December 20, 19[illegible]

Project No. 79-M6
Project Leader: D. E. Kester, Department of Pomology
Cooperators: R. M. Asay, L. Liu (also see listed in text)

Annual Report

Tree Research: A. Noninfectious bud-failure

Objectives: The overall objectives are a) to modify BF expression in orchards, b) to clarify BF-resistant plants in BF-susceptible varieties, and c) to develop BF-immune varieties.

Interpretive Summary:

The concept of classes of propagation stock has been developed utilizing visual symptoms and vegetative progeny tests to separate among them. As applied to BF material, class I refers to source trees without visible symptoms and which produce no BF vegetative offspring under specified conditions and within specified time periods. Class II refers to source trees without visible symptoms but which produce BF in vegetative progeny trees under specific condition in specific time periods. Class III refers to source trees with visible symptoms and which produce BF offspring under specific conditions.

Selection has been made for Nonpareil clones corresponding to each of these classes and these are being used in research. Nine apparently Class I clones have been selected which have also been virus-tested. Vegetative progeny trees of these clones are now being compared for yield and performance in the RVT trials begun in 1974 along with clonal selections of Peerless, Ne Plus Ultra and Mission. In general, all clones of a variety were similar in performance but this year some individual clones consistently have produced somewhat higher yields in the 3 plots. More data is required to confirm this.

Comparisons have been made of vegetative progeny trees grown at Winters originating from Nonpareil and Jordanolo trees, normal (symptomless) and BF, following six annual cycles of repropagation and grown either at low (75°/65°F) or high (100°/86°F) temperatures. These were planted in Winters in 1977. Results show cool temperatures masked BF symptoms but did not reverse them. High temperature killed out the BF clones. Cool temperatures maintained normal condition, but high temperature exposure during the cycles resulted in gradual shift to BF.

Tests are beginning to duplicate orchard vegetative progeny tests with clones of each of the 3 classes utilizing in vitro shoot tip cultures in test tubes as a method of "genetic indexing".

Seedling progeny tests procedures have been previously described where Nonpareil, Jordanolo and other almond varieties crossed to an early blooming peach produced 50% BF offspring in 2 years. In contrast, Mission and certain late blooming almonds have not produced BF offspring. First results this spring from tests using a late blooming peach with Nonpareil and Jordanolo also showed no transmission and supports the concept that a relationship between earliness of bloom (chilling requirement) and sensitivity to BF exists.

Tree Research: A. Noninfectious bud-failure (continued)

The study of seasonal changes in vegetative buds on normal and BF plants confirmed previous observations that vegetative buds of BF plants differ physiologically from buds of normal plants and do not become as dormant in fall and winter. The distinction between flower buds and shoot buds by bud size can be made by the middle to the end of August and coincides with the apparent sensitive period for symptom development. Less numbers of flower buds develop on BF shoots.

Normal and BF Nonpareil trees grown in growth chambers at temperatures of about 100°F required nearly 3 weeks of exposure before symptoms could be produced. It appeared that during these 3 weeks the buds became less dormant and evidently more sensitive. Class II plants were not affected in 5 weeks under the conditions of this test. Buds of Harpareil required only 7-10 days to develop bud failure.

In parallel studies (Dr. S. Weinbaum and Z. Even-Chen) the stomates of bud failure plants differed from those of normal plants in their response to increasing temperature. In normal plants, the stomates were found to open as ambient temperature increased to 100°F. Stomates on BF plants opened less efficiently and the leaf temperatures were found to actually be about 5°F warmer than cooling plants at a given air temperature.

Overhead sprinkling for cooling BF plants was studied at West Side Field Station (Fresno Co.) with Dr. Brewer (KFS) and L. Brown, (Coop. Ext., Kings Co.). Temperatures were measured in various parts of tree, but data is not available at this time. However, high salt scorch on the foliage of sprinkled trees indicate that the procedure has hazards even if effective for reducing temperature.

