPORT OF RESEARCH ON NONPRODUCTIVE SYNDROME

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(NPS) ("BULL" MISSION) IN ALMOND

By

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Carried out in part under Almond Board of California Projects on "Bud-failure disorders" and "Almond Variety Evaluation".

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February 1988

## REPORT OF RESEARCH ON NONPRODUCTIVE SYNDROME (NPS) ("bull" Mission) IN ALMOND

by

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## Carried out in part under Almond Board of California Projects on "Bud-failure disorders" and "Almond Variety Evaluation".

<u>Collaborating personnel</u>: Many individuals collaborated on various aspects of this research, either directly or indirectly. Major collaborators included Dr. George Nyland, Warren Willig, Leon Cory, Dr. M. Habib, Richard Asay, Don Rough, Warren Micke, Lonnie Hendricks, Norman Ross (deceased). Special thanks is given to Dennis Campos for legal council, to various commercial nurserymen who provided information, in particular Wm. Burchell for his encouragement and cooperation, and various Farm Advisors and growers for their cooperation but too numerous to list.

### INTERPRETIVE SUMMARY

This report summarizes research carried out primarily between 1976 through 1984 with some continued activity into 1987 on the problem commonly known in the industry as the "bull Mission" problem. The purposes of this research were to understand its nature, cause and control and to resolve some controversy that arose as to its origin.

The problem was expressed most prominently by the appearance of highly vigorous, nonproductive ("bull" ) Mission trees with highly distorted, soft-shelled, enlarged, elongated nuts with large "wings". These variants began to appear in orchards primarily in the 1970's but their origin was found to occur much earlier. During the same time period growers reported that nuts on many Mission trees were not the traditional small, hardshelled type but were larger, narrower and softshelled. At first these aberrant trees were only reported in Mission and from one nursery. With time, a similar problem developed in Mission with other nurseries and with Nonpareil, Carmel, and Fritz with reports of other varieties.

At first it appeared that the primary cause of the condition might be due to something causing sterility which secondarily resulted in large tree size, enlarged nuts and softer shells. This explanation was found to be erroneous and in fact the misinterpretation of nut characteristics produced on normally vigorous, low producing plants as in a young orchard or in severely pruned trees in a scion orchard can lead to misdiagnosis of the problem.

Instead the distortions and malformations in the flowers, nuts, leaves and the vegetative structure are systemic within the plant and indicative of the presence of varying proportions of abnormal cells apparently resulting from varying capacities for growth and physiological activity. Their presence causes disruptions in the normal patterns of vegetative and reproductive development of the plant. Variation in these patterns give rise to the conditions referred to as "bull", "ugly", "peanut" "narrow leaf", "softshell", "winged", etc. There is a more or less continuous range of potential variation with the most vigorous, nonproductive ("bull") on the one end with the nearly normal producing but distorted and softshelled condition on the other. Because of this variation in expression, the condition is being referred to as a "syndrome". Expression of nonproductivity results from various combinations and degrees of defective ovaries, ovules, and inability of styles to allow pollen tube growth. Reduction in pollen viability may occur.

Variation not only involves difference in expression of the syndrome but also to variation among branches of the same tree, between the base to the top of the tree and from tree to tree. There may also be variation in shell hardness and growth within the same nut. More or less normal branches can be found on severely affected trees and vice versa. These patterns show a strong chimeral nature which occurs because the affected plants are composed of genetically different kinds of cells in particular layers and sectors.

Extensive indexing led to the conclusion that the systemic condition of the "bull" syndrome was not a virus although there may be specific virus conditions that are associated with other somewhat similar "bud-failure" conditions.

Extensive propagation studies showed that the various forms and variants occurring in particular trees are perpetuated during vegetative propagation although the capacity for continued variation is also maintained. Nursery trees are produced by single buds removed from single budsticks. This operation makes possible the perpetuation of any expressed or latent form of the disorder that exists in any small part of the budsource tree and can account for the great variability that exists within and among orchards There is some indication that variation can extend to the size of nursery trees associated with the relative vigor of the source tree. This relationship can account for the concentration of "bull" trees within a single bundle following size grading in the nursery and can explain the prevalent distribution of "bull" trees in groups of ten and as replants.

Epidemiology of the "bull Mission" disorder involved its occurrence in two separate and independent PROGENY ORCHARD SOURCES in the upper San Joaquin valley near Modesto. In one case the primary initiation occurred prior to 1960 covering a time with no available records. The other apparently occurred within a specific orchard in the Delhi area. These were followed by secondary distribution through nursery propagation in the sequence of progeny orchards until their prevalence in commercial orchards signaled that a problem existed. At least two cases were observed where "bull", "ugly", or "peanut" Nonpareil and Carmel developed at the same time in the same orchard with "bull" Fritz coming from the same nursery operation. All originated in the upper San Joaquin Valley.

The combination of research and epidemiological studies leads to the conclusion that specific primary mutational events have occurred not once but a number of times in specific orchard or nursery trees located in a specific location in the upper San Joaquin valley. These occurred during approximately the same time period either spontaneously or through induction by some mutagenic agent. These were not single mutations as has occurred before in the industry but can best be described as involving an "explosion of mutations" whose effects are sublethal but which drastically affect the growth and physiological functions of the cells. When such affected trees become part of a PROGENY ORCHARD SOURCE sequence, the budding technique and management procedures used in modern fruit tree nurseries not only becomes an extremely efficient method for discovering any latent genetic variant occurring in the source tree but also provides a mechanism by which any variant based on vigor could be concentrated and preferentially selected. Detection first occurs, however, in the PROGENY TREES perhaps one or two removed from the site of the original problem. scion generations

The investigation provided an opportunity to evaluate nursery operation procedures in relation to source selection. Three kinds of sources were differentiated: ORCHARD SOURCES (many trees of a selected single orchard), PEDIGREE ORCHARD SOURCES (a sequence of consecutively propagated orchard sources) and SOURCE-CLONES (vegetative progeny from a single selected tree). Orchard sources and progeny orchard sources produce commercial progeny trees which not only reflect any genetic variation in the source trees but also may maintain these variants in sequential progeny orchards. VISUAL INSPECTION of the source orchards was found to be inadequate to detect the NPS problem. However, if source identity is maintained during the nursery operation, SOURCE-PEDIGREE ANALYSIS can trace the problem to any specific source. Once the specific problem source is identified as the origin of a problem, NPS can be readily controlled by change in budwood source. The problem is how to reliably identify a new source that can be used with confidence.

Evaluation of SOURCE-CLONE SELECTION, a specific source- clone MIssion 3-6-1-65, and the potentiality of Registration and Certification to control NPS was also possible from the study. Evaluation of the genetic potentiality of a specific single tree source-clone requires both the VISUAL INSPECTION OF THE SOURCE TREE(S) under suitable growing conditions but also the VISUAL INSPECTION OF THE PROGENY TREES. Adoption of such a dual program of genetic verification was shown to be a fundamental principle in long term sourceclone selection not only in relation to the NPS disorder but also for the somewhat parallel situation in noninfectious bud-failure.

Mission source-clone 3-6-1-65 was released in 1968 and subsequently was found to be free of NPS as were two other source clones also available through FPMS, UCD. This conclusion was reached through source-progeny analysis of usage by at least four commercial nurseries and by long-term performance in 5 RVT plots. An early charge that 3-6-1-65 was a source of

the "Bull Mission" problem was refuted by vegetative progeny tests of the trees in question.

A Registration and Certification program was found to be an effective method for distributing NPS-free Mission providing that the principle involved of maintaining source identity even into the progeny orchard are strictly followed.

The general conclusion is reached iN that it is improbable that all the changes in source material described, involving several progeny orchard sources and several varieties within the same time period and general location, could have occurred by chance. Also during this same period and locality a parallel problem known as the "green peach" disorder developed in various cling peach propagation sources. Here there was a circumstantial linkage to treatment of the source orchards with a soil fumigant DBCP. Although the evidence is so far speculative, the possible connection among these situations should be considered.

Elimination of the problem sources should reduce if not eliminate, the current problem with NPS in new orchards. On the other hand, it should be recognized that the "triggering" agent that results in NPS is not clearly identified. Consequently, there continues to be the potential for additional outbreaks in the future should the same conditions develop. The potential of NPS in Nonpareil, Carmel, Fritz and other varieties is not clear. Consequently it seems important that serious attention should be given by the nursery industry to develop or adopt selection and distribution practices that will minimize the potential for NPS and quickly detect any sudden occurrence. by

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### INTRODUCTION

During the 1970's and earlier, highly vigorous nonproductive variants began to appear prominently in the Mission variety in various California almond orchards. These were referred to as "Bull Missions" by growers and nurserymen. Initially these variant trees were associated with propagation material from a single nursery. Later, these trees also began to appear from other nurseries but not as prominently.

Prior to this time, nonproductive trees similar to this description occasionally occurred particularly in the Sacramento and upper San Joaquin valley and were associated with infection by the "calico" strain of the Prunus Ring Spot Virus (Nyland, Virus Handbook, 1968).

During the same period that the "bull Missions" began to appear, some growers reported that the Mission variety had changed and that many of the nuts produced in the San Joaquin valley were atypical, being large in size, soft shelled, narrow, and with prominent wings. These contrasted with the small, hard- shelled round nuts that they had been used to. Also toward the latter part of the 1970's, limited numbers of "bull" trees appeared in Nonpareil, Carmel and Fritz with unconfirmed reports of "bull" trees from other varieties.

In 1968, distribution began from a virus tested source-clone of Mission (FPMS 3-6-1-65) in the Foundation Plant Materials Service (FPMS) orchard of the University of California to be used in the newly initiated Registration and Certification program administered by the Nursery Service of the California Dept. of Food and Agriculture (CDFA). Some nurserymen concluded that this source was the origin of the "bull Mission" problem. The problem eventually became embroiled in litigation involving a private orchard corporation, a private nursery and the University of California. The cases were settled out-of-court by 1985.

#### PLAN AND SEQUENCE OF RESEARCH

Records show that the first confirmed cases of "Bull Mission" were reported in 1970 in single orchards at Modesto and Kern Co. planted in 1964, 1965 and 1967. Initial observations suggested that the "calico" virus to be the cause but the diagnosis was not confirmed by indexing tests. Intensive research on the problem began in 1976 when the incidence of the problem in commercial orchards became intense. In 1987, an apparent new outbreak of the problem in commercial orchards necessitated further observations which made possible the clarification of unanswered questions about the problem.

The objectives of these researches were the following:

- a. to establish the basic nature of the problem and how it was expressed.
- b. to establish the epidemiology of the disorder and how it could be controlled.
- c. to determine the relationship between the Mission source clone 3-6-1-65 to the problem

In this report, a summary of the principal findings are presented with some details to be provided in further reports or research papers. A description of the problem and methods for control are given in the UC IPM Manual (1985).

### NAMING OF THE DISORDER

Because of the variety of symptom manifestations and the range of variability, the disorder is referred to as "Nonproductive Syndrome" or NPS. Although growers commonly refer to these vigorous nonproductive trees as "Bull" trees, this phrase does not take into account the total range of variability that is involved.

### PART I. NATURE OF THE PROBLEM (Objective 1)

<u>Abstract</u>. Almond trees showing a characteristic disorder of <u>nonproduc-</u> <u>tive syndrome</u> (NPS) have been found in California almond orchards predominantly in 'Mission', also in 'Nonpareil', 'Carmel' and 'Fritz'. These variants have appeared from specific nursery sources and can be traced to their independent initiation in a specific time period and location in central California. The pattern suggests that specific primary mutational events have occurred in orchard trees either spontaneously or through induction by some mutagenic agent. These variants have then been secondarily perpetuated through vegetative propagation.

### DESCRIPTION OF EXPERIMENTS AND RESULTS

1. Surveys and description.

Initial surveys were made in a number of orchards in San Joaquin, Stanislaus and Kern Co. to establish the range of expression and variability of affected trees within and between orchards.

Later, orchard surveys were extended to bud source orchards as well as to additional progeny orchards throughout the San Joaquin and Sacramento Valleys. "Bull" Mission trees also appeared in specific UC test plots from trees obtained from specific commercial sources.

Initial surveys were able to identify by visual inspection a number of distinctly "bull" trees based on their vigor, size and lack of production. These trees had nuts which were distinctly abnormal, being large in size, elongated, and soft-shelled, with more or less extended wings on the shell (Fig. 1). At the other extreme, there were trees which were productive and with nuts that were essentially normal for Mission. In between these two extremes, were trees with moderate to good production which also had atypical nuts but which could not always be identified as "bull" with certainty. Some trees were productive and essentially normal but had limbs which showed varying degrees of "bull" characteristics. Or trees with severe "bull" characteristics had limbs with essentially normal production. There was an indication in some trees of gradients from branch to branch and from base to top.

The Luder orchard near Manteca was utilized for intensive observation in which morphological and phenological characteristics of individual trees in an entire row of trees were monitored from 1976 through 1982. Table 1 provides data on various characteristics within individual trees in observations started in 1977. Quantitative nut characteristics included kernel size, shell hardness, shelling percentages, and kernel shape.

Table 1. Nut characteristics from individual trees in Luder orchard in 1977 and 1978. Arranged in order of "bull" characteristics and kernel weight. "Bull" characteristics based on 4 years observations.

Tree	Kernel wt (g)		Shell	Shell %	ling	W/	L	Tre 77	e Yie 77	eld 78	Maturity 78	Rating
				77	78	77	78					
6-29	1.02	28	H-SS	46		67		 N?	M	M	Е	1*
6-23	1.03	28	Н	50	-	69		N?	Н	M	L	1
6-19	1.03	28	н	53	53	68	71	N?	Н	M	L	1
6-12	1.08	26	Н	54	-	68	-	N?	H	H	?	1
6-24	1.12	26	н	49	-	68	-	N?	Н	М	L	1
6-27	1.20	24	S	57	-	63	-	В	М	M-H	E	3
6-28	1.22	23	S	56	-	65	-	в	M	H	?	3
6-22	1.22	23	H-SS	56	-	65	-	N	Н	M-H	?	2
6-20	1.28	22	SS-S	58	-	65	-	В	М	M	м	3*
6-21	1.29	22	SS-S	57	-	65	-	N	Н	M-H	?	2
6-25	1.38	21	H-SS	58	-	65	-	В	M	M	M	3
6-30	0.77	37	H-SS	51	-	70	-	N?	L-M	M-H	E	3*
6-18	1.34	21	SS-S	56	-	62	-	В	VL	L	?	5
6-36	0.96	30	S	55	-	65	-	В	VL	-	E	5*
6-33	0.98	29	SS	58	63	63	59	В	L	M	Е	5*
Expla	nation	of ta	ble:									
	Shell:	H <b>-</b> har	d, SS-s	semi-so	oft, S	S=sof	t					

Shell: H-hard, SS=semi-soft, S=soft Shelling percentage = kernel weight/in-shell weight x 100 W/L = kernel width/kernel length x 100 Tree type (visual inspection): N-normal; B="bull" Crop density: H-heavy, M-medium, L-light, VL-very light Maturity: E=early, M-medium, L-late, ?=not observed Final rating for NPS after 4 years observations:

1=(normal) to 5 (severe). \* = variable within tree

The table is arranged in order of increasing "bull" rating and average kernel size. This character appeared to provide some basis for distinguishing different degrees of the "bull" characteristics among these samples. In the last column, the overall rating for "bull characteristics" is based on the composite accumulated observations on that tree from 1977 through 1980.

