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DAVIS, CALIFORNIA 95616

April 28, 2000

Chris Heintz, Research Director
Almond Board of California
1150 Ninth Street, Suite 1500
Modesto, CA 95354

Dear Chris:

Enclosed find the Annual Report for the **Almond Variety Development** project.

It is my understanding that this report may be placed on the ABC Web-site and I have tried to write the report to be more Web-compatible (less technical and more emphasis on application. [~~File copy sent earlier by E:MAIL~~]. If more data is desire do not hesitate to contact me).

File attached - Too Big to E:mail!

Sincerely,

Tom Gradziel

Tom Gradziel
Assoc. Professor/Geneticist

Project Number 99-TG-o0

Almond Board of California

Annual Report - 1999

Project Title: **Almond Variety Development**

Project Leader: Tom Gradziel

Cooperating Personnel: W. Micke, D.E. Kester, M.A. Thorpe, J. Adeskaveg, J. Connell and C. Walters

Location: Department of Pomology, University of California at Davis

Objectives:
Develop improved pollenizers for '*Nonpareil*', and develop the next generation of California almond varieties which possess self-compatibility, improved disease and insect resistance, and a range of bloom times and maturities.

Summary: Current trends in almond production practices will require future varieties to produce uniform and economically sustainable yields with reduced chemical inputs. Greater uniformity or year-to-year production consistency is being pursued through the improvement of initial crop-set at flowering as this has been shown to be the principal determinant of final crop yield. Short term goals to improve crop-set involve the development and release of improved pollenizers for the principal California variety, *Nonpareil*. Longer term goals involve the development of self-compatible and eventually self-fruitful *Nonpareil*-type varieties requiring fewer honeybee pollinators and amenable to single variety plantings. Reduced grower inputs are also being pursued through the development of varieties resistant to important insect pests and diseases. Breeding program achievements include the selection, testing, and release to the California industry of a low-Bud-Failure clonal source of the important *Nonpareil* pollenizer variety *Carmel;* the release of *Nickels*, a hybrid rootstock for almond with improved disease resistance; and the preparation for release of a productive and high quality pollenizer (field tested as UCD 13-1) for the crucial early *Nonpareil* bloom. Advanced self-compatible almond selections with good horticultural characteristics should be ready for initial grower testing within 5 years. A wide range of resistance sources for important almond diseases has been collected and is undergoing field testing. Rapid progress in breeding appropriate resistances into future varieties will depend on accurate grower and processor identification of the almond diseases where genetic resistance is essential (i.e. chemical/cultural control will not be economically viable in future California almond production practices).

Introduction.

A major challenge facing the California almond industry is the stabilization of crop production at a time of reduced petrochemical inputs. The last 15 years has seen a consistent increase in overall production, often based on increased plantings in more marginal production areas and the use of chemical pesticides to control insect and disease. This same period has witnessed sizable year-to-year fluctuations in the almond crop size (Fig. 1), which has frustrated orderly market expansion as well as strategic planning by growers and processors. As in previous years, statewide almond production correlates well with regional production in the UCD Regional Variety Trials [RVT] (Fig. 1). Experience at the RVTs has shown that the principle determinant of crop production is the level of cross-pollination and so flower set during the often stormy flowering season, and the level of associated blossom and foliar diseases (as summarized in previous reports). Thus, The principle objective of the breeding program has been the stabilization of production at profitable levels through the development of improved cross-pollination and the incorporation of improved pest resistance in new varieties and rootstocks. Improved cross-pollination is being achieved through the development of improved pollenizers particularly for the major variety *Nonpareil* and through the development of the next generation of almond varieties with self-compatibility (which should allow consistent set even in inclement weather conditions and with reduced honeybee pollenizers activity). Pest resistance is being pursued through the collection and characterization of genetic resistance, and its incorporation into new varieties. Genetic recombination has been achieved through traditional controlled crosses between selected parents as this strategy allows for the simultaneous selection for the multitude horticultural traits required for variety success. Recent advances in the new biotechnologies has been actively employed to develop molecular markers allowing the more efficient identification and so selection of genes controlling key traits. Although the almond breeding program is less than 10 years old, (extensive new crossings for variety development was initiated in the early 1990s following the retirement Professor Dale Kester), it has achieved consistent growth both in a number of progeny trees generated (Fig. 2) and in the wealth to of new germplasm incorporated. In 1999 over 8000 new