Tree Research: B. Variety evaluation

Project Leader: D. E. Kester
Cooperators: R. M. Asay (Pomology), W. Micke (Extension Specialist), Jim Doyle (KFS), D. Rough, T. Aldrich, M. Viveros (Farm Advisors), R. Baldie (CSU, Chico), Delta College

<u>Objectives</u>:

1. To coordinate management and evaluation of selection block and commercial material in Regional Variety Trial (RVT) orchards.

2. To evaluate new germplasm materials with the <u>thin stone-shelled</u> character for NOW resistance and to determine its inheritance.

Tree Research: B. Variety evaluation (continued)

3. To continue to study cross-pollination requirements as needed.

<u>Interpretive Summary</u>:

Comprehensive yield data were obtained in 3 RVT plots in cooperation with cooperators at each of the locations (Kern, Arbuckle, Chico). These are beginning to show trends in variety comparisons. Other information in regard to bloom and pollination have been obtained pointing toward need to maximize cross-pollination. Comparative quantitative data on nut characteristics as well as other tree characteristics were obtained. Yield depends on both nut size and number and preliminary examination of factors affecting each was started.

A comprehensive <u>variety evaluation schedule</u> was developed for comparing varieties in the following four categories: 1) kernel characteristics - raw product, 2) kernel characteristics - processed product, 3) tree characteristics, and 4) tree resistances. Evaluation scales of 1-10 or 1-5 are established for 40 individual traits using the 5 varieties - Nonpareil, Merced, Mission, Peerless, and Ne Plus Ultra as standards. Summations of evaluations give a <u>selection index</u> for overall value and for separate categories A publication of the method is in preparation. A second publication evaluates the 5 standards and a second group of 6: Butte, Carmel, Price, Fritz, Ruby and Thompson. The process will eventually extend to other varieties currently being grown or under test. These selection indices provide comparative scores which, when supplemented by measurement data from orchard tests, should provide more comprehensive information on variety selection than we have previously had. This project has been a joint effort of project personnel and the technical staff of Almond Board with contributions by many industry and Cooperative Extension.

Last year we reported that various hybrids we have previously produced with wild almond species have individuals producing <u>thin stone shells</u> with moderate shelling percentages. This characteristic is potentially valuable for NOW resistance. Among the interspecies hybrids are additional characters as late bloom, self-fertility, compact growth, etc. In addition, Dr. Soderstrom has measured hull resistance in some of these materials. Crosses were made this spring to study inheritance of this shell character and to incorporate it with the other characters listed.

Cross-pollination requirements have been determined among various almond varieties. We can now list the following incompatibility groups:

Tree Research: B. Variety evaluation (continued)

Group 1. Nonpareil, IXL, Profuse, Jeffreys, Tardy Nonpareil
Group 2. Mission, Ballico
Group 3. Merced, Price, Norman, Ne Plus Ultra, Ripon (?)
Group 4. Thompson, Harvey, Granada, Robson
Group 5. Sel. 2-62, Sel. 2-71, Carmel (?)

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COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
DEPARTMENT OF POMOLOGY

DAVIS, CALIFORNIA 95616

February 6, 1980

Dale Morrison
Almond Board of California
P.O. Box 15920
Sacramento, California 95813

Dear Dale:

I am sending over a continuation of my annual report. I still will plan to add a short section on Part B - Variety Evaluation.

Sincerely,

Dale E. Kester
Professor of Pomology

DEK/gr
Enclosure

Tree Research: A. Noninfectious bud-failure

Sub-Project I. Physiology of bud-failure.

Expt. 1. Seasonal bud development in BF and normal buds.

Expt. 2. Affect of location and irrigation treatment on bud-failure (correspond to points 1 and 2 in plans 1979).

Procedure. The same general procedure as in 1978 was followed involving shoot collections every 2 weeks beginning in May and continuing through December and January. Data included bud sizes, Petri dish tests, and evidence of shoot tip necrosis. The plots involved normal and BF plants at Davis and Winters. The blocks of BF trees at Winters and Davis were divided into 3 subplots; A. irrigated through season; B. non-irrigated and; C. 1 irrigation in late July. Soil moisture was monitored with a Neutron probe. Yield records were obtained.

