# **Almond Board of California**  Correct Project Number: 95-M8

## **Annual Report - 1995**



# **Objectives:**

Develop replacement varieties for 'Nonpareil' and its pollenizers which possess self-fertility, improved disease and insect resistance, and a range of bloom times and maturities.

- A. Identify the most promising parental combinations resulting in self-fertility, high quality and yield, and later flowering period. Continue studies on the underlying control and inheritance of these traits.
- B. Develop crossing strategies that can consistently generate large progeny populations from crosses between selected parents regardless of weather conditions, labor availability, etc.
- C. Test genetic strategies for developing improved production consistency, including selffertility, and resistance to Bud-failure, Navel orangeworm and aflatoxin contamination, and other disease and insect problems.
- D. Develop rapid yet accurate evaluation guidelines for characterizing nut and tree quality, and yield potential, to allow rapid and efficient elimination of inferior seedlings from breeding populations.

### **Summary:**

Germplasm has been identified which shows good promise for stabilizing almond production while reducing grower and handler costs. Specific crossing-parent combinations are being pursued to promote the most rapid and cost-efficient breeding of this germplasm, including genes for self-compatibility and pest resistance, into high kernel and tree quality varieties adapted to the changing California conditions. Early field trials of progeny resulting from crosses incorporating the new germplasm into a Californian almond tree and nut type, support their ultimate vale to Californian crop improvement. Advanced selections now producing in regional trials have shown some of the best cropping potential to date, though further improvements in tree and kernel quality may be necessary for specific selections.

# A. Identify the most promising parental combinations resulting in self-fertility, high quality and yield, and later flowering period. Continue studies on the underlying control and inheritance of these traits.

The objective is to generate both a high quantity and high quality of almond seedlings. The high quantity or large progeny population size is necessary due to the overall improbability of obtaining an individual seedling possessing the large number of desirable tree and nut traits necessary for a new variety's success. The large size of progeny populations from controlled crosses also allows better genetic understanding of specific parental combinations [i.e. parent quality] which work best for targeted goals (for example, pollen self-compatibility, flower self-pollination and good nut size and tree yield), thus leading to improved program efficiency. Early crosses made in this breeding program (1990, 1991, & 1992) had been directed towards the testing of parents and progeny for Bud-failure potential. Almonds tested included established varieties (including the *Carmel* clones presently used by nurseries), advanced selections and promising breeding lines.

Information provided by the evaluation of progeny from these test-crosses have helped to identify low Bud-failure potential lines that are now used either directly by industry (as in Carmel source-clones), are under consideration for release as new varieties (13-1, 2-19E & 2-43W), and/or as parents for new crosses (Fig. 1 & Table 1). Subsequent crosses (1993 to present) have concentrated on bringing needed genetic improvements in cropping efficiency into a high quality nut adapted to Central Valley conditions. Priorities include the development of self-compatible and/or self-pollinating almonds to decrease the present crop vulnerability to cross-pollination problems at flowering, and disease and insect resistance to reduce grower and processor costs. Following an initial screening of over 300 almond breeding lines, 30 lines were selected as possessing the most



Figure 1. Kernel size comparison for self-compatible and selfincompatible breeding lines.

promise as parents for future crosses. Important attributes of these lines are summarized in Table 1. Their average kernel weight and self-compatibility response are presented in Fig. 1. All selfcompatible lines have smaller kernels than the *Nonpareil* standard. [An exception is seen in USDACP05-330P, though the large kernel observed in this line may largely be due to the very low crop loads obtained on these trees). The smaller size results from the very small kernel size typical of the wild sources of self-compatibility (wild almond, *Prunus webbii,* P. *mira,* P. *argentia*). Three to 4 generations of backcrossing to Californian almond types have improved both nut size and overall nut and

tree quality. Present understanding of the genetic control of nut size, however, suggests that continued rapid progress towards improved kernel size depends on the identification and utilization of lines having significantly larger kernel size while still retaining local adaptation. Three possible sources of very large  $(>1.5g)$  are being pursued: (a) progeny from crosses oflarger, European varieties to Californian almonds, (b) wild (roadside, riverbank, etc.) almonds in California, and  $\{c\}$ progeny from controlled crosses within the breeding program. The first progeny populations from crosses directed towards new



variety development (1993 crosses Figure 2. Distribution of high-cropping potential current-season lateral <sup>=</sup>1994 Progeny Block) will come branches in a selected breeding population. into production this (1996) season

and intensive selection for size, quality and high yield potential are planned. Other opportunities for stabilizing cropping potential have already been identified in novel vegetative growth and bearing habits (summarized in previous Annual Reports). One potentially useful bearing habit derived primarily from *Prunus webbii* lines is the ability to produce flower-bud bearing currentseason lateral branches on current season shoot growth. The distribution of the number of current-season laterals in a 1995 breeding population is shown in Fig. 2 with the performance of a *Nonpareil* tree of similarly maturity included for comparison. Most of the very high lateral bearing lines are only 1st or 2nd backcrosses from the wild sources and so of poor commercial quality. Many later generation backcrosses have achieved commercially acceptable levels of nut quality (as indicated by the extent of the label in Fig. 1). The best of these selections will be chosen over the next few years for further crosses and possibly larger field trials to determine yield advantage of this growth pattern.