- Trees 6-29 through 6-24 (in the table) were judged to be normal. Yet their shelling percentage varied from 46 to 54. Otherwise, kernel size ranged from 26 to 28/ounce within the range often shown by Mission. Shape index (W/L) varied from 67 to 69, again typical of most Mission nut samples.

- Trees 6-27 through 6-25 (in the table) represent a more or less intermediate class of "bull" trees in which the productivity was reduced somewhat but the amount varied with year. Kernel sizes were larger than in the "normal" trees, the shells were soft or semi-soft, some times with both hard and soft areas within a single nut. The shelling percentages were 55 or more and the kernels tended to be narrower on the average than the nuts on normal trees.

- Tree 6-30 represents a class based on very small (37/ounce) kernels which were also broad in shape (W/L=70), and more or less hard shelled. These have been called "peanut" and are found to be produced often on the same limbs or trees with abnormal nuts of the other description.

- Trees 6-36 and 6-33 represent severe "bull" trees in which the kernels are more or less normal in size but so shriveled that the weight is abnormally low. Nuts are soft-shelled, shelling percentage is 55-63, crop was light, and the nuts matured very early.

- Tree 6-18 (along with trees 6-33 and 6-36 above) were the most severely affected trees of the group. Nuts were soft-shelled, kernels large in size, shelling percentage high and the kernel shape narrow.

Vegetatively, trees showed increased size and vigor, more or less in direct proportion to the degree of nonproductivity. Narrower, elongated leaves were sometimes observed on affected trees. Occasionally very narrowleafed nonproductive branches were observed.

During 1978, overall crop yields were less than during 1977 and it was somewhat difficult to distinguish as "bull" those trees with less severe characteristics. There was at first a general supposition that the primary manifestation of the problem was sterility with the nut abnormalities a consequence of the reduced crop density and increased vigor. In 1979 and 1980, weather conditions were excellent for set and investigation became focused on the variation in set and nut characteristics. Some limited studies on chromosome number (Willig, W., M.S. thesis) and stomate size to find evidence of ploidy differences were negative.

It became apparent during this period that the primary cause of the disorder could not be attributed to sterility of reproductive cells with

other characteristics arising as secondary manifestations. Instead the distortions and malformations of the vegetative and reproductive structures themselves became the focus as the primary expression of the disorder resulting from abnormal patterns of growth and development of the fruit, leaves and vegetative parts. These came about either by differential growth rates, different physiological capacities or both in the cells of different sectors of the stem, flowers and fruit.

Studies were made on the dropping and set pattern (Willig, W., M.S. thesis; S. Habib) of trees with different levels of nonproductivity. With the more severely affected trees, the drop began at bloom and continued steadily until the end of April. With less severely affected trees, drop of apparently normal flowers did not begin until several weeks after bloom and continued until the end of April, following the usual pattern for normal plants. The final percentage was in proportion to the degree of the "bull" condition.

In 1980, it was found that flowers of the severely affected "bull" trees had varying proportions of undeveloped ovaries and ovaries (Fig 2). These traits, as well as other yield and kernel characteristics, are listed in table 2 and 3 for two UC plots containing trees obtained from commercial nursery (Nursery A, see Part II). In a separate study, Habib (M.S. in

Table 2.	Yield and	nut chara	acteris	tics of	group	of 8	trees	of sin	gle
	planting a	it Kearney	Field	Station,	Fresno	Co.	Trees	planted	in

Tree	19	79		1	980				
No.	Crop	"Bull"	Undev. ovary	Undev. ovule	0vu/ ova	Set	Nuts/ tree	Kernel wt.	% kernel
			(%)	(\$)	(%)	(%)	(no.)	(g.)	(%)
	I. "Bu	ill" tree	s at Kear	ney Field	Sta.,	Fresno	Co. Plante	ed 1976	
1	low	B+	5	40	25.3	5	1,062	1.24	57
2	var	B-	3	20	31.4	35	5,045	1.14	55
3	low	?	13	10	42.0	24	4,032	1.21	47
4	?	OK	11	10	42.6	22	4,583	1.24	49
5	low	В	5	10	38.3	21	4,754	1.19	47
6	М	В	8	0	42.7	17	3,946	1.20	47
7	?	В	9	40	32.1	16	2,123	1.23	51
8	?	В	16	50	28.1	7	368	1.26	53
	II.	Normal	tree in s	eparate b	lock. K	Kearney	Field Stat	tion.	
26-10	-	N	12	0	55.6	31	-	-	-

### III. Normal trees. Foundation Orchard, UC Davis.

4-9(P)	N	5	0	54.5	6	-	-	-
4-10	N	2	0	44.0	23	-	-	-
14-11(P)	N	2	0	53.5	22	-	-	-
14-12	N	6	0	49.0	9	-	-	-
12-19	N	2	0	47.7	22	-	-	-
12-20	N	2	0	44.8	22	-	-	-
16-7	N	2	0	50.1	16	-	-	-

P = pruned

Table 3. Yield and nut characteristics of Mission trees in Hench RVT plot. Kern Co. 1980. Planted in 1974.

Tree	"Bull"	Undev. ovary (%)	Undev. ovule (%)	Ovule/ ovary (%)	Set (%)	Nuts/ tree (No.)	Hardshell (%)	¥ kernel (१)
65-1	 B+					1,956	20	53
65-2	B+	-	-	-	-	2,340	0	56
65-3	B+		_		-	4,884	20	57
Compos						4,004	20	57
ite	N	-	-	-	-	8,357	100	47
77N-15	B+	30	70	23.6	1	276	10	57
77N-18a	a B+	40	80	22.2	5	708	0	57
1	b B-	0	0	-	-	-	30	52
77N-20a	a B+	0	80	22.0	1	4,884	30	58
1	b B-	0	-	=	-	-	90	49
Compos	-							
ite	N	8	5	45	24	7,404	40	48
		II. Mission	n/Nemagua	rd in oth	er sec	tion of h	olock	
R21	N	(SC 3-6	-1-65)			10,570	100	48
R27	N	58	Ħ			11,920	100	45
R31	N	(Nurs.	A scion O	rchard?)		9,320	100	47
R23	N	(Nurser	y A)			10,930	100	52
R33	N	98				9,730	100	46
R35	N	88				10,090	100	48

preparation) found that pollen tubes from pollen of normal trees would not grow down the styles on affected "bull" trees. Thus, "bull Mission" includes a combination of manifestations, including (a) increased vigor, (b) reduced production resulting from a combination of defective flowers, which drop at or soon after bloom, and excessive drop of seemingly normal flowers and small nuts, (c) abnormal morphological development of nuts, (d) inhibition of the shell hardening process, and (e) enhancement of ripening. All of

the characteristics taken together exhibit a SYNDROME of expression showing a "dosage" response with a continuum from very severe to relatively mild correlated to productivity and vigor (Tables 4 and 5).

( )

Table 4. Relationship between yield and nut distortion in trees in Luder Orchard. Based on 49 tree visual inspection tree ratings in 1979.

Crop rating	Numb none (1)		ees with the or nut disto moderate (3)	rtion	ng very severe (5)
1 Very light				1	2
2 Light				2	
3 Medium			2	1	3
4 Moderately heavy	2		6		
5.Heavy	28	1			

Table 5. Relationship between yield and nut distortions in Morrison orchard. Escalon, 1979

Crop		for nut distor			rating
	none	slight	severe ver	y severe	
1 Very light				3	
2			1	10	
3				7	
4			3	2	
5		1	2		
6	3	3	2	1	
7	4		1		
8 Heavy	10	1	1		

### 2. Variability.

Variability is a key element in the expression of the NPS disorder and is expressed in the following ways: a. "Dosage" and time relationships in productivity, vigor and morphological abnormality.

The previous paragraph described a continuous range in vigor, productivity and abnormality (Tables 4 and 5). In the more severely affected trees, the adverse effect on the reproduction system occurs early and results in depressed development of the entire ovary or just the ovule or the style. Such defective flowers drop early or, if not defective but not fertilized, may continue to enlarge but drop later. If the effect is less, delayed in time, or affects different sectors of the flower, different parts of the the fruit and nut may develop at different rates and the shape is distorted. If the normal physiological development of the nut is affected, the shells do not harden and the nuts tend to mature early.

Similarly, variation in the rate and/or amount of vegetative growth may affect the patterns of leaf development and rate of shoot growth in different parts of the tree.

- b. Chimeral patterns. A chimera results when different parts of the plant exhibit significantly different genetic characteristics which can be identified by obvious change in appearance or other characteristics. Such a pattern is widely in evidence with NPS as described in the previous section. Plants exist with branches that are severely "bull" on otherwise productive trees; or, plants exist where there are "normal" branches on otherwise "bull" trees. There are examples of apparent gradients within trees among branches and from base to the top of the tree.
- c. Variability within orchards. A key pattern of variability is shown in the distribution pattern within orchards. It has been consistently observed that the distribution of "bull" trees within orchards (Fig. 3) has a relationship to the bundles of ten trees which is the basic unit with which nurseries package and distribute trees and with which growers plant. After digging in the nursery, trees are graded by caliper diameter and growers buy a certain size. It appears that factors are at work that tend to concentrate trees of a given level of "bull characteristics" within the same group of ten trees of a given size grade.

In one orchard, Carmel trees of two nursery sizes were planted. All had arisen from a single source orchard according to the nursery. "Bull" Carmel appeared only in that part of the orchard planted with the larger size grades.

## 3. Vegetative propagation

Vegetative propagation tests were conducted to determine the bud-perpetuation pattern from affected trees to their vegetative offspring. Experiment (1).Different nonproducing source orchards.

Budwood was collected from three separate orchards in San Joaquin County. Bud wood was collected by Don Rough, FA, propagated by the Stuart Nursery and trees were grown at the UCD orchards, Davis. Trees were planted in 1978. The source trees were:

- (a). An old "bull" tree (planted in 1942) in an orchard near Escalon which also had mosaic leaf symptoms thought to indicate the presence of "calico" virus. (red)
- (b). A severely nonproductive "bull Mission" tree. Original tree planted in 1971. (white)
- (c). A low producing orchard without distinct "bull" symptoms. (blue).

Experiment (2). Propagation from different levels of "bull" trees.

Budwood was collected from every tree in an entire row of Mission trees in the Luder orchard, Manteca, in order to capture the entire range of NPS variants from normal to very severe. Trees were propagated in the UCD nursery and planted in the UCD orchard. Trees planted 1978.

Experiment (3). Propagation from chimeral branches.

Budwood was collected from (a) nonproductive branches and (b) from productive branches on "bull" Mission trees at the Reed orchard, Chowchilla. Trees were propagated at the UCD nursery and planted in the UCD orchards in 1978.

Experiment (4). Nursery propagation tests.

Budwood was collected from (a) nonproductive branches and from (b) productive branches but with nut abnormalities in trees in Expt. 1b and from Expt. 3. June budded trees were propagated in two consecutive years to test the size of the nursery trees they produced.

Experiment (5). Vegetative propagation of "peanut', "ugly" and "bull" trees of Carmel, Nonpareil and Fritz.

Budwood was collected from (a). nonproductive and "peanut" and "ugly" fruit Carmel and Nonpareil trees from the Enos orchard, Merced, and from nonproductive Carmel from the Veira orchard, Modesto. These were planted with normal trees of the same varieties. Later additional "bull" trees were propagated of Nonpareil as well as "bull" Fritz trees, some with bitter kernels and some without. All trees were grown in the UCD orchards. The three Mission sources of Expt. 1 were code named "red", "white", and "blue" and represented three different orchard sources.

<u>Blue</u>: All of the progeny trees were positive for Prunus Ring Spot Virus (see section 3), the trees grew well although not over vigorously, and began to produce some flowers during the second year (Table 6). The trees had high percent set (44 to 54) during years from 1979 to 1982 and pollen was good. The nuts were relatively small and hardshelled, (Fig. 4) shelling percentages averaged 45% and shape index ranged from 55 to 65. These data compared closely with that (both % set and nut characteristics) obtained during that same period from normal check trees at UCD, which were virus-free.

Table 6.	Characteristics	of	Mission	trees	propagated	from	different
	nonproductive sour	ces	in San Joa	aquin Co	. Planted 1	978. U	C Davis.

Source Trees		1979		1980		<u>1981</u>	1982	
Source	Trees	Flowers /tree		Flowers /tree		Set %	Set %	Pollen %
	(no.)	(no.)	(%)	(no.)	(%)	(%)	(%)	(%)
Red	13	8.3	31.5	175	24.8	17.7	10.8	14.2
White	10	30.5	16.4	424	8.7	6.7	3.2	20.0
Blue	19	34.3	44.5	375	47.6	41.1	54.2	65.2
check	12	-	-	•	-	40.0	53.1	-

I. Reproductive capacity

Source	Samp year	les no. (no.)	Kernel weight (g)	Shell ave. (%)	ing % range (%)	% Hard	Width ave. (%)	l/length range (%)
Red	1981 1982	13 13	0.96	48.2 50.3	42-53 44-53	45 31	56 59	52-62 54-63
White	1981 1982	10 10	1.01	51.5 49.3	47-55 43-51	56 15	56 59	54-59 57-61
Blue	1981 1982	12 12		46.8 42.7	42-49 44-	100 100	59 65	55-64 64-68
Check	1982	8		44.5	41-49	100	67	65-68

### II. Nut characteristics (1981 only)

Comments:

<u>Red</u> - trees had considerable "muletail" growth; slight mosaic leaf patterns in 1980. Some limbs with better crops

<u>White</u> - all nuts showed some distortion of fruits and much variability. Some small peanut type kernels. Shells varied, some too hard to break with fingers, others shell thin but hard. Invariably all had a narrow, thin prominent wing. Some branches with higher production. <u>Blue</u> - All small hardshelled nuts.

White: All of the trees were negative for PRSV. Trees were typical vigorous "bull"type trees. Flowers began to appear in the second year at the same amount as the "blue" but percent set was consistently low with a decreasing trend with age. Pollen produced significantly lower set when used in a controlled pollination test. Nuts produced were large, soft-shelled and consistently narrower in shape than the nuts on the "blue" trees (Fig. 4).

<u>Red</u>: These trees were propagate with the objective of evaluating the relationship of "calico virus" to the "bull" condition. All of the progeny trees tested positive for PRSV and some early season mosaic like symptoms were observed in 1980. Trees were vigorous and there were significant numbers of shoots with "bud-failure" - like shoots resulting from "blind nodes". Trees of this source were slower to come into flowering than either the "white" and "blue". Average percent set was intermediate between the "blue" and "white" and also decreased with age as did "white". Nut characteristics were intermediate between the "blue" and the "white".

Specific tests for "calico virus" were negative for trees of the "red" source (Nyland, Lowe, Kester, 1983). However, some of the virus-like symptoms of this source - mosaic leaves, delay in coming into flowering, and "blind node bud-failure" - may be related to the particular strain of PRSV. The main cause of nonproductivity, however, appeared to be due to an intermediate strain of NPS unrelated to virus. Since the source tree was originally planted in 1942, it raises questions about when NPS first appeared, the reason for its occurrence, and the nature of other "old" bull trees usually associated with "calico virus".

<u>Expt. 2 results</u>. A direct association was shown between the level of "Bull" symptoms in the individual source tree and the individual progeny trees that were propagated from it. This pattern indicates the presence of subclones of different levels of NPS that are becoming more or less stable.