Results. Not all of the tests of the seasonal curve has been completed since these will continue through January or until the dormant season is over. Likewise, the comparison from shoot samples measuring BF symptoms collected in December is not yet complete. Consequently details of experiments will not be presented since a publication is being planned. In general the results reported in 1978 still hold.

Expt. 3. Temperature patterns in orchards and modification by overhead sprinkling (with Dr. Robt. Brewer, KFS and Lyndon Brown Kings Co. Farm Advisors).

Procedure. Mature trees were subjected to overhead sprinkling during July and early August. Thermocouples were attached to plant parts and other areas in orchard to establish temperature patterns.

Results. The work was carried out largely by Dr. Brewer and the data is still being analyzed by him. However, observations made during August and September showed severe leaf scorch (sodium or chloride?), hull rot (on Jordanolo) and defoliation. Trees had been under considerable moisture stress prior to harvest. However, this experience shows that the practice of overhead sprinkling could be hazardous even if temperature is controlled.

Expt. 4. Effects of high temperatures to BF development in container grown plants in growth chambers.

Procedure. Almond and peach seedlings growing in 1 gallon containers were budded with Nonpareil and Harpareil trees with and without BF. These were propagated in June, grown in the lathhouse and greenhouse until October. In a first experiment, plants were placed into growth chambers at high temperatures (about 100^{o}F) and a group of normal and BF plants removed each week for 5 weeks. Shoots were removed, cut into single nodes and buds forced in Petri dish tests (described elsewhere). Later experiments modified this basic procedure to keep a constant number of plants in the growth chamber.

Results. Maintaining a constant temperature was found difficult until it was realized that plants were themselves acting as air conditioning agents and reduced the air temperature within the chamber. Temperature stress increased as plants were removed. Thereafter a constant number of plants was retained in the chamber. Also defoliation and mite infestation was a constant problem.

At the start of the experiment buds showed some degree of dormancy, more in normal plants. Dormancy decreased over a period of 3 weeks and, with defoliation, shoot sprouting often occurred in the chamber. Immediately after the 3rd week, buds on BF Nonpareil began to show bud-failure as shown in Petri dish tests. Buds of Harpareil, required 7-10 days to develop bud-failure under the conditions of the test.

Expt. 5. Stomatal resistance in BF plants (Dr. S. Weinbaum and Z. Even-Chen). A research paper is in preparation.

Sub-Project 2. Within-variety selection (clonal selection) for freedom from BF.

Separate clones of Nonpareil corresponding to the following 3 classes have been identified.

	Source Tree	Progeny Trees
Class I	No symptoms	No symptoms
Class II	No symptoms	Individuals develop symptoms under given set of time and temperature conditions
Class III	With symptoms	Individuals develop immediate symptoms

The basic test procedure is the vegetative progeny test and a given time-temperature regime must be specified.

Expt. 1.

Procedure. Tests for BF among various clones and source materials are continuing at West Side Field Station. Such trees were dehorned in spring 1978 to produce new cycles of shoot growth.

Results. Four virus-tested clones from FPMS have produced no BF plants at West Side Field Station since their 1972 planting. Variability among other sources, mostly commercial, show 0 to about 10% indicating that mixtures of trees of clone Classes I and II are present. Late dehorning changed the pattern of BF symptoms on shoots the following year, but the pattern returned to typical one the second year.

Expt. 2.

Procedure. Nine new Nonpareil clones, including the 3 in Expt. 1 have been planted in RVT Trials (see later in report) for comparisons of BF and yield.

Results. Nonpareil trees of these clones in the Kern plot (planted 1974) have produced no evidence of BF whereas same aged trees of Merced and Harvey have begun to develop symptoms this year. Yield has been obtained. Some yield differences among clones were found but we cannot confirm that this difference is real. Similar results have been obtained at Arbuckle (1975 and 1978 planting) and Durham (1976 planting). Some clones showed somewhat higher production in all 3 plots but more years data are needed to confirm this.