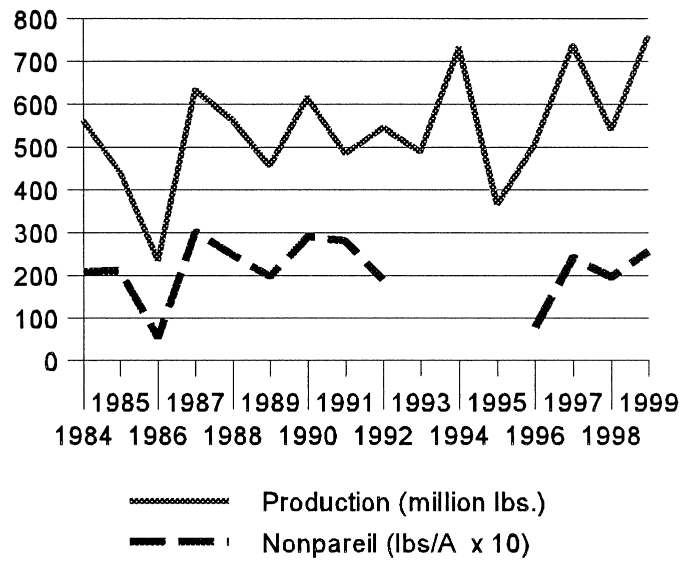

Figure 1. Fluctuations in California almond production from 1984 to present, with average yields for *Nonpareil* at the Manteca College RVT (1984-1992) and younger RVT at Kern Co. (1996-1999) provided for comparison.

seedlings from controlled crosses were generated from over 30 different parental combinations. The aggressive roguing of inferior seedling trees from these and earlier field plantings during the last year has kept the total field seedling population to a more manageable size of approximately 20,000 seedlings. Achievements include the patenting and release of the disease resistant Nickels rootstock; the identification, testing, and release of an improved, low Bud-Failure source of (the *Nonpareil* pollenizers variety) *Carmel*; the selection, testing and preparations for release of an improved pollenizers for early *Nonpareil* bloom (designated as UCD, 13-1 in field trials), and the identification, and initial characterization of new germplasm conferring pest resistance and self-compatibility.

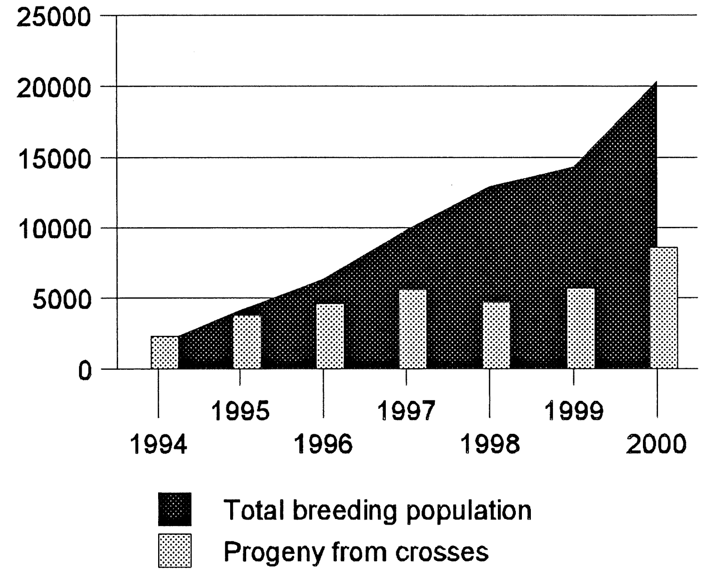

Figure 2. Summary of number of progeny from crosses by year and consequent total number of seedling progeny in the almond breeding program .

Nickels rootstock.

Peach x almond hybrids have been popular rootstocks for replant conditions as they confer added vigor to the scion making it more competitive with adjacent, older trees. Hybrid rootstocks have also become increasingly popular in plantings on more marginal soils. Several of these rootstocks, however, including the Hansen rootstock, appeared poorly adapted to nurseries storage practices particularly when extended cold storage is practiced (Fig. 3). *Nickels,* an almond X peach hybrid rootstock for almond developed and tested as *UCD,1-82,* is now nearing the completion of the UC patenting and release process. *Nickels* has many of the same characteristics as *Hansen 536* including ease of propagation, uniformity, vigor, resistance to root knot nematodes, resistance to calcareous and possibly saline soils, and potential for deep rooting. Recent trials have shown that *Nickels* is better adapted to nursery handling conditions,