Expt. 3 results. A principal characteristic described for the NPS disorder is chimeral variation among branches of individual trees with high producing branches observed on severely "bull" trees and severely "bull" branches on otherwise normal trees. Progeny trees propagated from different chimeral branches of the same tree (Table 7) not only tend to show the NPS of the source branch but are themselves unstable and produce chimeral distribution. This indicates the great instability of the vegetative material in these trees with the chimeras. Progeny from these separate branches do have different degrees of the NPS conditions and are "genetically" distinct.

Tree		rce "bull"		Trunk dia. (cm)	Crop rating		Bull"	Kernel weight (g)	(A)	rd (B) (%)	W/L
1-1	5N-10E	B+	1981	5.6	2	11	B+	0.99	54	0	55
	511 200	2.	1982	5.0	2	3	B+	1.07	53		58
-2		11	1981		3	8	B+	1.08	55	10	56
			1982			3	B+	(combi			
- 3			1981 N	7.5			B+	0.90	48	30	57
			1982 N		3	-	-		-	-	-
			1981 S			17	B -	1.21	48	80	58
			1982 S		8	-	B -	1.17	50	30	64
-4			1981	5.0		3	В	-	-	-	-
			1982		1	-	В	1.05	49	0	64
- 5	6N-10E	B-	1981 N	5.1	7	24	В	=	-	-	-
			1982 N		7	35	В	-	-	-	-
			1981 S		-	-	-	-	-	-	-
			1982 S		5	4	В	0.96	48	40	62
- 7	6N-10W	B+	1981 N	5.6	4	-	В	-		-	-
			1982 N		4	22	B+	-	-	-	-
			1981 S			35	-	1.07	48	70	58
			1982 S		8	30	В	1.09	45	100	64
				 P) & here		 ,					

Table 7. Productivity and nut characteristics of progeny trees from chimeral branches of different levels of "bull" characters. From Reed orchard, Chowchilla. Planted 1979.

(A) = Shelling percentage (B) % hardshelled

S = South N = North

<u>Expt. 4 results</u>. The distribution pattern of NPS trees in a number of orchards showed a grouping in multiples of ten (Fig. 3). This pattern suggests that nursery grading of same-sized trees into bundles of ten has the effect of concentrating trees with the same level of NPS on the basis of caliper diameter. This could occur if the trees with the most severe NPS and the most vigorous in the orchard came from largest caliper nursery trees.

In 1981, an experiment was conducted to compare the caliper size of nursery trees propagated from different sources of trees with different levels of NPS. The sources included (a) "normal" source trees from FPMS 3-6-5-67, which had produced no evidence of NPS in the source or its progeny (42% set), (b) trees of the "white" source from Expt. 1 (Table 6) in which the set (7%) was extremely low, and (c) trees of an intermediate NPS (32%) producing tree originating as a branch on an otherwise severely "bull" tree (Table 7).

Caliper measurements of nursery trees from the severely NPS source (b) trees were significantly larger on the average than those from the less severe NPS tree (c) but the ranges overlapped. The trees from the normal source (a) showed a wide distribution extending across the entire range of tree sizes of the other two sources.

In 1982 an attempt was made to repeat the experiment under more rigid conditions but poor nursery tree growth and low percentage stand made the results inconclusive.

Expt. 5. Tests with NPS Carmel, Nonpareil and Fritz.

In one case, "ugly" and "peanut" trees of Carmel nd Nonpareil associated with low production came from a single orchard in Merced Co (Enos). Both nut types appeared in progeny trees. Many of the Nonpareil trees were distinctly chimeral with branches of "peanut" and "ugly" appearing sporadically among the productive, normal branches in the same tree. This pattern indicates that the two types of nuts are manifestations of the same basic pattern as NPS found in Mission. Progeny trees growing at Davis show the same characteristics (Fig. 5, Table 8).

Table 8. Productivity and nut characteristics of progeny trees from "bull", "ugly" and "peanut" source trees of Nonpareil and Carmel. Planted 1979. Data in 1982.

I. Reproductive capacity

Source	Trees	1	1981		1982			
		8	% set		Poll	en		
		ave.	range		<pre>% germ.</pre>			
	(no.)	(%)	(%)	(%)	(%)	(%)		
Carmel								
normal		42,32	15-51	61.5e	46.1c	67.0d		
NP-la		12,13	2-30	18.5c	32.7Ъ	-		
NP-1b		-	-	19.4c	50.3c	-		
NP-2		2	1-5	4.2ab	12.8a	32.0a		
Nonpareil								
normal-1	25	18-35	55.9de	65.4d	53.7c			
normal-U	37	16-60	-	-	-			
NP-1 (pe	6	2-12	4.6ab	33.4b	36.7Ъ			
NP-2 (ug	gly)	15	8-28	-	-	-		
2 (u	19	12-32	-	-	-			
II. Nut characteristics								
		1981 (n	uts)	1982 (	mature f	Truit)		
Source	Kernel Sh	ell- len-						
		g % gth		-				
	(g) (	€) (cm)	(cm) (%)	(cm) (c	em) (cn	1) (%)		

Carmel normal 1.18 59.5 2.44 1.21 49 3.98f 2.54a 2.16b 64 NP-2 1.15 67.7 2.67 1.16 43 4.00f 2.52a 2.14b 64 NP-1A 1.05 68.9 2.08 1.21 58 3.22c 2.50a 2.22b 78 NP-1B short, peanut 3.18c 2.46a 2.18b 74

Nonpareil

normal-1 1.30 68.5 2.45 1.29 52 3.62e 2.42a 1.72a 67 normal-2 1.17 61.0 2.27 1.22 54 -NP-1 1.01 69.8 1.91 1.19 63 2.96ab 2.42a 2.14b 81 67.2 2.39 1.40 NP-2 1.35 59 --NP-2 1.27 67.8 2.36 1.32 56 -\_

Comments:

Carmel NP-2. Elongated type. From Stanislaus county Carmel NP-1. Peanut type. From Merced CO. Nonpareil normal-1 from Stanislaus Co. same orchard as Carmel Nonpareil normal-1 from UCD FPMS source Nonpareil NP-1. From Merced CO. "peanut" type source Nonpareil NP-2. From Merced Co. "ugly" nut source Similarly, Fritz trees coming from the same nursery have been found not only to be "nonproductive" but with "bitter" kernels. Progeny trees of these different "mutants" produced the same characteristics when propagated to the UCD orchards.

In another situation, trees of Carmel and Nonpareil that were severely "bull" were reported and in fact were located on facing sides of adjoining trees. Buds from a "normal" Nonpareil and the "bull" Carmel survived when brought to UCD for propagation but buds from the "bull" Nonpareil did not. Progeny trees of the "bull" Carmel were extremely low producing and the kernels were long and narrow with a high shelling percentage (Table 8, Fig. 5).

Carmel and Nonpareil indexed negative with the Shirofugen test.

4. Virus tests (with Dr. George Nyland)

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Several kinds of virus indexing tests were employed to determine the presence of virus infections. The tests involved collection of budwood material, including both one and two year wood, and budding to a "clean" indicator plant which has been selected as being free from any known virus. Healing between the budpatch of the donor plant to the indicator plant is needed to allow transmission of a virus (or virus-like pathogen) from the diseased plant to the healthy plant. Symptoms of disease then must show on the indicator under conditions known to bring about expression of the disease.

The following tests that were carried out:

- a). Indexing to "clean" almond trees. This test was initiated in 1970 from severe NPS trees from an orchard in Kern CO. (Nyland, field notes 1979). The test trees were Nonpareil, Mission, Peerless, Ne Plus Ultra, and Drake in the Plant Pathology Orchard, UCD. No evidence of NPS symptoms were produced by 1979 although there was some possible indication of Prunus ring spot (see below).
- b). Shirofugen cherry. In this test, buds are inserted sequentially in a vigorously growing shoot or Shirofugen cherry. This variety is extremely sensitive to all strains of the Prunus ring spot virus complex (including "calico virus", "peach stunt", "prune dwarf", "mild necrotic ringspot" and others but it cannot distinguish among the different "strains". Within 3-4 weeks, if the virus is present, the bud union becomes blackened and gummy and the bud is usually killed. If the virus is absent the, bud union heals and appears healthy although the bud may not grow. The shoots are immediately removed to prevent the spread from any infected bud.
- c). "Shiro plum" index. This test is carried out in the same way as described for (a) but requires two years to produce characteristic leaf symptoms on the indicator plants.

Extensive Shirofugen tests were carried out over a period of several years with trees affected and nonaffected with NPS. An initial test in 1970 (2) showed both positive and negative responses in Shirofugen cherry indexing tests from NPS affected trees. Subsequent Shirofugen cherry indexing carried out in conjunction with progeny orchard and scion orchard surveys in 1976 through 1982 showed no correlation between affected trees and trees that showed positive with Shirofugen cherry with as many positive as negative responses. These tests not only showed no relationship between virus infection and NPS but also that trees affected with PRSV are widespread in mature almond orchards without any particularly obvious effect on performance. This observation should not be taken to mean that no effect was produced or that specific strains may have more adverse effects than others.

Shiro plum index tests carried out in 1982 and 1983 with the "bull" trees thought to be related to "calico virus" in the Progeny Test No. 1 (Table 6) also turned out to be negative. These trees had additional characteristics, i.e., bare shoots and blind nodes as well as some leaf patterns which were not present in other trees with or without the NPS condition. They were, however, positive to Shirofugen indexing. It is possible that trees of this particular source have unique "strains" of PRSV which had undesirable effects.

- 5. Effect of age, vigor and tree management on nut characteristics: One of the questions at issue in the investigation was how to separate the normal nut variations expected under conditions of high vigor and low crop with the altered nut characteristics arising from the NPS condition. Nuts on young Mission trees just coming into bearing are often soft-shelled, large in size and somewhat elongated. Likewise, trees grown in scion orchards where they are pruned severely for budwood (dehorning) may produce nuts which are atypical and resemble those associated with "bull" Mission condition.
  - a. Scion source trees in the Foundation Orchard at FPMS, UCD, are maintained in pairs and are severely pruned each year to stimulate production of long shoots to provide scion or buds for propagation. In 1977-78, one tree of a number of pairs of source trees both Mission and other varieties - were left unpruned in order to fruit and the other was given normal pruning. These trees were maintained with this management for four years.

Comparisons were made of Mission, Peerless, Thompson and Nonpareil by measuring the size and shape of the immature fruits (July or August) before splitting. In addition mature nuts were collected and measured. In each case (all varieties), the pruned trees produced larger nuts and the shape was somewhat altered (Fig. 6B) although the difference was not always statistically significant (data not shown). The somewhat altered nut shape of the trees stimulated by vigor, although somewhat "ugly", was uniform in cross-section and did not show the gross distortions found with the nuts on trees with NPS (Figure 4D).

Table 9 shows data of the mature nut where it is shown that the nuts on the severely pruned trees were significantly larger in size, had thinner soft shelled nuts, higher shelling percentage and narrower kernels. The same response was shown by trees in a scion orchard maintained by Nursery B.

Table 9. Characteristics of nuts collected from normal Mission trees growing under different growing conditions.

Source	Year	Treatment	Kernel weigh (g)		W/L (%)	Remarks
		I	. FPMS	(1979)		
T4-9	1979	pruned "	1.22	56 52	59 58	Dehorned for S.W.
T4-10	н	unpruned	0.93	42	64	Heavy crop
OF016-7	Ħ	11	1.01	45	63	Moderate crop
II. Grayson Rd. Scion orchard, Nursery B (1980)						
3-6-1-65	1980 "	pruned unpruned		(high) 50	62 64	thin,soft, S.T. hard
S.W. = scion wood						

S.T. = sticktights

OFO = Old Foundation Orchard. Original source tree of 3-6-1-65

b. Effect of age. The teaching orchard at UCD contained a group of Mission trees (along with other varieties and species) planted each year to provide trees of different ages for student instruction. In 1968, budwood was obtained from the FPMS as soon as it was released and used exclusively in consecutive years. These trees provided not only provided a vegetative progeny test of this source but also the effect of age (and vigor) on nut characteristics. Data (Table 10) obtained in 1979 shows that during the first two bearing years (3 and 4), the nuts were significantly larger in size, shells slightly softer and kernels longer in shape than in the subsequent years. Nuts were not distorted but were uniformly larger in size.

Table 10. Effect of age of tree on nut characteristics of Mission

Year planted	Age of trees	f Ker wt Pch RS		Shell perc Pch RS	ent	W/L % Pch RS	
1970	10	1.20	1.24	50	50	59	60
1971	9	1.25	1.13	49	48	59	58
1972	8	1.25	1.10	49	50	59	60
1973	7	1.09	1.14	42	46	59	59
1974	6	1.19		46	-	59	
1975	5	1.18	-	45	-	58	-
1976	4	1.33	1.30	54	56	58	57
1977	3	1.35	1.33	52	-	57	55

I. Teaching Orchard, UCD. Mission 3-8-1-63.

Nuts collected from young Mission trees of all sources in the RVT plots invariably showed softshells, larger sizes and elongated kernels during the first and sometimes second bearing year.

#### 6. Fingerprinting and other genetic tests

A number of biochemical tests were made to compare nonaffected and affected Mission trees. These included various isozymes which were being used in other studies (Hauagge, et.al. ) as well as total protein. No consistent significant differences were detected between affected and noninfected trees in these tests.

#### 7. Inheritance studies

Open-pollinated seeds were collected from individual normal and "bull" Mission trees in orchards with known pollinizers (Thompson, Nonpareil or Ne Plus Ultra in Luder or Morrison orchards) and from known Mission bud source trees (Kester and Asay, unpublished research notes). The objective was to determine if typical "bull" trees could produced in the seedling progeny similar to the pattern that existed in inheritance studies with noninfectious bud-failure (Kester, D.E., Annual Report 1986). Seedlings were planted in 1978 and observed until 1987.

No pattern of "bull" type progeny trees were produced. Instead, a low percentage (around 1-2%) of stunted, chlorotic inferior trees appeared in seedling progeny from the affected trees suggested the existence of genetically defective sex cells (gametes) from the "bull" trees. In general the overall vigor was less in the progeny trees from the "bull" sources but this response could have come from the conditions in the orchard where the seedlings were grown.

#### DISCUSSION AND SUMMARY

The results of these studies taken together show a clear pattern. First, there does not appear to be a virus or transmissible agent involved although the difference between the NPS condition and the existence of older "bud-failure" like trees associated with the so-called "calico virus" or other strains of Prunus Necrotic Ring Spot Virus is not resolved. In practice, all of these virus strains can be readily identified by standard indexing tests and can be controlled in nursery practice.

Nonproductive Syndrome (NPS) in its various modes of expression, i.e., "bull", "ugly", "peanut", "softshell", etc. is concluded to be caused by mutations which disrupt the orderly pattern of vegetative and reproductive growth and development. Within the plant there are variable populations of cells that have different capacities for growth and physiological function. These translate into growing points in which the cells that lead to different parts of the shoot or a flower differ genetically among themselves, differ from one growing point to another and even may change in their proportions as the individual growing point develops into a new plant or flower and nut. The most conspicuous feature of the syndrome is the increased vegetative vigor of the plant when involving a whole tree but variations in vigor can also explain the distortions of fruit and elongation of leaves from the presence of "subclones" of cells of different vigor and physiological capacity within the same shoot, leaf, flower or nut.