Expt. 3.

Procedure. This is a continuation of an experiment started in 1971 and previously reported (Kester, et al., 1976). Buds were taken from mature normal (Class I or Class II) Nonpareil and Jordanolo and BF (Class III) of the same varieties and used to propagate plants in the greenhouse. The resulting plants were subjected to high ($100^{o}/86^{o}F$) or low ($75^{o}/65^{o}F$) temperature for a series of 6 consecutive growth and repropagation cycles. Surviving trees were then planted in Winters in Spring 1977.

Results. By Spring 1979, the various trees which had now been growing in the same relatively hot location for 2 years at Winters, showed marked differences in BF as influenced by the treatments to which the trees had been exposed during the previous six growth cycles.

When grown at cool temperatures during the prior cycles symptoms on BF plants were masked but when the plants were grown out at Winters the same severe symptoms as at the beginning were shown (i.e., plants did not revert towards "normal"). When BF plants were grown at high temperatures in consecutive cycles all were killed out. When "normal" plants of Jordanolo and Nonpareil were recycled at high temperatures under the conditions of this experiments they gradually reverted to BF with time, but when recycled at the cool temperature the process was prevented (or delayed). Whether this latter change represents the developmental shift of Class II to Class III plants or the change from a Class I to Class II cannot be established.

Expt. 4. Shoot tip culture (Mrs. Linda Liu).

Procedure. The general techniques were described in 1978 report. Two kinds of experiments were followed. One was a study conducted by a graduate student (Angeliki Papadatos) to excise the growing tip of Nonpareil embryos and multiply these consecutively in culture. The purpose was to develop a method whereby seedling progeny tests in inheritance studies could be shifted from orchard to test tube (see next section). Shoot-tip cultures were made with Nonpareil (Class I and Class III clones) with buds collected in July and October with different amounts of chilling in storage.

Results. Some problems in technique involving contamination, media, and handling were found but may now be worked out. Multiplying shoots in culture without roots seemed to be a feasible program. Difficulty occurs with almond when attempting to get root formation. However, roots are not essential to this procedure.

Shoot-tips of Nonpareil did not grow well when collected in fall without sufficient chilling. With chilling, and proper media shoot-tips are now beginning to grow well to make miniature plants in culture.

Expt. 5. Callus and cell cultures.

Procedure. Callus of different clone classes of Nonpareil have been established according to previously described procedures. In some experiments, differential temperature treatments were applied.

Attempts have been made to establish cell suspension systems of almond both by "batch culture" (shaker) system or in a continuous flowing culture system. Equipment has been prepared for the latter which would allow the continuous exposure of almond cells to differential temperature during cell division and growth (Lou Fenton, graduate student).

Results. A long term growth curve experiments lasting more than 2 months confirmed a growth rate difference between normal and BF material. Callus from the normal started to grow more vigorously but gradually slowed down in rate. Callus from BF tissue started more slowly but gradually increased in rate. An attempt to show differential effects of high temperature damage was not successful.

Suspension cultures of almond have not yet been kept growing continuously after their initial start. These studies are continuing.

Sub-Project 3. Inheritance studies.

The program is based on the concept that BF tendencies is a genetic characteristic of individual varieties and these can be studied by seedling progeny tests involving either crosses to another almond or to a peach but different patterns of transmission are shown. Identification of BF offspring is made either by bud-failure symptoms, rough-bark symptoms or both. Earlier results have shown 50% transmission from Nonpareil and somewhat different amounts by other varieties. No transmission was made from Mission and some other late blooming peaches.

Procedure. Methods of making crosses and growing seedling plants were described in 1978 report. In 1979 crosses were made on unemasculated blossoms covered by a cheesecloth bag.