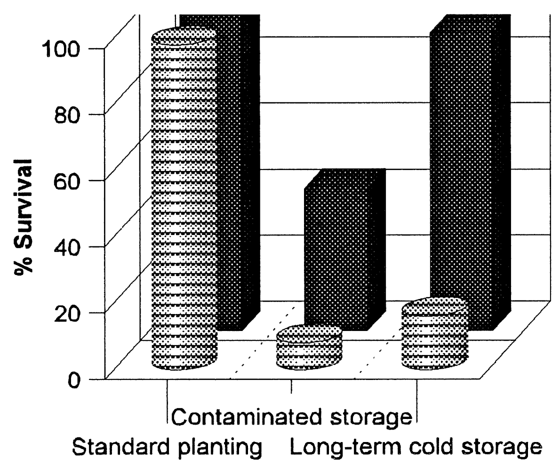

Figure 3. Survival of *Nonpareil* on the new *Nickels* (rectangles) vs. *Hansen* (cylinders) rootstock following different pre-plant treatments.

particularly cold storage and subsequent transplanting problems (Fig. 3) due, in part, to its higher dormancy and chilling requirements. Field trials have indicated that trees growing on this rootstock are longer lived than on *Hansen 536* and have a broader adaptation to areas where `crown rot' is a problem. Propagation tests showed rooting successes of greater than 60% for hardwood cuttings of this selection when treated with rooting hormones and fungicides and planted directly into the nursery row in late Fall, as is common practice. As an unbudded plant, *Nickels* is a large medium shape tree, more or less intermediate in growth form between the *Nemaguard* and Almond *Selection 5-33* parents. Growth habit tends to be like the peach with long vigorous shoots which eventually develop shorter spurs. Flowers are borne laterally on long shoots, usually 2 to 3 at a node with a single vegetative shoot. Nuts produced have a very hard peach-like endocarp. Bloom and leafing-out period is in early to middle March in California, which is later than essentially all of the almond cultivars, and corresponding to about that of *Nemaguard*. It is much later in bloom than *Hansen 536* and *Hansen 2136* indicating a higher dormancy requirement. The tree also goes into Fall dormancy in late October to November, making it considerably earlier than that of other almond cultivars and the *Hansen 536* and *Hansen 2136* rootstocks.

<u>Low Bud-Failure *Carmel*</u>.

The variety *Carmel* is a popular pollenizers for the *Nonpareil* mid-bloom and has become the second most important almond variety in terms of acreage planted. The high occurrence of Noninfectious Bud-failure in this variety has, however, led to severe losses in both production and tree survival. A large, multi-year project, involving the breeding program, Extension Specialists, Farm Advisers, and nursery and grower cooperators, has led to the identification and release of *Carmel* source clones having low potential for showing bud failure during the early, crucial years of tree development. Evaluations in 1999 conclude the continued stability of the principle low Bud-Failure *Carmel* source (designated D2 in Fig. 4) a as well as other low Bud-failure selections

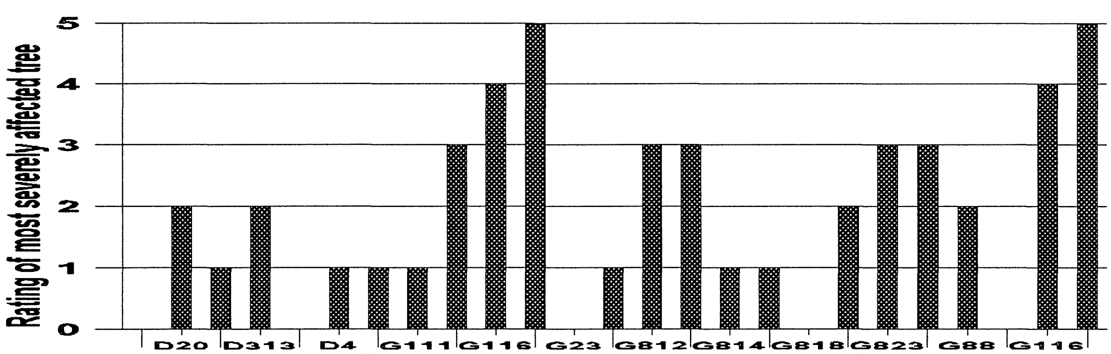

Figure 4. Level of Noninfectious Bud-Failure in progeny of Selections showing no symptoms as mature trees (0= no BF, 5=all scaffolds severely affected in some progeny trees).

undergoing vegetative progeny testing. While this strategy of identifying and maintaining low Bud-Failure sources should allow continued and profitable production from this variety, eventually some Bud-failure symptoms (typically expressed as the death of terminal buds followed by the growth of lower lateral buds) will occur in the upper growth regions of affected trees (where production loss will be more limited). For a more dependable, long-term control of this problem, new pollenizers varieties free of this affliction need to be developed.