Natural mutations occur as single genetic changes within cells which are reproduced during cell division in the growing points and whose cellular descendants may be maintained indefinitely within the plant without ever being recognized. If these cells come to occupy a major sector of the stem and if the effect of the change is sufficiently important to produce a recognizable change in the plant - whether desirable or undesirable - then the chances are the mutation will be discovered. However, the initial (primary event) change occurs long before the actual occurrence and then usually after the plant has been propagated vegetatively (secondary event). The nursery operations of budding fruit trees is a highly efficient method by which hidden mutations can be discovered by the sudden appearance of whole or part trees with the mutation. Most of these new plants are chimeras, in which the changed cells may occur only in specific layers of the plant. With repeated propagation, the progeny trees tend to segregate into (a) subclones which are more or less stable for the initial plant, (b) subclones which are more or less stable for the mutation and (c) subclones which continue to be variable.

Mutations occur frequently in fruit trees, including apples, pears, peaches, citrus, etc. and have given rise to new cultivars. In almond a number of mutations have occurred - late bloom (Tardy Nonpareil), double flowering, and red flowering, for example. These have affected a single trait and the number of trees has been very limited. Most mutations are harmful to the plant since they are apt to represent a loss in the normal functioning of the plant. Such mutations are eliminated and rarely receive the attention given to the relatively rare useful mutations.

The NPS disorder has the main attributes described for mutations. Not only is the disorder maintained during propagation but there is evidence of segregation into subclones with similar characteristics. However, there continues to be much variability within the NPS affected plants.

There are two general hypotheses that could account for the origin of the NPS disorder. One is that a single chance mutation occurred that disrupted the normal pattern to produce excessive growth and inhibition of various functions as shell hardening, etc. The patterns produced would be the result of different proportions and distribution of the mutant cells within the plants that occur during growth and development.

The second hypothesis is that the manifestations produced are the result of an "explosion of nonlethal mutations" that result in a whole range of cells with unpredictable effects as they become part of the growing points and later developmental patterns within the plant. Those that are more vigorous in growth could likely dominant the growing process such that there could be a sorting out of cell lines with different degrees of vigor. Such a situation would be most likely to occur if the plant has been exposed to high levels of a mutagenic agent either by accident or for experimental purposes. This procedure of exposure to a specific agent followed by forcing out of lateral branching or consecutive propagation is the basic method utilized in plant breeding programs using mutation breeding.

#### PART II. SOURCE PEDIGREE ANALYSIS AND VEGETATIVE PROGENY TESTS IN COMMER-CIAL NURSERIES

Abstract. Analysis of propagation sequences of budwood sources of six different commercial nurseries provided concepts of SOURCE ORCHARDS, PROGENY ORCHARDS, PROGENY SOURCE ORCHARDS, and SOURCE- CLONES. These were examined in relation to the epidemiology of the NPS disorder in Mission. Evidence was found that the NPS condition developed at specific times independently in two separate progeny orchard sources beginning in one case before 1960 and in the other case specifically in a single orchard of the sequence planted about 1960. These occurred in the northern San Joaquin valley in the region between Escalon to Merced. Similarly, NPS disorders were found to have developed in Carmel, Nonpareil and Fritz in limited amounts but more or less simultaneously in the same orchards at the same time. A pattern is described in which NPS results from initially latent mutations within propagation source trees which are screened out in the highly efficient nursery propagation system to produce an array of variable subclones which are not identified until they appear in progeny trees. These variants do not come to light until their frequency becomes sufficiently high in commercial orchards to produce economic effects.

An hypothesis is presented that the occurrence of these mutants, along with a comparable condition known as the "green peach syndrome" that developed at the same time in the same area under similar conditions is related to localized exposure in specific orchards to agricultural chemicals, in particular DBCP, a soil fumigant used extensively in the area between late 1950'S to late 1970's.

#### INTRODUCTION

The purposes of this part of the report are:

- (a). to describe the distribution of NPS within commercial orchards and propagation sources, and
- (b). to correlate the distribution of NPS with the pattern of propagation from specific source orchards.

This process of investigation is being called SOURCE-PEDIGREE ANALYSIS. It involves a combination of VISUAL SURVEYS OF SOURCE ORCHARDS and VISUAL SURVEYS OF VEGETATIVE PROGENY. It requires detailed information about the BUDWOOD COLLECTION HISTORY of the nursery, maintenance of SOURCE IDENTITY during nursery propagation and follow-up VISUAL INSPECTION OF PROGENY ORCHARDS.

#### MATERIALS AND METHODS

Data on distribution of NPS was obtained by surveys of commercial orchards where the problem was reported to exist. Knowledge of orchards with the problem came from reports of Cooperative Extension Service personnel, growers, and nurseries. Observations were also made of trees obtained from specific nurseries to plant in RVT plots or in other UC tests plots. Later, information on affected orchards was obtained from insurance claim reports and sales records made available through depositions, since the problem eventually became involved in litigation.

Identification of symptoms was made by visual observations of the trees and the nuts from them. These symptoms have been variously described as "bull", "ugly", and " peanut". Field observations were supplemented by the collection, visual inspection and measurements of nut samples. Measurements included the average kernel weight, shape index (width/length) and the shelling percentage (kernel weight/in-shell weight) which characterized the softness of the shell.

Once the pattern of symptoms was established, affected trees could be identified in most cases with more or less certainty. Very severely affected, nonproductive, large sized "bull trees" could be identified at almost any time of the year once the trees had come into production and had grown several subsequent years in the orchard. Trees that showed the "lesser dosages" of the NPS condition, including reduced fruit set and nut abnormalities, identification was best done after April or May when the set on the tree was established and the altered form of the mature nut could be observed. Examination of nuts on the ground immediately after knocking was a good time not only to determine yield potential but also to evaluate nut characteristics, particularly from the top of the tree.

Information about propagation source orchards and blocks was obtained from nursery records provided either voluntarily by cooperating commercial nurseries or as a result of legal depositions. Surveys were then conducted in the source orchards in the same way described for progeny orchards where the problem existed.

In this report commercial nurseries are identified by a capital letter and a specific source orchard by a number. Numbers are used to designate a particular source orchard (SO), a progeny orchard (PO) and a source-clone (SC). These designations are made to direct attention away from specific individuals or nurseries and toward the scientific relationships that are being described. A listing of the specific identities of the nurseries and orchards is available where such information is useful.

#### RESULTS

#### Nursery A.

Distribution of NPS in progeny orchards. A very large commercial orchard in eastern Merced Co. provided an extensive vegetative progeny test of nursery trees produced from Nursery A. Eight to 220 acres of almond trees with one-fourth Mission were planted annually from 1963 through 1971. All blocks but part of one originated from Nursery A. All blocks of Mission trees were reported by the owner to contain varying percentages of Bull Mission trees. Surveys in the orchard confirmed these observations (Table 11). Range of expression varied from very mildly nonproductive to severely "bull". (Table 12). Shirofugen cherry indexing produced positive results in some cases and negative results in others with no correlation to the problem.

Table 11. Pattern of planting and occurrence of NPS affected trees in progeny orchards in SO-5. Eastern Merced Co.

Year of plantin		No. of acres	NPS occurrence
1963	9	220	30 to 40 percent NPS trees of varying levels of severity
1964	10	160	Significant NPS; no exact count
1965	11	100	n n n
1966	3,4	320	17 18 TT
1967	1,2,8	640	One observation showed all 13,14 trees in limited area affected
1968	12,15,16	400	Part from different nursery
1970	39,40	440	significant numbers, no exact count
1971	30,31, 32	770	significant numbers
	41,42,4	•б	

Tree	Rating for "bull"	Kernel wt (g)	Shell- ing %	<pre>% hard     nuts</pre>	W/L	Remarks
66	N	1.13	48	100	65	sticktight
67	N	1.08	43	100	61	sticktight
68	N	1.09	48	100	61	-
69	В	1.03	54	40	54	
70	B+	1.02	52	30	55	shrivel
71	B++	1.13	59	0	54	
72	B-	1.00	37	100	65	wing; peanut
73	В	1.05	50	100	57	wing; peanut

Table 12. Tree and nut characteristics of representative trees in SO-4 of Nursery A, Block 9

Table 13 was derived from Nursery A sales records and insurance claims for affected trees in orchards planted from 1967 through 1975. These records

Table 13. Distribution by year planted of Mission trees affected by NPS from Nursery A as shown by insurance damage claims.

Year	Miss:	ions s	old	Claims	reported	Recalculations	
planted	Trees	Growe	rs Ave.	Trees	Growers	Trees	Growers
	No.	No.	No.	No.	No.	No.	No.
1965	nr	nr	nr			65	1
1966	nr	nr	nr			33	1
1967	55,560	110	505	168	3	563	7
1968	23,614		347	232	3	125	5
1969	21,603	70	302	841	15	-	-
1970	56,156	105	585	2952	50	-	-
1971	87,491	93	910	796	21	-	1 <b>—</b> 1
1972	63,767	108	590	1175	19	-	-
1973	76,435	102	749	1248	17	285	10
1974	91,130	88	1036	9249	21	-	-
1975	73,120	129	590	5254	40	-	-
1976	NR	NR	NR	0	0		

do not account for all the "bull" trees produced (Table 11 and 15). Nevertheless the analysis provides a clear pattern of time sequence and the incidence of NPS trees in progeny orchards during a key period when the problem was occurring. Production of NPS trees apparently ended in 1976 with a change of budwood source. The table does not show when the problem began but only the period when records were provided. Table 14 lists additional commercial orchards propagated from trees which were examined at various times and which showed various percentages of NPS trees. The planting dates (1964 through 1975) overlap the same range of planting years (1962 through

1971) of PO-4 (Table 11) as well as those (1967 through 1975) listed in Table 13.

Table 14.	Progeny orchards w from nursery A.	ith significant numbers of NPS trees originating
Year planted	Orchard designation	Occurrence of NPS trees in orchard
1964	Kern CO.	18% reported. 1970 was a source for virus indexing
1965	Wasco, Kern Co. Modesto	25 to 30% in 1984 Estimate 20% or more in 1970.
1967	eastern Merced Co.	About 70%, varying degrees of severity. Also a source orchard
	Modesto	Estimate 20% or more
1969	Escalon	190 trees affected; about 20%
1971	western Kern Co.	High per cent but no actual count
1974	RVT plot,Kern Co. trees plan	One bundle of 10 trees out of 26 ted
1975	Kearney FS,Fresno	All of an 8 tree block

A pattern emerging from this analysis shows that NPS trees were appearing in significant percentages in 'Mission' orchards planted with trees from Nursery A as early as 1963 (1962 propagation year) and continued with annual fluctuations through 1975.

<u>Commercial budwood source orchards</u>. (Table 15). The primary collections for budwood propagation would be made in May and June for commercial production of trees to be produced during that season and sold the following winter-spring season. A lesser amount would be collected in July, August or September for "dormant" budding to produce trees to grow the following season and to be planted the second winter-spring season.

Table 15. Budwood source orchards used by Nursery A.

Year Budwood collected		Location	No. of Buds	NPS occurrence in budwood orchard
1962 1963	1963 1964	no record no record		
1963	1964	no record		
				cionificant NDC charged
1965(d)			nr	significant NPS observed
	1968		nr	significant NPS observed
1967	1968			18% NPS
		SO-5 B1k 9 (?		
1968	1969	SO-4 Modesto		18% NPS
1969	1970	SO-5 Blk 12 1,2,3,4	109,000	up to 30% NPS
1970	1971	SO-5. Blks 9,10,11	58,000	up to 30% NPS
1971	1972	SO-5 Blks 9,10,11	45,000	11 11
1972	1973	SO-5 Blks 9,10	15,000	n n
1973	1974	SO-6 SO-7 Modesto	172,000	Around 70% trees affected No information
1974	1975	no record		
1975	1976	Famosa		
1976	1977			
1977	1978	11 17		
				•••••

(d) = dormant buds

The 1965 and 1966 records are incomplete and show only sources for dormant (fall) budding onto yearling trees to produce a limited number of trees for 1967 and 1968 planting. Although the same sources would have likely been used for June budding, this is not certain. Observations made in orchard SO-2 and SO-3 at various times subsequent to their use for budwood showed varying percentages of NPS trees.

The record for budwood source orchards used from 1967 through 1978 is complete from nursery log books except for 1974. Examinations of scion orchard sources revealed significant numbers of NPS trees in SO-4, SO-5 (several different blocks), and SO-6. All of these orchards were surveyed in 1980 and 1982, including the exact trees used for budwood collection. Percentages of NPS trees in these blocks ranged from 18 to 70 per cent. (Tables 12 and 16).

Tree	Size	crop	"bull"	Av.kerne wt.(g (g)	l % )kernel (%)	Hard shells (%)	W/L 5 (%)	Remarks
			02		 E E	••••	<i>c i</i>	T
2	Med	Med	0?	1.10 0.76	55 38	80 100	64 62	L.wings;2p
1 3	Med	Heavy	0	1.15		100	62	L.wing
	Large	Med	1		47			L.wing
7	VL	Light	5	1.02	52	40	59	L.wing
8	VL	L	5	0.60	43	10	55	shriv.
10	VL	L	5	1.06	54	0	55	L.wing
4	VL	L	5 5	1.14	53	10	49	L.wing
9	VL	L	5	1.10	54	0	52	L.wing
5	VL.	L	5	1.10	57	20	51	L.wing
6	VL	L	5	0.78	52	0	49	L.wing
				II. Ro	w 13.			
10	OK	OK	0	1.16	47	100	64	
11	small	OK	0	1.00	48	100	62	L.wing
5	М	M	1	0.76	49	100	51	Wing, peanut
8	L,M	M		1.08	50	40	56	Peanut
	L	М	2 3	1.42	53	30	56	
2 1	M	M	3	1.20	53	20	52	L.wing
9	L,M	L(1)	4	1.30	48	20	57	0
7	L	L	5	1.00	48	20	58	
4		VL	5	1.20	52	20	56	
3	L	L	5	1.24	50	0	54	
6	L	L	5	1.23	49	0	50	
Tree	<pre>(1) narrow leaf Tree Size: Very Large=VL; Large=L; Medium= M; Crop: Heavy=H; Medium=M; Light=L; very light=VL</pre>							

Table 16. Representative tree ratings and nut samples from SO-6, Nursery A. Samples collected in 1980. Trees planted in 1967.

Bull rating: 0-none, 1 (slight) to 5 (very pronounced

Figure 7 is a flow chart connecting nursery source orchards and progeny orchards originating with Nursery A as far back in time as records were available. All of these source orchards are known to have themselves originated from Nursery A and all represent consecutive propagation of a single PROGENY ORCHARD SOURCE originating in sequence from some original source (SO-1?) prior to 1960. NPS trees have been present in all orchards of this sequence, indicating the origin of the problem to have occurred before 1960.

Use of Certified source material. Nursery A obtained a limited supply of budwood of FPMS 3-8- 1-65 at the time of its general release in 1968 and used it to establish a 53 tree scion orchard in spring 1969 (Figure 8). This orchard was then used to produce large numbers of non-certified nursery trees in 1972, 1973,1974 and possibly 1975. However, during this same period, an equally large numbers of buds were collected from SO-5 and SO-6 and another not observed. Budsource identity was not maintained during the digging, size sorting and selling operation.

In the interim between 1968 and until scion orchard trees were old enough for varietal verification, propagating material of 3-6-1-65 was obtained directly from the Foundation Orchard trees at FPMS, UCD. Certified trees were produced in 1970 and 1971. In 1970 400 certified Mission trees were sold to a single Modesto grower (GL) to produce one block of trees and two other other orchards were identified as from "certified" trees. No NPS trees were observed in these orchards by 1982. In 1971 only 400 certified trees were available and all sold to grower GL who supplemented these with 170 additional non-certified commercial trees (two nurseries). Some replants also replanted later. Twenty nine "bull" trees were tentatively identified in 1982.