Results. Seedling of 1976 almond x peach crosses have been grown for 2 years in the orchard, results obtained in Spring 1979 and plants discarded this summer. These results are shown in Table 1. Again, Nonpareil of all sources produced approximately 50% transmission of BF offspring. The 3 varieties Milow, 5A3, and Vesta showed some transmission but at a lower rate than Nonpareil. Another group produced no BF offspring. Numbers were low or were late blooming.

1977 crosses are shown in Table 2 in which comparisons were made between effect of late blooming peach parent and early blooming peach parent. All progeny including Nonpareil and Jordanolo crossed to late (J.H. Hale) produced no BF offspring. Nonpareil, however, in crosses with an early blooming peach produced, 14 and 11% at first year, the same as in previous tests. All of the seedling plants from 40 A-17 peach were very early leafing indicating very short chilling. In contrast, all the seedling population with J.H. Hale were very late leafing indicating long chilling requirements. These results are consistent with our earlier hypothesis.

New crosses in 1979 were made with several objectives in mind. First, Group 1 continued some studies with BF transmission with peach. A second group utilized a genetic dwarf peach, first, to continue BF transmission studies and second to incorporate a recessive genetic dwarf gene into F_1 hybrid populations. F_1 will be normal size. We want to know if the BF factor shows up here. If so, we hope to screen these out as F_1's then study the segregation of "normal", self-fertile, genetic dwarf individuals in the next generation.

Group 3 and 4 involve the inheritance of a thin walled, stone-shell type (1-29) discovered previously. Group 5 involves crosses of a group of the same hybrid plants to a large size, high quality (5 A-20) almond selection. Group 6 includes crosses of productive almond with a group of very precocious, highly productive, self-fertile hardshelled complex hybrids involving almond, peach and *P. webbii*.

Group 7, is a series of crosses designed to study transmission of BF in relation to chilling requirement of peach. Titan is known to transmit about 50% BF F_1 hybrid offspring from earlier studies. The 4 peach varieties represents a range of chilling requirement from short to very long. This test should demonstrate the role of chilling background in the BF expression in hybrids.

Discussion. Results obtained to date show significant trends but the story is far from complete. First, we need more precise laboratory procedures to test for the presumed BF offspring in progeny. Evidence is that the character of RB, BF we are looking at in the field is being expressed very early and we expect to test out the test tube system of growing shoot tips of young seedlings and subjecting them to controlled high temperature very soon after germination. Furthermore we could follow individual clones in consecutive propagation in controlled tests to analyze the time required for development. Secondly, it seems that many individual varieties have a BF factor that segregates in seedling offspring but this occurs with both Class I or II clones of that variety. Thus, the phenomenon control that triggers BF to develop may be a separate phenomenon from that of the presence of the problem.

Thirdly, there is developing a relationship between the background chilling requirement of the variety and the ability to produce BF. We can only speculate at present what this means but we can direct research now to answering specific questions. The concept is particularly significant since it means we may be able to manipulate the genetic "background" and thus control BF both for experimental use and possibly practical use.

Table 1. Crosses made in 1976. Data in Spring 1978 and 1979. Each cross involves the almond variety shown crossed with 40 A-17 peach.

Almond Parent	Phenotype	No. of sdlgs.	% BF 1978	% BF 1979
GROUP 1. Nonpareil crosses				
761 4-49 Nonpareil	BF	100	38	55
762 5-49 "	"	96	26	44
763 1-1 "	Normal	81	25	29
764 Wells "	"	96	40	50
765 B4 "	"	52	31	46
766 B6 "	"	49	28	33
767 B18 "	"	79	27	46
All Nonpareil	"	55	31	44
GROUP 2. Other selections with BF transmission				
7613 Milow		23	10	22
7614 5A-3		54	10	30
7616 Vesta		45	17	32
GROUP 3. No transmission to date				
768 Mission		3	0	0
769 WEO F-7 1,2-15		7	0	0
7610 Merced		6	0	0
7611 Sel. 5-33		26	0	0
7612 Sel. 5-58		2	0	0
7615 3-24E		21	0	0
7617 2-62		17	0	0

Table 2. 1977 crosses. Results obtained in 1979.