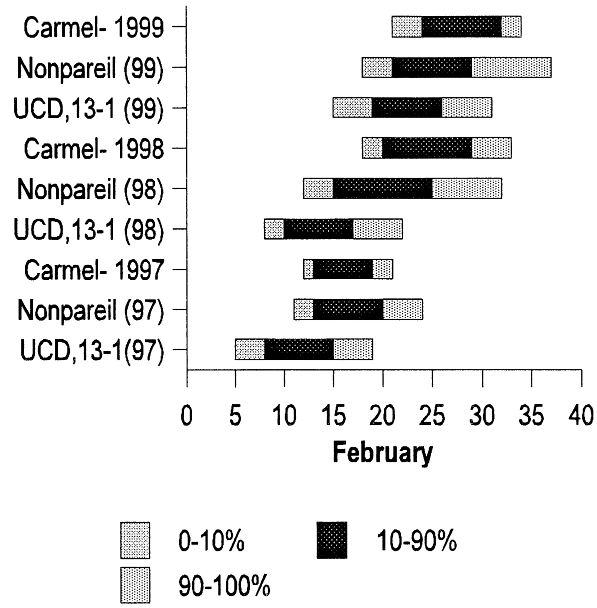

Figure 5. Bloom overlap of UCD,13-1 and *Carmel* with *Nonpareil*.

<u>UCD 13-1 Pollenizers for *Nonpareil*.</u> The early *Nonpareil* bloom contains the highest proportion of viable flowers and so is crucial to maximum crop set. It is also the most vulnerable to poor cross-pollination since traditional pollenizers such as Ne Plus Ultra and Solano often bloom too early to have good overlap with *Nonpareil*. Following extensive field testing (summarized in earlier reports), UCD Selection 13-1 has now been selected for patenting and release to the industry. Bloom of mature trees in Regional Variety Trials consistently showed good overlap with early *Nonpareil* bloom (Fig. 5). In addition, it has been one of the most productive varieties in Butte in Kern County RVTs, with lower production at the Delta college RVT resulting from cultural rather than variety causes (as summarized in 1998 report). Kernel and shell qualities are good as compared to both *Nonpareil* and *Carmel* (Fig. 6). Selection 13-1 is free of Noninfectious Bud-failure but is susceptible anthracnose (see Fig. 11) and to a lesser degree to Alternaria. Because of the high incidence of anthracnose in the Northern

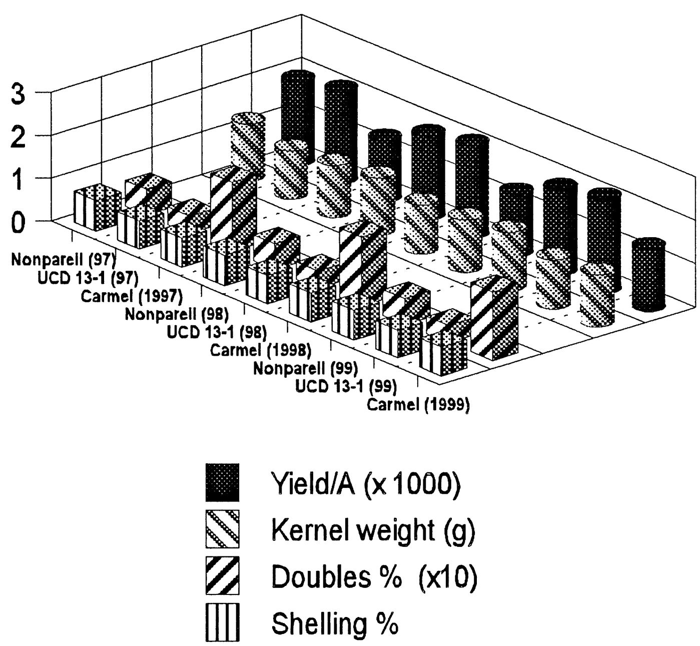

Figure 6. Kern RVT performance of 13-1 relative to *Carmel* and *Nonpareil* over the last 3 years.