In 1976, nursery personnel observed trees in the scion orchard that they believed showed NPS symptoms and concluded that these were the source of the NPS trees coming from their nursery. At our suggestion, a vegetative progeny test was started with trees propagated from each of the suspect trees. The test block near Waterford,CA, when subsequently observed revealed no evidence of NPS trees.

A new scion orchard of Mission 3-8-1-65 trees from separate source trees (OFO and NFO Foundation, UCD)(see later) had been established in 1973. No evidence of NPS trees was present.

Nursery B.

<u>Mission</u>: Nut samples from various sources examined in 1977 showed some shell softness and abnormal nut shapes. A field survey of budwood source orchards in 1980 combined visual inspection and nut collections.

Budwood source records supplied by the nursery provided an opportunity to make a complete SOURCE-PEDIGREE ANALYSIS of an entire PROGENY ORCHARD SOURCE ("strain") of Mission from 1960 and before through 1987. (Table 17).

Source designation	Observations in 1980
SO-1	Trees and nuts normal; many replants Oldest existing SO orchard. Used 1965 and earlier
SO-2	Originated from SO-1 about 1960. Used from 1966 through 1971. Orchard had been removed by 1980. Reported as free of any off-type trees or nuts.
SO-3	Originated from SO-1 about 1960 or before. Used 1966 through 1969. Some severe NPS trees and others with varying degrees of "ugly" atypical nuts. Base to top gradients (?).
S0-4	Originated from SO-3 about 1966. Used in 1970,71, and 1972. Some NPS and off type nuts nuts when examined in 1980.
S0-5	Originated from SO-4 about 1970. Used in 1974 and 1975. NPS off type nuts present in various trees.
SO-6	Originated from SO-3 about 1967. Used in 1971 to 1973. No definite NPS trees but varying numbers of trees with softer shells and elongated nuts.
S0-7	Originated from SO-6. Used from 1973 through 1985 except for 1977. Varying numbers of trees with softshells and elongated nuts in 1980 survey but no definite trees with obvious "bull" characteristics. In 1987 5-8% of trees with definite NPS symptoms ranging from severe "bull" to off type nuts.
SO-8	New orchard planted 1982 from SO-7. In 1987, some definite NPS trees.
SO-9	Source-clone started from single tree in SO-1.
so-10	New Source-clone started from 15 year old orchard in Stanis- laus Co. with characteristic hardshelled Mission nuts.

Table 17. Mission budwood source orchards used by Nursery B and some vegetative progeny orchards. 1960 to 1987.

Visual inspections of these source orchards supplemented by nut sample measurements in 1980 and again in 1987 were used to identify NPS trees (Figure 9).

The entire sequence originated with the Adrian orchard, Escalon,CA., (no longer in existence) from which SO-1 in the sequence was propagated. In the 1980 survey, this orchard was old with many replants but without evidence of NPS or off type nuts. (Table 18).

N	ursery B	. 198	0 sam	ples.					
Source orchard						sample 6 56-57 58			62-63
	I. N	urser	y B s	ource	orcha	ard survey	y y		
SO-2	1(1)								
SO-3	4	5	8	1	4	1	1	2	
SO-4	2	3	11	15	18	10	6	4	3
SO-6	3	4	15	13	6	1		×	
SO-7	13	2	8	14	6	1 10 1 4	3		
II. Nut	samples	from	Kern	RVT	plot.	Includes	both	N and	В
R64S (N)	4								
R64S (N) R64S (N,B) R65S (N,B) Comp.(N)	1	1	2	1	2				
R65S (N,B)	5	2	1	1			2		
Comp.(N)	13	2	1						
R77N (N)	1*								
(B)			1		2		1		
<pre>N= normal; B = "bull" *Composite (1) Representative of orchard</pre>									

Table 18. Distribution of shelling percentage as a measure of shell softness

in Mission nut samples collected from budwood source orchards from

Two progeny orchards, SO-2 and SO-3, were used as budwood orchards. SO-2 was owner operated and provided budwood for many years before removal. With close personal supervision, nursery personnel were certain that no abnormal trees were present and nuts were the small, hardshelled type.

SO-3, on the other hand, when examined in 1980 showed strong evidence of NPS trees and abnormal nuts (Table 18). It was the first of the sequence where NPS trees could be identified with certainty (Fig. 10). This orchard was used as a bud source for four years and gave rise to two consecutive progeny orchards - SB-4 and SB-5 - in which significant numbers of NPS trees were identified both by tree and nut characteristics. There was a complication in that nursery trees were also obtained from Nursery A during 1970 and 1972 for distribution, including the SO-5 orchard.

The other sub-source sequence is identified as SO-6 and SO-7. In 1980, no distinct "bull" trees were observed in either orchard, including the specific trees used as budwood sources. However, there were varying numbers of trees with softshelled and elongated nuts with high shelling percentages (Table 18). SO-7 was the budwood source orchard exclusively from before 1980 through 1985.

A survey made in SO-7, September 1987, showed approximately 5 to 8 per cent of the trees with distinct "bull" characteristics either of the tree or nuts. In 1987 a number of commercial orchards (PO-9) planted in 1984

(propagated in 1983) were reported to have significant numbers of NPS trees, in at least one case 8% of the trees being affected.

<u>Progeny tested sources</u>. Budwood of Mission 3-8-1-65 from FPMS, UCD, was obtained in 1968 and planted into a scion orchard (Grayson Road). The nursery reported that at three years the nuts were larger and softer shelled than expected but were smaller and hardshelled with slight tendency to appear "ugly" in the two subsequent seasons. Part of the trees were left unpruned and part were severely pruned in 1978. Visual inspections and nut sample analysis in 1980 confirmed the general conclusion that the difference in nut characteristics was related to the degree of vigor and cropping in the source tree. During 1977 through 1980, search was made for other commercial source orchards with typical small, hardshelled nuts. In 1980 a single tree was selected from a productive 15 year old orchard near Modesto with typical small, hardshelled nuts.

In 1982, a vegetative progeny orchard test was established with single rows of trees from the following sources: a. source-clone 3-8-1-65 from the Grayson Road scion orchard; b. source-clone (SC-1A) from single tree of SO-1; and c. source-clone (SC-10) from the a new source. Remainder of the orchard (PO-8) was planted with trees from the SO-7 source (Figure 11). Visual inspection and nut samples obtained in 1987 in this plot showed no evidence of NPS trees from 3-8-1-65, SC-1A and SC-10. Some of the nuts on a few trees from SO-1A were somewhat atypical but no distinct "bull" tree was observed. NPS was detected in the PO-8 trees (from SO-7 source) the percentage of trees was relatively low.

In 1987 trees with NPS were reported in commercial orchards planted in 1984. These would have been propagated in 1983 from buds collected from SO-7. In one instance 8% of the trees were reported sufficiently affected to necessitate removal.

"Bull", "Ugly" and "Peanut" trees in other varieties than Mission. "Bull" trees of Carmel have been produced in low percentages in some commercial orchards. Also off type trees of Carmel, Nonpareil and Fritz have been observed sporadically in certain orchards. These are sometimes whole trees and sometimes part trees. No detailed study has been made of their distribution but what is known fits the patterns described for NPS Mission. In at least two cases, Nonpareil and Carmel developed in the same orchard at the same time. Vegetative progeny studies are described in Section I.

#### Nursery C.

Nursery C located in the Sacramento valley reported that essentially no NPS trees had been produced from their material except in specific instances. These occurred in either of two situations (a) when trees were obtained from Nursery A (often for replants) or (b) in one instance, when buds were collected (1968) in a commercial orchard near Modesto. This orchard was later identified as SO-4 of Nursery A (Table 14). Trees from this source planted in an orchard near Durham produced approximately 30% moderate to severe NPS.

Although some budwood material was produced from FPMS 3-6-1-65, the use was very small and there was no information as to distribution.

#### Nursery D

Nursery D also located in the Sacramento valley had used a Mission budwood source orchard near Chico for many years. They reported the occurrence of 5 or 6 cases of NPS trees in progeny orchards, all of which were in low percentages and located in the area from San Joaquin Co. to Merced Co. and occurred in 1971 to 1976 plantings. No cases had been reported in other orchards supplied by them in other parts of the Sacramento or southern San Joaquin valley.

#### Nursery E

This nursery located in the upper San Joaquin valley reported the occurrence of several orchards with significant numbers of NPS trees but all were resales from nursery A. These orchards included the Luder orchard, Manteca, where early research on the problem was conducted (see Part I).

Budwood was obtained of FPMS Mission 3-8-1-65, UCD, in 1968 and a scion orchard was established. This source was used exclusively from 1972 through about 1976 and a number of thousands of trees were sold without indication of problems. Examination of two representative commercial orchards grown from trees of this nursery, one near Fresno (1000 trees) and one near Escalon, confirmed this conclusion, Only in one instance was there an unexplained and unconfirmed incident in which a grower claimed that about 15 trees in a single orchard had NPS symptoms.

#### Nursery F

This nursery in the Sacramento Valley obtained FSPMS 3-6-1- 65 in 1968 and have used it exclusively as a propagation source from about 1971 or 72 to the present. Visual observations and nut examination of these trees showed no evidence of NPS and no commercial buyers of the nursery reported problems.

#### DISCUSSION AND SUMMARY

The research described in this section provided an opportunity to characterize source selection practices in commercial nurseries. The primary procedure involves sequential selection of ORCHARD SOURCES which develop into a system of PROGENY ORCHARD SOURCES involving a "budline" that may be unique for the particular nursery. Since fruit and nut varieties are vegetatively propagated and represent a genetic clone one should theoretically not expect variability to develop among the progeny trees of a particular variety. In almond, specific variants can develop within these clones (varieties) including (a). viruses (b). genetic mutations and (c). variants of the noninfectious bud-failure type. The frequency of these variants in any progeny orchard trees depends upon their frequency in the source orchard from which the budwood came and in the ability to select against them.

It was concluded in Part I that NPS is related to mutations. The analysis in Part II concludes that these were initiated more or less simultaneously in more than one source and more than one variety in a relatively limited area and time span. In some cases adjoining trees of the same orchard were affected. It is unlikely that this situation could occur by chance without exposure to some external agent. The possibility of an infectious agent, such as a virus, was tested with negative results. Furthermore, the chimeral patterns shown by NPS variants within and between trees and in consecutive propagations argues for a condition that is characteristic of the cells within the tissue. The expression of NPS is described as like an "explosion of mutations" that would occur if the plant were exposed to a mutagenic agent which results in a variety of sublethal mutant cells, each with a different potential for growth and physiological expression. Initially such sublethal mutant cells would be latent in the plant and would not become visible in the plant until these mutant cells occupy important segments of the plant.

During the same period as the NPS was occurring, there was a parallel development of a somewhat similar condition known as "green fruit" in cling peach varieties. It also developed in a chimeral pattern, occurring in a number of varieties. In this case, there was a circumstantial linkage to exposure of the source orchards to DBCP, a soil fumigant widely used in that particular area during the period of late 1950's to late 1970's. With the almond there is less direct evidence of such an association and this material was not used in nursery fumigation. However, testimony concerning nursery A stated that some of the original experimental trials with DBCP was conducted in their home orchard near Modesto. As regards Nursery B, SO-3 was surrounded by peach orchards literally saturated with DBCP although that particular orchard itself had no record of treatment.

Nursery propagation by single buds (budding) is an extremely efficient method of selecting latent mutations since entire new plants are produced. With large scale nursery operations, even a relatively few such plants in the source orchard could be screened from the larger numbers of nonaffected plants and appear in progeny orchards. The wide variation in vigor and expression within the NPS syndrome and their concentration into particular size grades during the nursery operation provides a mechanism toward selection in consecutive scion generations. As trees grow older and larger, there is some indication that NPS may become more pronounced in upper branches of the tree. Bud wood collection from older source orchards depends more and more on upper branches and in particular on vigorous shoots and watersprouts.

Once the NPS condition can be associated with a particular budwood source, control appears to be easily accomplished by shifting to a new budwood that does not have a history of NPS production. Furthermore, control in the orchard is accomplished by replacing the affected trees either by replanting or by grafting. The main problem is how to identify new sources which do not have a latent potential for NPS. This study shows that there are numerous commercial nursery sources as well as Registered source-clones (see Section III) that can be utilized with some confidence. However, since the hypothesized "triggering agent" is speculative, there is no assurance that new cases of NPS might not arise if currently NPS-free sources are exposed to the same or similar agent. The fact that sporadic occurrence of a NPS-like condition occurs in Nonpareil, Carmel and Fritz should perhaps continue to be a matter of concern.

The problem of verification of trueness-to-variety and freedom from latent genetic problems in a nursery production system is a general one that involves not only NPS but also the somewhat parallel but genetically different problem of noninfectious bud-failure and in fact any other kind of mutation that might develop. With all of these problems, VISUAL INSPECTION OF THE SOURCE PLANTS is shown to not be completely reliable as a method of selection but needs to be combined with VISUAL INSPECTION OF THE PROGENY PLANTS. For source orchards, selection needs to be based on source-progeny analysis of the entire sequence of source and progeny orchards, a process requiring careful maintenance of source identity. Visual inspection of the whole orchard is needed and not just the specific trees that are to be used for budwood collection. If a significant percentage of consistently off-type trees occur, it would be best to avoid that orchard entirely since it could be expected that there would be other unidentified trees that would have some latent potential for the observed disorder. The advantage of the system is that through mass propagation the frequency of any chance variant to develop would be relatively low and would not constitute an economic problem even though present. With natural occurring mutation, this argument could likely hold. With NPS the high percentages that developed before detection argues against the particular procedure.

#### PART III. PROGENY TESTING AND NURSERY USE OF REGISTERED SOURCE-CLONES

Abstract. SOURCE-CLONE selection involves the identification of single plants which are tested free of specific viruses and are "true-to-type". This process can be effective for control of viruses because one can apply tests directly to the source plant. It has been limiting for selection against latent genetic disorders because no direct tests are available to predict potential for latent genetic disorders, such as noninfectious bud-failure and nonproductive syndrome. However, research now shows that a combination of VISUAL INSPECTION OF SOURCE PLANTS (phenotypic selection) combined with VISUAL INSPECTION OF PROGENY PLANTS (genotypic selection) can be an effective method to identify source-clones free of NPS. Thus, sourceclone selection combined with principles of distribution which includes maintenance of source-identify in the propagation process and is embodied in Registration and Certification schemes can be effective in production of virus tested, true-to-type nursery deciduous nursery trees. Mission 3-6-1-65 was shown not to be a latent source of NPS trees.

#### INTRODUCTION

Registration and Certification programs have been developed throughout many parts of the world to produce "virus-tested" source materials of fruit, nut and vine varieties for the production of "clean stock" of nursery materials. In California this effort had the combined input of the California Dept. of Food and Agriculture (CDFA), University of California, and USDA. There are three parts of this program (Figure 12):

I. Selection of sources of propagating materials which are "free of known harmful viruses" and which are "true-to-type", i.e., true for the variety selected.

These selections originate from single source trees and their vegetative progeny is referred to as a CLONE. (In this report these selections are referred to as SOURCE-CLONES to distinguish them from the original variety which originated from a seedling and is itself a clone). These differ fundamentally from SOURCE ORCHARDS and PROGENY SOURCE ORCHARDS as described in Part II.

II. Maintenance of the source-clone in a FOUNDATION ORCHARD under conditions to prevent reinfection of viruses.

The maintenance and distribution from the Foundation Orchard is the function of the FOUNDATION SEED AND PLANT MATERIALS SERVICE (FSPMS) located at the University of California, Davis.