♀parent		♂parent	No. of plants	% BF offspring
1. 771 J.H. Hale	x	Jordanolo	50	0
2. 772 "	x	BF Jordanolo	74	0
3. 773 "	x	Nonpareil (Wells)	50	0
4. 774 "	x	BF Nonpareil	50	0
5. 775 "	x	Mission	50	0
6. 776 Wells Nonp.	x	40 A-17	33	14
7. 777 BF Nonp.	x	"	42	11
8. 778 Mission	x	"	8	0
9. 779 Merced	x	"	41	0
10. 7710 Merced BF	x	"	51	2 ?
11. 7711 CP 5-58	x	"	28	0
12. 7712 CP 5-33	x	"	1	0
13. 7713 2-62	x	"	13	0
14. 7714 1-69	x	"	2	0

Table 3. 1978 crosses

			% set	No. of seeds
GROUP 1. Crosses with 40 A-17 peach to study BF transmission				
791 Butte	x	40 A-17	32.5	65
793 Carmel	x	"	28	44
795 Price	x	"	46.5	44
				No. 153
GROUP 2. Crosses with 40 A-17 peach to study BF transmission and to incorporate a genetic dwarf peach gene into hybrids				
792 Butte	x	Dwarf peach	15	21
794 Carmel	x	"	19	25
7921 Wells Nonp.	x	"	4	15
7922 BF Nonp.	x	"	29	104
7925 6 A-11	x	"	5	8
7926 5-58	x	"	14	80
7929 1-46	x	"	6.5	5
7930 2-17	x	"	-	3
7939 Titan	x	"	5	19
				No. 285
GROUP 3. Crosses of thin stone type 1-29 (Mission x *Prunus argentea*) with almond				
7927 Alm 5-58	x	1-29	34	100
7932 Alm 2-1E	x	1-29	31	100
				No. 200

Table 3. 1978 crosses (continued)

			% set	No. of seeds
GROUP 4. Crosses of 1-29 to other hybrid selections				
798 (Texas x hybrid B)				
1-13	x	1-29	2	6
7910 (Texas x P. tangutica)				
1-17	x	1-29	2	2
7912 (Texas x P. bucharica)				
1-18	x	1-29	11	12
7913 (Texas x P. webbi)				
1-19	x	1-29	43	54
7915 (Texas x P. webbi)				
1-21	x	1-29	35	100
7917 (Texas x P. webbi)				
1-23	x	1-29	36	100
7919 (Texas x P. argentea)				
1-28	x	1-29	1	1
				No. 275
GROUP 5. Crosses of hybrid selections with almond				
796 (Texas x Hybrid A)				
1-5	x	5 A-20	18	82
799 (Texas x Hybrid A)				
1-13	x	5 A-20	8	31
7911 (Texas x Hybrid A)				
1-18	x	Carrion	2	2
7914 (Texas x Hybrid A)				
1-19	x	5 A-20	34	34
7915 (Texas x Hybrid A)				
1-21	x	5 A-20	31	106
7918 (Texas x Hybrid A)				
1-23	x	5 A-20	37	100
7919 (Texas x Hybrid A)				
1-28	x	5 A-20	29	1
				No. 362

Table 3. 1978 crosses (continued)

			% set	No. of seeds
GROUP 6. Crosses of dwarfed, possible self-fertile of *P. webbii* x almond				
7933 Sel. 2-1	x	3-48	43	100
7934 Sel. 2-1	x	3-52	36	100
7935 Sel. 2-1	x	3-60	33	100
7923 Milow	x	3-60	62	100
7924 Milow	x	3-39	35	100
			No.	500
GROUP 7. Crosses of peaches with Titan for time of bloom study				
7936 Titan	x	40 A-17	5	24
7937 Titan	x	Springcrest	9	38
7938 Titan	x	Red Cal	5	16
7940 Titan	x	Late peach	5	34