# B. Develop crossing strategies that can consistently generate large progeny populations from crosses between selected parents regardless of weather conditions, labor availability, etc.

The major bottleneck to breeding progress is the size of the populations which we can manage. This is presently limited by (a) our ability to accurately analyze the large number of individual seedlings at important development times, and (b) our ability to generate large numbers of progeny from desired parents during the very limited and often stormy flowering period. Several crossing strategies are being developed to overcome the later limitation. Crossing results for the

1994 & 1995 crossing season are summarized in Fig. 3. The 1994 season was a very good one for crosses with over 10,000 viable Reserves seed recovered from controlled crosses. Even though approximately 40% of this seed was lost during the severe weather of winter, 1995, enough seed was produced to achieve our goal of 5,000 new seedlings planted in the field with additional seed held in reserve. The 1995 crossing season was very difficult due to poor weather prior to and following bloom, with a low final set. Poor flower fecundity was recognized in early in the crossing season such that an additional 10,000 crosses over 1994 levels were blocks, [where the seed parent is 1995 (poor crop year). isolated from potential pollinizers





so that only the pollen supplied to isolation block bee hives is effective in setting seed], were used to a greater extent as were controlled crosses where entire trees were caged and mini-bee-hives with desired donor pollen place inside at flowering. Both isolation blocks and caged trees allow large numbers of controlled crosses to be obtained at low costs. The number of different parental combinations is limited, however, resulting in continued dependance on controlled hand crosses for testing new crossing combinations. Approximately 5,000 seed recovered using these techniques is now being prepared for planting with 70-90% of resultant seedlings selected for field transplanting.

C. Test genetic strategies for developing improved production consistency, including selffertility, and resistance to Bud-failure, Navel orangeworm and aflatoxin contamination, and other disease and insect problems.

#### Self-compatibility

Most year-to-year variation in crop production appears due to differences in initial seed set, which in tum is believed due to differences in honey-bee cross-pollination efficiencies. A goal of the almond variety development program is the breeding of self-compatibility into future varieties,

thus allowing self-pollen to be effective for seed set. While selfcompatibility appears to be controlled by a single gene and relatively easily transferred to new lines, the ability to self-pollinate (self-transfer of pollen from anther to stigma without insect vectors) is much more complex both developmentally and genetically). Self-compatibility, however, could dramatically increase honey-bee crossing efficiencies since bees prefer to work the same tree and variety with little bee movement between varieties. Consequently, the great majority of pollen on bee vectors is self-pollen; the small quantity of cross-compatible pollen apparently originating from some pollen mixing within the hive. With the use of selfcompatible almond virtually all bee visits will successfully provide (self- )compatible pollen to the stigma. This increase pollinator efficiencies is



Figure 4. Proportion of total flowers setting crop on selected genotypes.

supported by preliminary 1995 findings that the ratio of seed set/total flowers on measured branches is higher in the four self-compatible advanced selections tested relative to the *Nonpareil*  standard under standard open-pollinated conditions (Fig.4). The highest set was obtained in the self-fruitful (self-compatible plus self-pollinating) line 25-75. Although this finding supports the potential of self-compatibility for increasing honey-bee pollination efficiencies, it is based on relatively few observations. Larger field trials are needed, particularly given our present lack of understanding of how cross-compatible pollen transfer actually occurs in almond orchards.

#### Resistance to Navel orangeworm and aflatoxin producing *Aspergillus* spp.

Previous work had identified resistance to aflatoxin producing *Aspergillus flavus* in the intact seed coat of almond. Breakdown of this resistance resulted from damage to the seedcoat between the time of hull-split and harvest primarily by navel orangeworm infestation of the kernel. Strategies for controlling *Aspergillus* infection have thus shifted to controlling the Navel orangeworm (NOW) 'vector'. Continuing research towards the preharvest control of aflatoxin contamination in almond is supported by a \$50,000 grant from the USDA. The search for genetic resistance to other fruit and foliar diseases is continuing in cooperation with Dr. Jim Adeskaveg (UC-Riverside), Dr. Beth Teviotdale (Kearney Ag. Center), and Dr. Noreen Mahoney (USDA, Albany, CA).