Sacramento Valley, Selection 13-1 is not presently recommended for planting in this region despite the fact that it has been the highest yielder of all varieties at this RVT over the past five years. [Yield in 1999 was 2700 pounds per acre with an accumulated yield for the first four years of production of 6000 pounds per acre, as compared to 1900 pounds per acre for *Nonpareil* in 1999 giving it an accumulated yield of 5000 pounds per acre, and 1700 pounds per acre for *Carmel* in 1999 for an accumulated yield of 4900 pounds per acre; see 1999 RVT report]. Selection 13-1 is now being prepared for patenting with release expected within the next year. Virus tested budwood is presently available from FPMS under the Provisional release category.

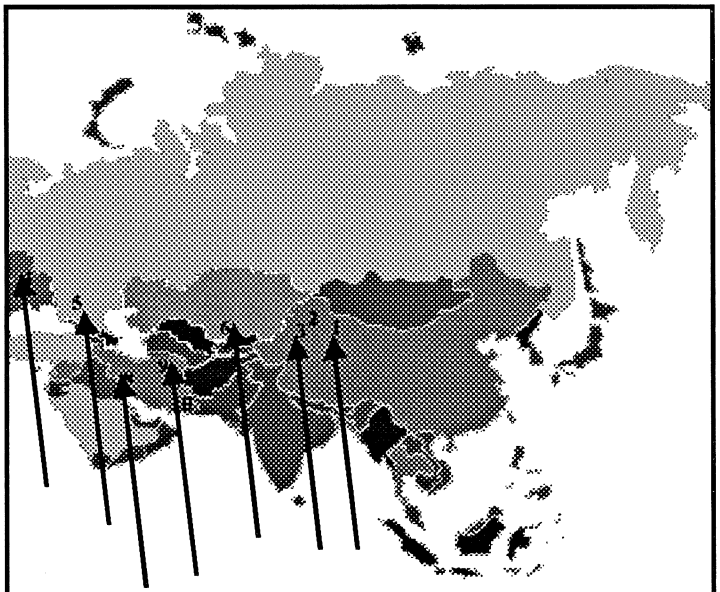

Figure 7. Map of Asia showing the diverse and often severe environments from which resistant and occasionally self-fruitful almond germplasm has been collected and evaluated at UCD.

Breeding for Pest Resistance. The lineage of most commercially important California almond varieties can be traced back to 2 parents: *Mission* and *Nonpareil*. While this germplasm has proven well adapted to California growing conditions, its general susceptibility to insects and diseases has required the widespread use of chemical pesticides to maintain production. Grower access to effective pesticides has been dramatically reduced in the last several years and this situation its expected to only worsen in the future. Genetic sources of pest resistance offer an alternative that satisfies both economic and environmental concerns. Almond has a very rich, though largely untapped genetic diversity in the European cultivars and land races and in the related wild species. Thus, in addition to the initial objectives of replacements for *Nonpareil* pollenizers and rootstocks, a major goal the breeding program has been the collection and characterization of new almond germplasm conferring both pest resistance and self-fruitfulness. As it enters the second phase, that is the generation of the next generation of improved almond varieties, the almond breeding program has incorporated into locally adapted almond (*Prunus dulcis*) breeding lines new germplasm from a wide range of almond and wild almond sources. A large collection of almond germplasm from Central Asia, (Fig. 7;- which is the center of origin as well as the center of diversity for almond), including the related almond species *Prunus webbii, Prunus argentia, Prunus mira, Prunus persica, Prunus fenzliana*, and *Prunus scoparia*, has now been established at UCD breeding plots and at the USDA germplasm collection at Davis California. The breeding program was particularly fortunate to have for its use a collection of related almond species and backcrosses to California almond types, which had been developed by the previous geneticist Dr. Dale Kester. This germplasm is presently being screened for resistance to the almond pests Navel orangeworm and ants, and the diseases anthracnose, *Alternaria*, and *Monilinia* blights, and aflatoxin producing *Aspergillus* species. In addition to identifying the different sources of resistance, we are also examining the mode of action of resistance genes, and their compatibility with commercial almond production practices.