III. Distribution of the "clean" materials to nurserymen and ultimately to consumers.

REGISTRATION AND CERTIFICATION is a voluntary procedure administered through the Nursery Service of the CALIFORNIA STATE DEPARTMENT OF FOOD AND AGRICULTURE (DFA). The program involves use of registered Foundation sources, production under guidelines specified in Nursery Service Regulations, nursery inspections, and identification of nursery stock as "Certified" by specifically colored tags.

#### RESULTS

#### SELECTION OF MISSION SOURCES

Three selection source-clones on Mission have been selected as "virus-free" and "true-to-type".

1. Source-clone 3-6-1-65 was selected as "virus-free" from trees in a Dept. of Pomology Irrigation Research Plot by Dr. George Nyland and used for a number of years in virus research. It was eventually grafted into the Foundation Block (September 1965) and when variety verification was completed was distributed to commercial nurseries. (Figure 13).

- Source-clone 3-6-2-70 was submitted originally by John Wynne, Dave Wilson Nursery. Found to be virus infected, it was heat treated to remove the viruses present and placed into the Foundation Orchard in 1970. Essentially no commercial distribution has been made of this source.
- 3. Source-clone 3-6-5-67 was obtained from the IR-2 Repository, Washington and originally came from the same trees in the Dept. of Plant Pathology, UCD, as 3-6-1-65. This source had been used experimentally at UCD since its acquisition in 1967 but no commercial distribution had been made.

#### DISTRIBUTION OF MISSION 3-6-1-65 TO COMMERCIAL NURSERIES.

Bud wood was first distributed in 1968 in limited amounts to establish scion orchards. These could be used to produce CERTIFIED nursery stock once the identity of the trees in the scion orchards had been verified, regulations met and trees tagged with specific labels to identify them as "certified stock". However, since Registration and Certification is voluntary, nurseries also could utilize this source without going through the Registration and Certification process.

Research began in 1976 when NPS became recognized as a new problem with no basic understanding as to its nature and no guidelines as to its diagnosis or handling.

- a. Visual inspections of the Mission 3-6-1-65 source trees for trueness-to-type were positive (Figure 14) but, at that time, there were insufficient numbers of authenticated vegetative progeny trees of sufficient age for reliable verification.
- b. In one nursery, where nursery trees of 3-6-1-65 had been produced, trees were distributed along with other nursery trees produced from source orchards with NPS (Figure 7). Trees were distributed as non-certified and separate identify was not maintained (Figure 8).
- c. Attempts to verify trueness-to-type in scion orchards was largely unsuccessful (see Section I) and in fact resulted in misidentification of some trees as "bull". However, vegetative progeny tests from these specific trees demonstrated their freedom from NPS (Figure 8).
- d. Distribution by two nurseries (Nos. E,F,) involved significant numbers of trees in progeny orchards and has been highly successful. Progeny orchards examined were verified as free of NPS.
- e. Approximately 1000 trees were produced and sold as certified. Three orchards with these trees had no evidence of NPS trees. A fourth included NPS trees but was found to be a mixture of certified trees planted with nursery trees from commercial nurseries with history of NPS (Figure 8).

#### VEGETATIVE PROGENY TESTING

Mission 3-6-1-65 has been used in the Dept. of Pomology teaching orchard since 1969. The orchard involves consecutive annual plantings and results are described in Part I.

In 1970 a new Foundation Orchard (NFO) was established including all three Mission Source-clones. Subsequent distribution was made from these younger trees. A new scion orchard from budwood of Mission 3-6-1-65 of both the Old Foundation Orchard (OFO) and the New Foundation Orchard (NFO) was planted by Nursery A. Trees were pruned for crop and not for budwood. No evidence of NPS was shown.

Beginning in 1974, a series of Regional Variety Trial plots was established first in Kern Co. and subsequently at Arbuckle (1975, 1977), Durham (1976, 1978), Manteca (1978), Fresno (1981) and McFarland (1981). In addition to providing comparative tests of variety performance, they included comparative tests of different propagation sources, primarily of the FSPMS source- clones but also some commercial nursery sources. Data of yield, phenology, visual inspections of potential disorders and nut evaluations were made from all trees of the plots. Annual reports of this data have been made to the Almond Board of California who sponsored the research (Figure 15).

Kern plot: The first nut collections made in 1978 from all the Mission sources showed considerable tendencies toward softness of shell, large size and narrow shapes. In 1979, and thereafter, however, nuts from each of the three Mission FPMS source-clones, as well as several collections of commercial sources, have produced typical small hard-shelled nuts. The exceptions were specific trees with strong "bull" characteristics which came from single bundles of trees obtained from Nursery A (described in Section I) and from Nursery B.

Colusa Co. and Butte Co. plots: Only the three Mission Source clones were grown and none have produced any evidence of NPS affected trees.

San Joaquin Co. plot: This plot includes not only the three FSPMS source-clones but also trees of three commercial nursery sources. No significant differences in yield or nut characteristics have been produced among five of the six sources. One of the five showed some variation in nuts in the early years but differences have not been significant. Yield from the sixth source, however, has been significantly less than the others each year of the test. Furthermore the kernels are significantly larger, the shells significantly softer with higher shelling percentages and the kernels narrower. The appearance of the nuts tends to be "ugly" but there is no presence of the distinct "bull" trees that characterizes the problem. Shirofugen tests were negative for the three FPMS sources and one commercial source.

Fresno Co. and second phase of Kern Co. plots: Trees were planted in 1981. No NPS affected trees have been observed.

#### DISCUSSION AND SUMMARY

The research described in this section is important in that it was able, first, to verify that Mission Source-clone 3-6-1-65 as well as other specific sources from FSPMS, UCD, do not have a potentiality to produce NPS trees and, secondly, to evaluate the concepts of SOURCE-CLONE SELECTION, VEGETATIVE PROGENY TESTING and REGISTRATION AND CERTIFICATION in nursery practice.

#### a. Verification of trueness-to-type of Mission 3-6-1-65

PEDIGREE SOURCE ANALYSIS combined with VEGETATIVE PROGENY TESTING were able to demonstrate that the Mission 3-6-1-65 source-clone, as distributed from the Foundation Orchard, UCD did not carry latent NPS and was not a cause of the "bull Mission" problem. Similarly absence of NPS was verified in various other sources. Instead, the NPS problem was found to have developed within specific commercial PROGENY ORCHARD SOURCES, as described in Section II. Adoption of new Mission sources by the nurseries involved should reduce the immediate threat of NPS.

However, since the specific "triggering" agent for NPS induction in clonally propagated fruit tree varieties is still speculative, the possibility for new cases of NPS to occur in the future in any source of Mission or other varieties is not eliminated. In fact, the sporadic occurrence in Carmel, Nonpareil, Fritz and perhaps other varieties should continue to be a matter of concern.

b. Selection of source-clones for improvement of nursery stock

"Clean stock" programs are based on the concept of CLONING from a single "virus-free", "true-to-type" selected source plant followed by multiplication through a limited number of scion generations under close supervision and isolation. With proper selection and handling of this system, the FREQUENCY of progeny plants that are free of the tested viruses, true to the variety selected and free from any genetic disorder should be 100 per cent. On the other hand, if the source plant is the wrong variety or has a high potential for various kinds of latent genetic disorders, the frequency of off-types or incorrect varieties in the progeny plants also could be 100%.

These situations contrast to current propagation systems using orchard sources or progeny orchard sources. There the frequency of any latent virus infection, genetic disorder, or off- type plant in nursery stocks depends upon two factors. One is the frequency of any of these conditions existing in trees of the source orchard; the second is the ability of nursery personnel to select against these conditions.

Latent viruses have been found to be widespread in mature California almond orchards (this report and others) although the significance of their effect on yield and other characteristics may be disputed.

Part II of this report shows how the various manifestations of NPS have developed in significant numbers in various source orchards and progeny source orchards. These became propagated despite the best efforts of each of the nurseries involved and the problem was not discovered until aberrant trees appeared in growers orchards. This took place long after the key events had occurred in the source trees and five years or more after the budwood had been selected and propagated.

In parallel research we have shown that potentiality for noninfectious bud-failure is likewise widely distributed within California almond orchards and exists in a wide range of BF- potential among different trees of the same orchard. The principles of selection, maintenance, and distribution to combat BF parallel those that are described for NPS although the basic nature of the two problems are different.

Successful selection of source-clones depends upon the ability to detect problems in the source plant. For example, the presence of most harmful viruses in almond trees can be detected with current indexing technology although improvement in speed and sensitivity to various strains would be helpful. Nevertheless, verification can be made directly on the source plant. Continued freedom from specific viruses can be monitored to provide a system for producing nursery stock that can be certified as free of specified viruses. This assumes that this principle can be translated into cost-effective nursery management systems.

On the other hand, measurement of "trueness-to-type" and detection of latent genetic disorders or mutations in the source plant has been hampered by lack of suitable diagnostic procedures. This lack has been a limiting factor for improving nursery production systems for fruit trees and nuts in general and for almonds in particular. The research on NPS and the parallel research on noninfectious bud-failure provides fundamental knowledge that can be a basis for establishing the principles and applications whereby these genetically related problems can be controlled in nursery programs.

VISUAL INSPECTION is the key to selection for trueness-to- type and freedom from latent genetic disorders. There are two essential phases involved in visual inspection:

(a). Visual inspection of the SOURCE PLANTS under proper conditions of growth and management at a stage where the plant is in bearing. When a source plant is being managed for the maximum production of propagation material (cuttings, bud or scion wood) the plant is not under proper condition for visual inspection and in fact may lead to misidentification of problems.

This operation can be called PHENOTYPIC SELECTION because it is based only on the PHENOTYPE (appearance) of the source plant. It provides promise of "genetic suitability" but does not prove it.

(b) Visual inspection of VEGETATIVE PROGENY PLANTS. Progeny plants must be grown under commercial or required conditions to allow for expression of any special characteristics of significance. This operation can be called GENOTYPIC SELECTION because it tests the actual GENETIC POTENTIAL (GENOTYPE) based on the performance of its offspring. be called GENOTYPIC SELECTION because it tests the actual GENETIC POTENTIAL (GENOTYPE) based on the performance of its offspring.

Once this second step is completed, considerable confidence could be placed on the trueness-to-type of a selected source- clone but the system of propagation should also include the ability to trace the origin of any chance variant from progeny to source quickly and efficiently.

c. Registration and Certification systems.

These programs to combat virus diseases are now in operation for nursery propagation of many fruit crops including citrus, strawberry, and grapes (in California) as well as with deciduous fruit crops in other states, Canada and Europe. A program for deciduous fruit and nut crops (Prunus) was introduced into California in the mid 1960's (Figure 12), but has been little used in practice. Although there are many reasons cited for lack of use including need perception, management problems, economics, etc., one of the primary reasons has been the lack of confidence in the genetic quality of source-clones that have been available particularly related to noninfectious bud-failure, NPS, or other potential genetic problems.

It is suggested from this research on NPS as well as parallel research on noninfectious bud-failure that addition of a <u>genotypic</u> <u>selection</u> step into the procedure (in addition to a <u>phenotypic</u> <u>selection</u> requirement) would do much to offset this concern (Figure 16).

Research described in this report as well as additional programs on noninfectious bud-failure have as their goals the establishment of the basic principles of source selection in relation to genetic problems of nursery stock in general and of these two problems in particular. These principles would be applied to the selection and evaluation of particular source materials and to the development of nursery production systems which will control these problems. The latter goals could be accomplished either through more efficient, acceptable and effective Registration and Certification programs or through equivalent programs managed through the nursery industry itself.

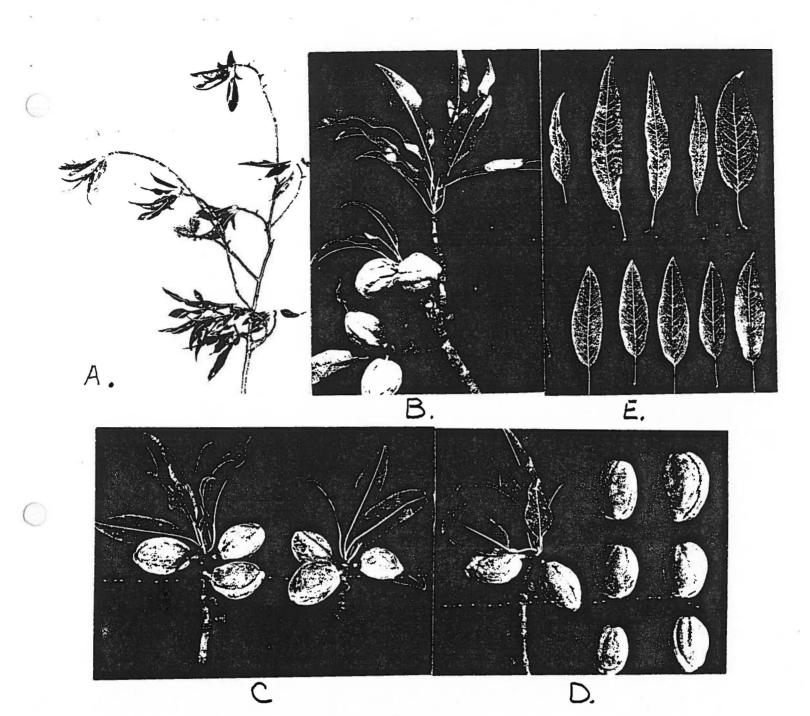


Figure 1. Expression of NPS in Mission almond.

- A. Severe "bull" condition. See consecutive terminal shoots which initiate flowers but which fail to set.
- B. Branch with good production but with strongly "ugly" fruits with distortions.
- C. Shoots with nuts nearly normal in same orchard as "bull" trees. Note single nut in center with some distortions.
- D. Nuts on "bull" tree showing variation. Note small "peanut" type lower left.
- E. Upper row: elongated leaves sometimes seen on "bull" tree. Lower row: leaves on normal tree.

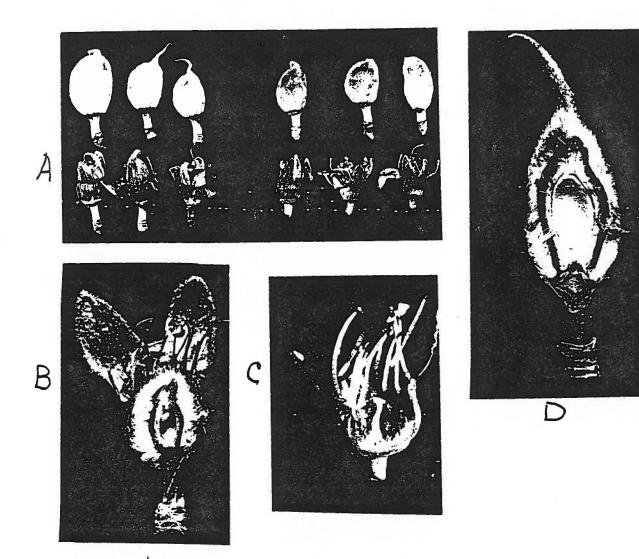


Figure 2. Examples of flowers on highly nonproductive "bull" tree.

- A. Left, young fruits on normal tree; right, abnormal appearing small fruits on "bull" tree. Note elongation.
- B. Defective flower with undeveloped ovule.
- C. Defective flower with undeveloped ovary.
- D. Apparently normal small ovary on a "bull" tree. Note elongated shape. B and C flowers will drop. D may develop but be abnormally shaped.

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Figure. 3.