Previous work with NOW has identified differences in field susceptibility within the California germplasm due to differences in both shell seal and kernel composition. Controlled feeding trials have now been completed for NOW response to both kernel and hull tissue. Preliminary analysis of kernel data (Fig. 5) show

differences in the days for NOW development from 1st instar (egg hatch) to emergence from pupae of the adult moth, as well as proportion of eggs successfully developing to mature moths. *Merced* and *Ballico* continue to show a kernel based resistance expressed as an apparent suppression of NOW development leading to a longer development time. A notably reduced value for per -cent adult moth emergence is seen for *Peerless* though this finding cannot be compared to earlier field studies since the high shell seal eliminated most NOW access to the kernel. [These data also summarize only the first two of four replications so that different conclusions may result from final analysis of the complete data]. Controlled feeding studies on hull samples from the same varieties resulted in full development from egg to adult moth only on the relatively



**Figure** 5. Susceptibility to navel orangeworm for selected varieties following controlled infestations of kernel tissue.

fleshy hulls of *Jordanolo* and the Spanish variety *Tarragona,* with the per-cent emergence being <20% for both. Thus, while NOW infestation of hulls is observed in the field, such infestation may not normally lead to adult moth development unless access to the more nutritious kernel meat is achieved. Earlier field and lab studies had suggested a distinct antibiosis of *Mission* hulls compared to *Nonpareil.* Lab studies rearing NOW on insect diets supplemented with either *Mission* or *Nonpareil* hulls show a dramatic reduction in adult moth emergence on diets supplemented with *Mission* hulls following 112 days of rearing (Fig. 6). Again, only the first 2 of 8 reps have been analyzed at present and these findings may change, but they are in agreement with 1994 observations. Attempts to identify the



Figure 6. Suppression of navel orangeworm development on insect rearing media supplemented with Mission Hulls relative to Nonpareil hull tissue (preliminary data).

chemical constituents conferring this apparent *Mission* hull based antibiosis continue in cooperation with Drs. R. Plath and R. Teranishi (USDA, Albany, CA). Specific volatile compounds commonly found in mature almond hulls and kernels have been identified which appear to repel 1st instar

NOW larvae at concentrations  $(\sim 2\%)$ commonly found in some wild almond and almond relatives (Fig. 7). [Volatile were diluted in light mineral oil, thus pure mineral oil is used as a control. The different fractions are hull constituents purified by Dr. Terranishi]. The compounds linalool, carvomethenol, and gamma-decalactone appear to have the most promise as repellents and work to verify this finding and if confirmed, test against other almond pests is continuing in cooperation with Drs. Bruce Cambell and Doug Light (USDA, Albany, CA).



# D. Develop rapid yet accurate evaluation guidelines for characterizing nut and tree quality, and yield potential, to allow rapid and efficient elimination of inferior seedlings from breeding populations.

Preliminary guidelines for characterizing nut and tree quality have been organized (Table 2) with performance of several important almond varieties provided for reference. Major selectable components of yield have been identified as kernel weight, nuts/tree and shelling ratio.

Performance in these three categories is shown in Fig. 8 for the 6 advanced selections now in Regional Variety Trials. [Data is from the new Paramount RVT with performance of *Nonpareil*  and *Mission* included as references.] UCD advanced selection 13-1 showed the best overall yield of all varieties and selections tested in 1995. These high yields appear to be the result of a moderately good kernel size combined with a very high number of nuts/tree. Shelling ratio (crack-out) of all advanced selections were found to be between those for Nonpareil and Mission. Selections 2-19E, 2- 43W, 1-87, 1-102W, and 25-75 flowers at the Mission time or later. Selection 25-75 is the only self-fruitful selection in the RVT plantings yet showed one of the lowest yields as a result of a relatively small nut size combined with a low number of nuts/tree of avallable flowers (FIg. 1). Variety Trials. Results are from 3rd leaf trees



despite having a high per-cent set Figure 8. Crop characteristics for advanced selections in Regional

and final tree performance may change in response to bearing habit of the mature tree form. Selection 25-75 is also distinct in that it possesses a 'peach' growth habit with a relatively weak, weepy branching habit. The willowy habit is most pronounced in the sandy soils of the Manteca RVT. The heavier soils combined with judicious pruning at the Paramount and Chico RVT sites have produced trees with growth habit more compatible to present industry practices. Detailed tree and nut data is being collected in the present (1996) season for these advanced selections.

# **Relevant Publications for 1995-96.**

- 1. T. Gradziel and D. Kester. 1996. Variety Development. In, J. Coats, (ed.) Almond Production Manual. 6pp.
- 2. D. E. Kester and T. Gradziel. 1996. Almonds. In J. Janick and J.N. Moore (eds) Advances in Fruit Breeding (2nd ed.) 30pp.

Table 1. Summary of breeding parents used in almond variety improvement.







Source:Adapted from California Agriculture 34:7.

\* The higher the rating-on a scale of 1 to 3, 1 to 5, or 1 to 10-the better the variety's performance. t Estimated.

### UNIVERSITY OF CALIFORNIA, DAVIS

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COLLEGE OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES AGRICULTURAL EXPERIMENT STATION COOPERATIVE EXTENSION (916)752-0122 FAX: (916) 752-8502



April 1, 1996

Kandi Cruz Almond Board of California 1104 12th St. Modesto, CA 95354

Dear Kandi:

Enclosed find the Final Report for the 1995 projects:

Almond Variety Development Project No. 95-M8

Genetic Engineering of *Nonpareil* almond,

I'm sorry this is a bit late but the due date caught me by surprise, (I'm still thinking it is February), Contact me at the above address or Tel. (916) 752-1575 if additional information is desired.

Sincerely,

Tom Gradziel Assoc. Prof./Plant Breeder