Navel orangeworm and ant resistance. Earlier work (as summarized in previous annual reports) has identified biochemical components of the almond hull which, when present, provide resistance to navel orangeworm. While research in this area continues in cooperation with the USDA/WRRC laboratories in Albany, CA, future breeding work in this area will emphasize the development of a well sealed shell or endocarp as this trait conveys resistance to both navel orangeworm, ants, and the aflatoxin contamination associated with their feeding, and appears to be more easily manipulated by genetic and cultural means. An important finding, initially reported in 1998 and confirmed this past season, is that high levels of lignin commonly associated with well sealed endocarps are not necessarily associated with lower tree productivity as previously thought (Fig. 8). Lignin is the woody substance in the shells of almond and other stone fruit which acts as a physical barrier to insect and pathogen attack of the underlying seed. Interestingly, the lignin content of hulls, as determined by biochemical analysis, was occasionally higher than that of the shell of that variety. Lignin is also part of the vascular strands required for water and nutrient transport in plant issue. While moderate levels of lignin in almond hulls used as animal feed may be desirable (as it serves as a source of roughage), higher levels may be undesirable since many end-users, such as the dairy industry, set upper limits for the proportion of lignin allowed. Recent work has also demonstrated that almond shell seal integrity is determined not only by the quantity of lignin but by the process of lignification. The process of endocarp lignification in almond appears similar to that of peach and other stone fruit, in that lignification initiates at the styler and dorsal sides of the maturing endocarp, with the suture and suture seal being the last area to lignify. As a consequence, almond, like peach, is vulnerable to pit-splitting when the attached mesocarp or hull tissue expands too rapidly following heavy

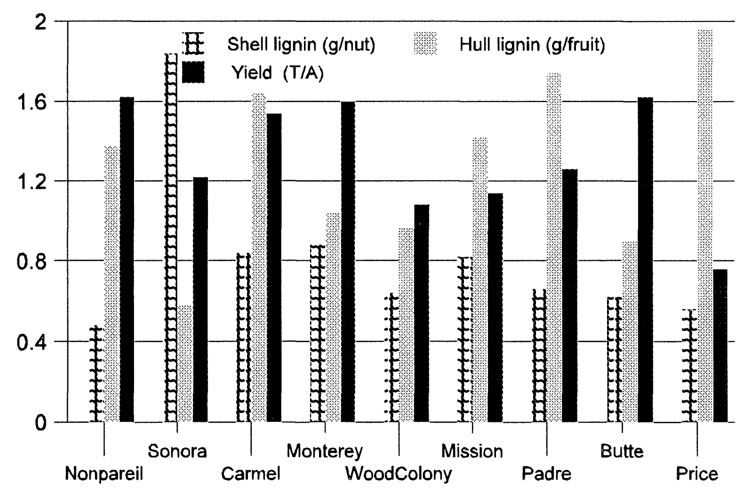

Figure 8. Comparison of hull and shell lignin contents and yields/A (1997 & 1998) for selected varieties showing an absence of any penalty of high lignin (shell hardness) on yield potential in almond.

irrigation or nitrogen application prior to the final lignification and so cementing of the suture seal. Unlike peach, almond appears vulnerable to a second area of shell seal breakdown which is adjacent and parallel to the suture seal and corresponds to the area of the vascular strands connecting fruit tissue with the developing seed (Fig. 9). During early development almond has 2 ovules, though one usually aborts. Subsequent degradation of the vascular attachments feeding the aborted embryo appears associated with the second, and often more common, point of endocarp break-down (Fig. 9). If confirmed to be widespread in almond, knowledge of the early stages of such endocarp breakdown might allow more efficient screening for new varieties with relatively thin yet well sealed endocarps (shells) as well as cultural management options for minimizing shell splitting in current varieties.

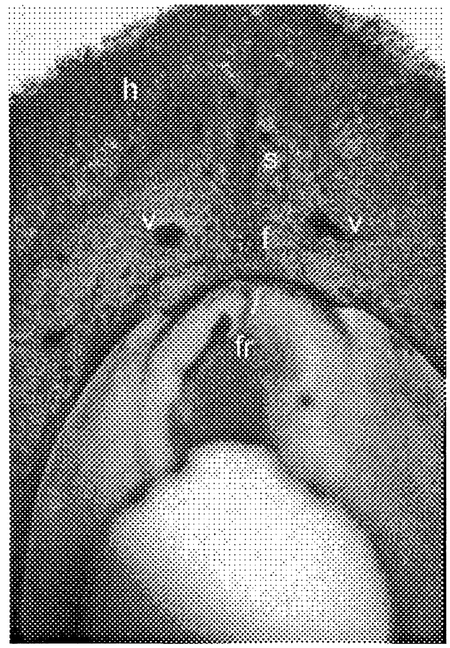

Figure 9. Cross-section of young developing almond fruit at the site of the carpel suture showing early degeneration of funicular region of aborted ovule. (Seed removed; h-hull, s-suture, v-vascular bundle, f & fr -funicular region).