Aerial view of orchard of two Thompson rows and 1 Mission row. Bull trees are outlined. Note grouping. Photograph courtesy of Wm. Wildman, UC Cooperative Extension Specialist.

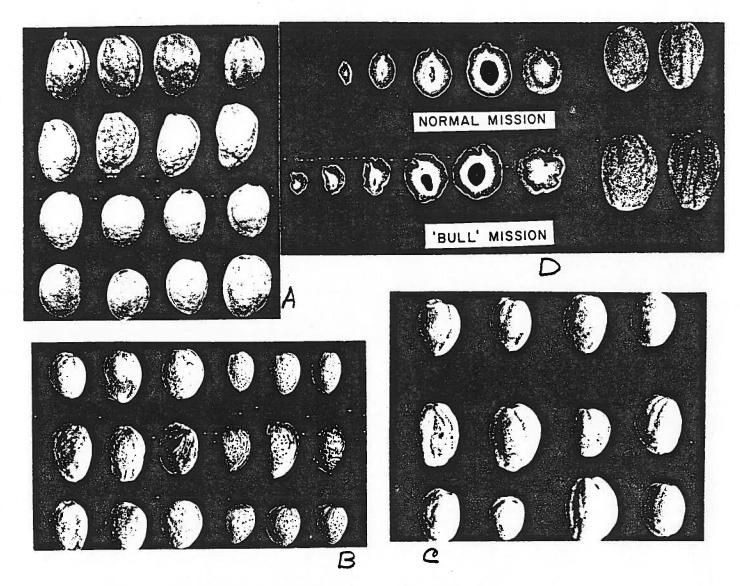


Figure 4. Nut expression in Nonproductive Syndrome of Mission.

C

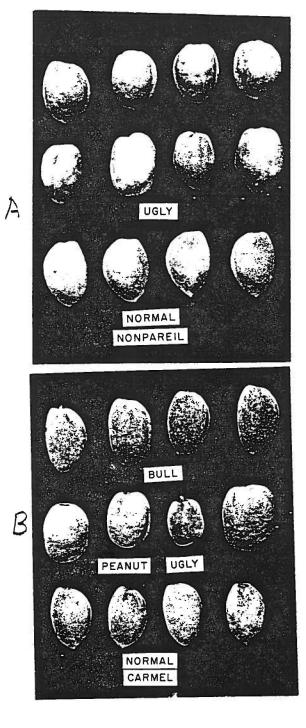
- A. Top row: immature nuts of Mission of "red" source. Next to top row: immature nuts of "white" source. Next to bottom row: immature nuts of "blue" source. Bottom row" immature nuts of a normal Mission. A and B show elongated shape and distortions.
- B. In hull nut on left; hulled nut on right Top row: Mission showing some elongated hardshaped nuts. source. Middle row: nuts on "bull" tree. Note elongation; wing. Bottom row: typical Mission nuts. Small. round.
- C. Top row: immature nuts on normal plant. Bottom two rows: shows range of variation from large, elongated to small "peanut"
- D. Nuts cut crosswise in consecutive sections.
  Upper: shows the uniform development of nut of the normal nut.
  Lower: shows the distortion and uneven growth of the different sections of the fruit. Shows unequal growth that has occurred in different sectors.

Figure 5. Abnormal Nut characteristics of Nonpareil, Carmel and Fritz.

Α.	Lower: immature nuts of normal Nonpareil.
	Middle and upper: examples of "ugly" nuts. Note shortened,
	rounded nuts.
Β.	Lower: immature nuts of a normal Carmel.
	Middle: immature nuts from peanut and ugly nuts. Same
	orchard as Nonpareil from 5A.

Upper: immature nuts from typical "bull" Carmel. Different origin than those in middle row.

- C. Normal and "bull" Fritz at maturity
- D. Upper: immature nut of "bull". Normal: immature nut of normal.
- E. In-shell nuts of normal and "bull" Fritz.



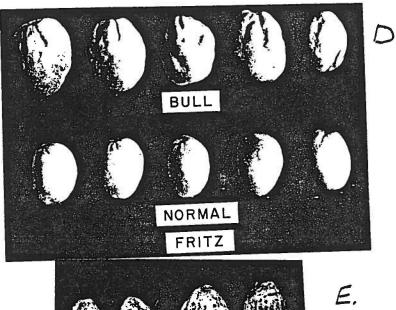
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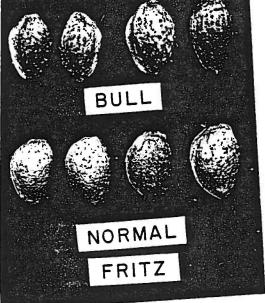
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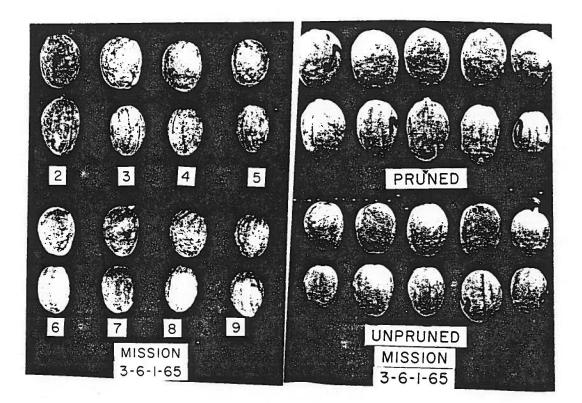
Fig.5



FRITZ







- Fig. 6. Normal variation of Mission immature nuts as a result of age and pruning.
  - A. Representative nuts of trees of different ages from 2 to 9 years of age. Note larger sizes and slightly "ugly" look of nuts on 2 and 3 year old trees.
  - B. Comparison of immature nuts on pruned trees and unpruned trees. Note larger nuts on pruned trees.

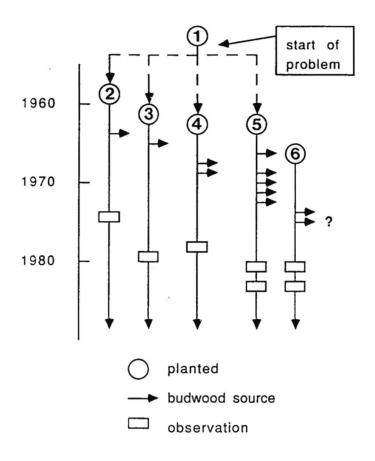


Figure 7. Source-pedigree analysis of budwood sources of Nursery A. Numbers refer to different budwood orchards. Arrows are the years used for budwood production. See Table 14 for description of orchards.

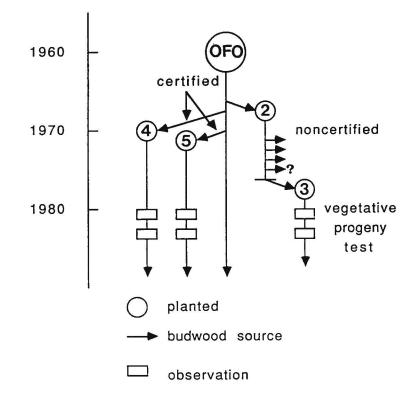
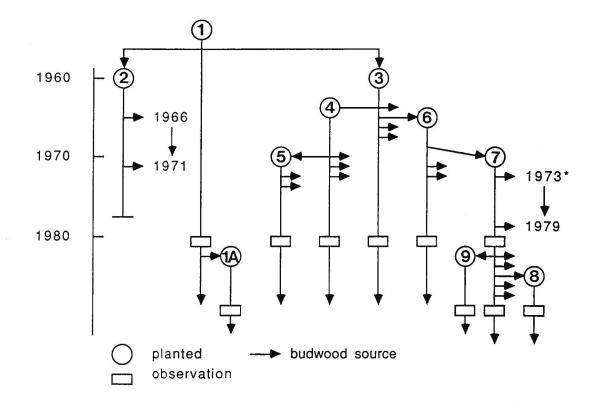
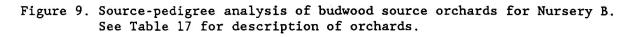


Figure 8. Use of Source-clone Mission 3-6-1-65 to produce certified and uncertified nursery material from Nursery A. Budwood was obtained from FPMS Foundation Orchard (1) in 1968 to produce a scion orchard (2) from which uncertified nursery trees were produced during three or four years. Question of whether block contained "bull" trees solved in negative by "vegetative progeny" test (3). In meantime a limited number of certified trees were produced in 1970 and 1971 to produce non-bull trees (4,5).



\*1977 excluded



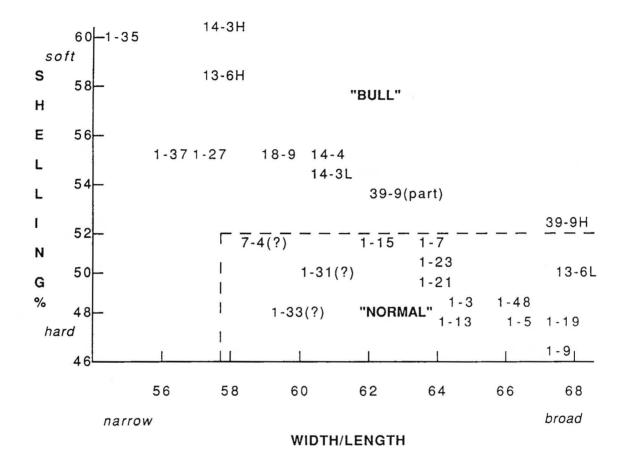


Figure 10. Nut distribution of SO-3 in relation to shelling percentage and shape as a measure of "bull" characteristics. Each number represents a nut sample from a separate tree. Samples collected in 1980.

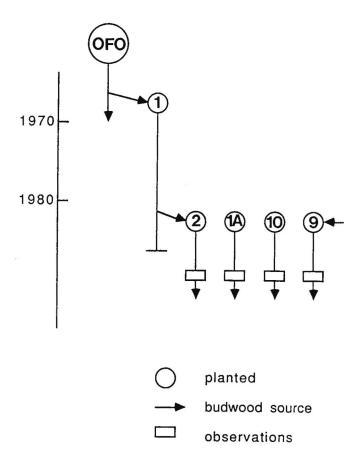


Figure 11. Selection and vegetative progeny testing of source-clones of Nursery B. FSC-1 is Mission 3-6-1-65 trees at the Grayson Road scion orchard. FSC-2 is test block established in 1982. SC-1A is a source clone started from SO-1, Nursery B. SC-10 is a new source developed by Nursery B. PO-9 is the progeny of SO-7.

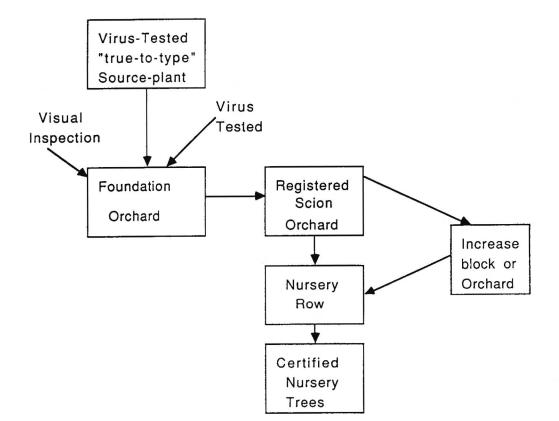
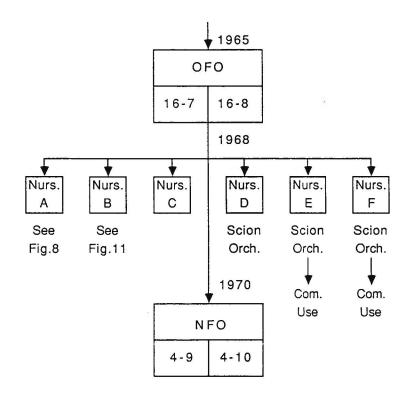
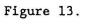


Figure 12. Distribution of source-clones from Foundation Plant Materials Service, UCD, for the production of "virus-tested" nursery stock to meet regulation of Registration and Certification.





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Distribution of Mission 3-6-1-65 to commercial nurseries from 1968.

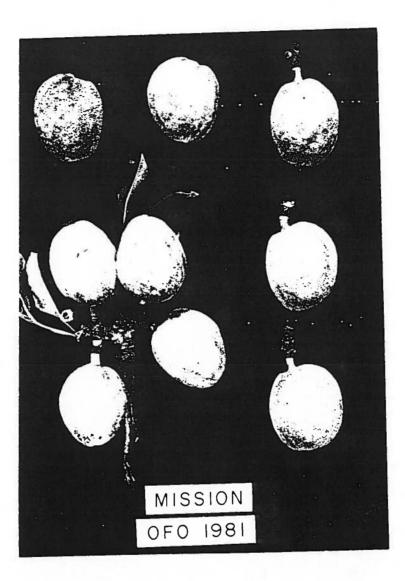
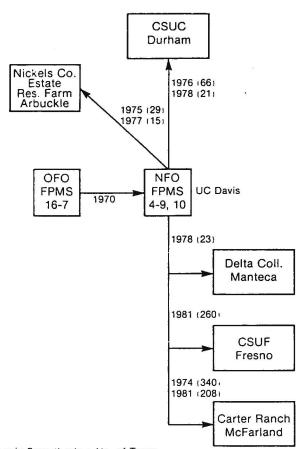


Figure 14.

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Nuts collected from Registered source tree in Old Foundation Orchard in 1981. Shows typical small, round, hardshelled nuts typical for Mission. Distribution of Mission Clone 3-6-1-65: RVT Progeny Tests

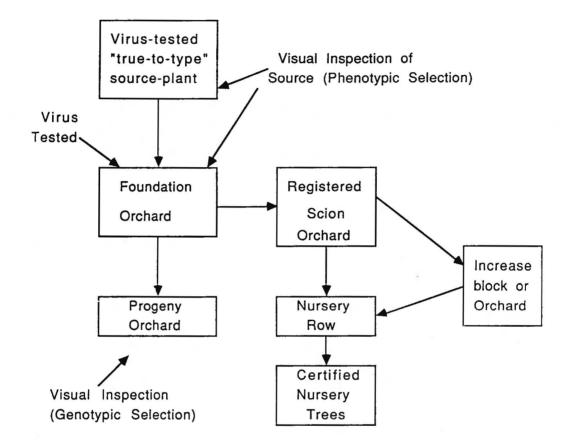


Number in Parenthesis = No. of Trees

Figure 15.

C

Distribution of Mission 3-6-1-65 for vegetative progeny testing.



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Figure 16. Addition of a "genotypic selection" step in the selection and distribution process for source-clones in the production of "genetically tested" nursery stock.

# Going Crazy

Crazy Top in Carmels seems to be getting worse, but nurserymen say there is no reason to panic

lat

Nurseryman Joel Hall examines rough, "alligator" bark on a two-year-old Carmel with noninfectious bud failure, commonly known as crazy top.

#### by Dan Campbell

ike a schoolyard bully who has to be faced down, the almond industry needs to stand firm and fight the genetic tree disorder known as "crazy top," according to Modesto-area nurserymen Joel Hall and Robert Woolley.

Crazy top has already "bullied" several other premier almond varieties into an early grave, and is now locked in combat with the popular Carmel variety. But Carmel won't go down without a fight. With over 49,000 acres of Carmels around the state, it ranks as the second most popular almond variety. Carmel trees are prized for their high production, ability to pollinate Nonpareil, worm resistance and quality almonds.

Whether crazy top — the common name for noninfectious bud failure — will be able to bring down the curtain on Carmel is currently a hot topic of debate in the almond industry. While some farm advisors are telling growers to phase out Carmels, Hall and Woolley say it's too soon to write off a good producer like Carmel.

Crazy top causes the growth of bare, elongated shoots that leave trees looking like they're stuck in a no-man's land between winter dormancy and summer growth cycles. It can remain relatively isolated, or rapidly engulf most of the tree. Once considered to be a problem primarily only in the south valley, bud failure is showing up with greater frequency and intensity in all parts of the state. Worse, it's striking younger trees than it has in the past, now showing up in second leaf trees in some orchards.

That trend has left some growers shaking their heads and muttering that Carmel "will be the next Merced," a reference to the way the once-popular Merced variety was made obsolete by crazy top. The Merced variety was itself a replacement for Jordanolo, another variety "done in" by crazy top (see related story).

Noninfectious bud failure was also once an extremely serious problem in Nonpareil. While it is still found fairly often in older Nonpareils, the nursery industry was able to select budwood in such a manner that crazy top is now a much smaller problem in Nonpareil than it was 25 years ago.

#### A treadmill problem?

To keep jumping from one new Nonpareil pollinator to another is like running on a treadmill, Hall and Woolley feel. Better to weed the problem out of Carmels, as occurred in Nonpareils, than to keep leaping into the dark and trusting to some new, unproven hope, they say.

But it won't be easy. Crazy top in Carmels is a problem of such magnitude that it will take a concerted effort by the entire almond industry to conquer it.

"It's not just a nursery problem, it's an almond industry problem that should be addressed by the entire industry," says Hall. "Everyone needs to contribute to finding a solution, right down to the farmer."



What's wrong with this picture? Nothing if you're more interested in growing fishing poles than almonds, courtesy of crazy top.

Despite the current problems, Carmel still has a lot going for it, the nurserymen say.

"I'd hate to see it thrown out the window over this. Bud failure is obviously a very big minus against it, but the industry needs Carmel," says Hall. "It's very productive and has made a name for itself. It took the industry years to build that recognition and demand."

"The basic message I'd like to get out to growers is not to panic over crazy top," Woolley adds. "In most cases there's no need to pull out all your Carmels. And Carmel should still be considered as a viable choice when planting new orchards. That's not to say that we have a crystal ball and can predict that Carmel will still be a standard industry variety in 20 years. But at this time, there's no reason to panic."

#### **Comparing Carmel to Merced**

"Many people are saying it looks like the Carmel is going to go the same way the Merced went," Woolley continues. "But there are important differences. At its worst, Merced shows a much higher percentage of crazy top with more severe symptoms than you find in Carmel. And Merced was never as widely planted as Carmel." With time, it is quite possible crazy top problems in Merced could have been reduced, he says.

The industry should continue to plant Carmel, Hall agrees. "I don't believe there is a nut on the market that can replace Carmel for good production and pollinating. Like Robert says, we probably could have rouged out (eliminated) the problem in Merced if the Carmel hadn't come along. We have got to give the Carmel a chance to cure itself."

Both nurserymen say they get calls from growers who are knowledgeable about most almond issues, but whom are obviously confused about crazy top. For starters, Woolley says it's easy to mistake crazy top for other problems, such as nutritional disorders and infectious bud failure, which is a viral disease with similar symptoms. Consult an expert before concluding that you have noninfectious bud failure, they urge.

"I don't think we really have a good handle on just how widespread noninfectious bud failure is in Carmel," Woolley says. "If you got 10 people in a room, you would wind up with at least a half dozen different estimates of how widespread it is." Based on conversations with other nurserymen, Woolley says infection rates seem to vary from a low of 2 percent to a high of 25 percent.

However, Merced County Farm Advisor Lonnie Hendricks recently reported that he has seen some isolated cases where 30 to 40 percent of the Carmels are affected. Hendricks blames heat from 1988, water stress and the heavy 1988 crop for bringing on the symptoms. He urged growers earlier this summer to tag and replace seriously infected trees.

Kern County Farm Advisor Mario Viveros has also urged growers to remove trees made nonproductive by crazy top. He compares the situation to that faced by a dairyman who can stay competitive only if he culls non-productive cows.

#### Replacing Carmel with Carmel

In order to avoid variety-mix problems at harvest, Viveros says the most logical variety to replant Carmels with is Carmel. For growers seeking an alternative variety in which crazy top has yet to be observed, he suggests Sonora.

"I say it is most logical to replant with Carmel again because handlers hate it when you mix your varieties, and no other variety that I know of will harvest with Carmel," says Viveros.

The highest incidence of bud failure Viveros has seen in Kern County is 20 percent. "Therefore, if you replant with Carmel, at worst the odds are one-in-four or one-in-five that you'll get bud failure again. So the odds are in the growers' favor when he replants with Carmel. He also eliminates harvesting problems and has a proven Nonpareil pollinator."

Viveros says many growers in Kern County are still planting new orchards with Carmel. "I don't have any problem with that, because Carmel has qualities I like and which are needed here. But I do think it's a mistake to continue planting 50 percent Carmel," says Viveros. He recommends planting new orchards with no more than 25 percent Carmel. "In the past, we've even had some growers plant two-thirds Carmel to one-third Nonpareil. That's asking for trouble."

"It's fine to plant up to 25 percent Carmel, then fight bud failure by replacing a tree with another Carmel as soon as bud failure is de-



Crazy top often starts in the crown of trees and spreads downward. How bad it gets is usually determined by the age of the tree when symptoms begin to appear.

tected," Viveros says. "Bud failure will be with us for a long time to come, and there's no guarantee that other varieties that have yet to show it won't start developing it. Any variety with Nonpareil "blood" in it has potential for bud failure. We just have to manage it."

#### Survey needed

Woolley says he hopes the Almond Board or University of California will undertake a thorough survey to determine just how many Carmels are infected with bud failure.

"If the range of infection is between 5 and 10 percent, we can definitely live with the problem," says Woolley. "Infected trees may still be producing some nuts. And the advantages of Carmels over other varieties will overcome losses to bud failure."

Even with a 20 percent infection rate, those trees — on average — will still give 50 percent of a normal crop, and probably 60 to 70 percent with good management, Hall says.

Grafting can be used to save a "crazy tree," but Hall and Woolley say that method is fraught with drawbacks. "Grafting sounds great because you get a tree back much faster than with replanting. But commercial orchardists have a tough time keeping up with all the follow up work needed to establish a healthy grafted tree," says Hall.

More often than not, grafting jobs are ruined by improper follow-up work, they say. Grafts are very susceptible to infestation by a number of different borer-type insects.

Viveros says he considers grafting a valuable tool for fighting bud failure, yet he has only one grower using grafting on a large scale. He thinks more Kern County almond growers will try it in the future.

"The Merced trees this grower topworked seven years ago are now full of beautiful growth. So even on Merceds, you can manage bud failure." However, Viveros says it "would be crazy" to plant new Merceds because of all the other problems associated with them.

Hall says growers must calculate their losses before deciding to replant an older tree which still is producing. "Most affected trees are not non-productive, they're lessproductive. That's an important difference. Consider how much production you will lose if you pull out a tree and replant," says Hall.

"Growers need to understand that different standards should be used in deciding how to react to bud failure based on a tree's age," says Hall.

#### Standards for replanting

As a rule of thumb, Hall's advice is to remove and replant trees if crazy top symptoms appear before the fourth leaf. The fifth and sixth years are a gray area. You could go either way depending on your situation. From the sixth leaf on, he says he wouldn't pull a tree if it still produces 50 percent or more of a crop. "By that point you've already reaped the early production, and even with only 50 percent of a crop for the next five years, you may be better off than with the losses you would suffer by pulling the tree and starting over," Hall says.

"When you replant, you can't treat the seedlings like 80 percent of the other trees in an orchard. Replants need to be watered, fertilized and pruned differently. Yet there are guys who replant the same hole every year for 10 years without ever correcting the problem."

Viveros agrees with Hall's replanting strategy. "If you see bud failure before the fourth year, there's no question that you'll be money ahead by eliminating that tree. After a tree matures, say sixth through ninth leaf, your action will depend on the severity of the infestation," Viveros says. "If you're still harvesting 50 percent or more, you'll probably want to keep that tree. But if you're

### "It's not just a nursery problem, it's an almond industry problem..."

- Joel Hall

much under 50 percent, it's time to remove that tree."

One suggestion has been made to fight crazy top by planting two-year-old instead of one-year-old seedlings. The most rapid shoot growth would then occur at a cooler time of year, and thus be less susceptible to the heat stress which brings on the disease. "If the entire nursery industry would convert to two-year trees, that might be practical," says Woolley. "But even then it would be difficult, because few growers know what they will be planting a year and a half in advance."

#### Alternative varieties

What are the best alternatives to Carmel for those growers who decide the risks of crazy top are just too great?

"Sonora looks good, but has certainly not been spectacular in trials, in large part because of a tendency for alternate bearing," says Woolley. "There are old standards such as Price and Fritz. But Price has a reputation for alternate bearing and mediocre tonnage over the long haul when compared to Carmel, Nonpareil and Butte."

Other possible alternative varieties he mentions include Wood Colony and Aldrich. Later blooming hard-shell alternatives include Padre, Ruby and Butte. "Butte is a strong producer, but when planted with Nonpareil it presents a risk because of the late bloom," says Woolley.

"And there's no guarantee that these new varieties won't eventually go crazy as well," Hall says. "This could turn into a Padre or Sonora problem down the line. We need to find out what causes it and how to stop it." Pull the rug on Carmel today, and you could be replacing it with another falling domino, he says.

"I love Don Rough's (a retired San Joaquin County farm advisor) old comment: If you're going to make a bet on a new variety, bet against it. More often than not, you'll win the bet," says Woolley.

Are they still selling many Carmels?

"A couple years ago I sold more Carmel than any other variety. But Nonpareil is No. 1 again now," says Woolley. "We haven't seen a wholesale abandonment of Carmel by any means. But it's a very emotional issue right now."

"Remember, trees aren't sacred," says Viveros. "You can replant them or topwork them. The only thing sacred in this business is your pocketbook."

Campbell is editor of Almond Facts.

## Bud failure haunts new varieties

Study the history of new almond varieties in California, and you'll also wind up studying the history of noninfectious bud failure. Unfortunately for the almond industry, the two often seem to go together like a horse and an unwelcome carriage.

In the first few decades after the turn of the century, Peerless and Nonpareil almonds were occasionally infected with bud failure, but on a fairly minor scale. "It wasn't widespread enough to cause much worry," says Dale Kester of the University of California, Davis. Kester has been wrestling with the genetic disorder growers call crazy top for most of his career.

When the USDA and UC started almond breeding programs in the 1920s and 1930s, the most promising new Nonpareil pollinators developed were the Jordanolo and Harpariel varieties. "They were tested extensively, planted by nurseries and then widely planted by commercial growers in the 1940s," says Kester. When Jordanolo and Harpariel reached

When Jordanolo and Harpariel reached bearing age, the time bomb exploded. "They started coming down with bud failure. When I went to work for the university in 1951, it was epidemic. Eventually it literally wiped out those varieties," says Kester.

Researchers knew crazy top wasn't caused by a virus because you could graft over an infected tree and it wouldn't spread to the scion. It was caused by a genetic disorder, and the early work Kester did in the 1950s helped show it was inherited.

After growers stopped planting Jordanolo and Harpariel, concerns about crazy top subsided. But in the mid-1950s, the Merced - the next "great new Nonpareil pollinator" - was released. By the time Merceds were coming into prime bearing age in the mid-1960s, they were showing severe symptoms of crazy top. History had repeated itself, and, as had occurred with the Jordanolo before it, the Merced variety was made obsolete by bud failure. The Merced also had problems with gum, worms and was hard to knock. The arrival of a hot new prospect - the Carmel variety - also contributed to the Merced's demise.

Carmel had originated as a chance seedling in Le Grand, where the original tree still stands. In the 1970s, the first commercial orchards were planted with Carmels. By 1980, bud failure was observed in sixth-leaf Carmels in Kern County. Now some growers fear history is repeating itself again as bud failure problems intensify in Carmels.

About the same time Merceds were coming down with bud failure, there was also an explosion of crazy top in Nonpareils in Kern County, where they had been heavily planted and pushed hard to bring them into early bearing. But for the most part, Kester says, crazy top is under control in Nonpareil.

A central question Kester and fellow UC researchers are grappling with is the relationship of budwood source, location, weather and orchard management in bringing out the latent susceptibility for bud failure, which appears to exist in all almond trees (although it hasn't yet been seen in some varieties).

To see if location plays a part, Kester took single source budwood material which had been propagated at the same nursery location, and planted it in eight locations around the state. That experiment proved that the rate of development was proportional to the amount of heat the trees were exposed to over time, accounting for why bud failure seemed to intensify progressively as one moved south.

"Studies on crazy top pattern development show that the incidence of bud failure in any one year is directly related to amount of heat in the prior year," says Kester.

Over the last five years, bud failure has become apparent in almost every nursery source of Carmel. The university is now developing a pedigree for Carmel, following it through the budwood sources to map how it has been spreading.

"In some orchards, Carmel only shows 3 to 5 percent infection. In others, 20 percent or higher has been observed. What's really upset people this year is the appearance of crazy top in second leaf trees," says Kester.

To understand how crazy top is triggered, we need to consider the seasonal growth pattern of an almond tree. A tree normally experiences the most rapid growth in April and May, then switches over to bud scale formation in June. In July a tree goes into a summer dormancy period. This is a biological necessity in Central California to protect new growth from the intense heat of summer. In August and September, flower bud growth is being initiated and the tree is making the transition to it's winter rest period. From October through January, the tree is in a winter rest period. Blooms emerge in February. Active growth begins again in March.

Bud failure appears to short circuit this growth cycle. Biochemistry studies show

that almond trees with crazy top don't go dormant in July, exposing new shoots to heat damage. "There's something wrong with the mechanism that triggers dormancy in affected trees," Kester says. Severe moisture stress also appears to be able to bring on symptoms. Frost has been cited as a cause, but Kester says that's fairly unlikely.

"Genetics, location, heat stress, management, moisture stress — all impact when symptoms appear. More vigorous trees are more susceptible, because it goes back to the amount of growth during the year." That's why orchards in hot areas that are pushed harder seem to succumb the fastest. Stress accumulates from one generation to the next and brings symptoms on sooner in progeny.

"If can you prevent crazy top over the first five or six years, you usually won't have that much to worry about. The most severe expressed symptoms show up earliest," says Kester.

He has developed tree diagrams showing how crazy top symptoms that occur in the first few years of a tree's life — when structural growth occurs — will probably be engulfed in the "mule tail" branches and rough bark which typify severe cases of bud failure. If no symptoms appear until the sixth year or later, bud failure is more likely to remain localized in smaller sections of the tree.

Kester says growers faced with bud failure have only three choices: live with it, remove the infected trees, or topwork infected trees with grafts. Topworking is useful in orchards not more than four to six years old but requires a lot of attention for success.

Pruning does not eliminate crazy top, because new growth again shows symptoms.

Because bud failure is inherent in Nonpareil, many new varieties now being developed also run the risk of inheriting bud failure potential, since most have Nonpareil as one of their parents.

Ongoing experiments are being conducted to find bud source material free of crazy top. "If we can make single tree selections, then maintain individual source trees and take progeny down into Kern County and see which ones have the lowest bud failure susceptibility, then we hope to be able to maintain those trees as a foundation orchard," Kester says.

But there are no guarantees of success. "It may be that Carmel is just too susceptible," Kester says. "Only time will tell."