Disease resistance.

Promising sources of resistance to a range of almond diseases including *Alternaria*, anthracnose, *Monilinia* Brown rot, Shot hole, and Hull rot are being evaluated in new breeding material. UCD Selection 1-87 has consistently shown useful levels of field resistance to several important diseases (Fig. 10). [A lower productivity and kernel quality for this item result in it being unsuitable for cultivar release at the present time. It may, however, be useful, along with the moderately resistant and higher quality selection 2-19E, in organic or similar farming operations where low levels of pesticide use is desired]. Higher levels of resistance, sometimes bordering on immunity, have been observed in many of the breeding lines derived from wild almond species, as demonstrated by the level of anthracnose damaged to hulls following controlled field inoculations (in collaboration with Dr. J. Adaskaveg UCR, -Fig. 12). Kernel and tree productivity are often marginal for this germplasm though

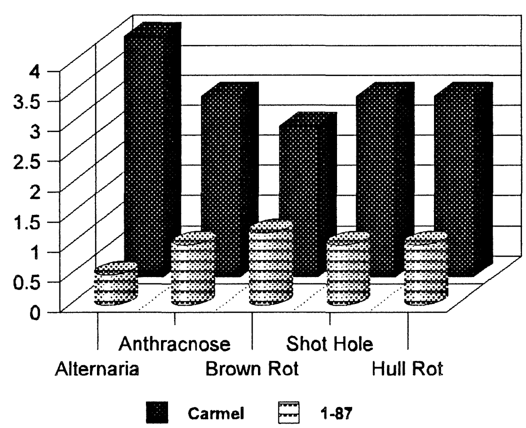

Figure 10. Resistance of UCD1-87 to various almond diseases as compared to the similarly maturing variety *Carmel* over the 1998-99 seasons. Ratings: (0-immune; 5- very susept.)

our previous breeding experience indicates the both productivity and kernel quality can be rapidly improved concurrent with the selection for disease resistance if the proper parental combination is identified, large enough progeny populations are generated, and the disease resistance can be efficiently selected from such large populations. We are presently testing the value of different parents and in different crossing combinations, and are attempting to develop molecular markers or tags for disease resistance of interest. However, the size of the of the breeding population required limits such efforts to the development of resistance for only one to 2 diseases. Thus, a crucial decision for the breeding program at this time, is the identification of one to 2 diseases were genetic resistance is most essential (i.e. effective cultural and/or chemical controls are not or will not be available in the future).

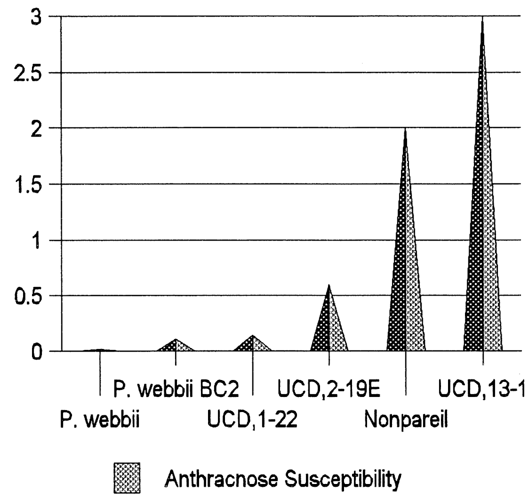

Figure 11. Resistance to anthracnose in selected almond breeding lines compared to standard varieties (following controlled inoculation of intact hulls with *Colletotricum* spp.). [0=immune; 3=susceptible]

Self-compatibility.

California almonds are normally self-incompatible, that is, pollen of a given variety will fail to fertilized flowers of that and other varieties in the same incompatibility group. For this reason, multiple pollinizer varieties are typically planted with the major variety (usually *Nonpareil*), and honeybee pollinators are brought into the orchard at flowering to move the pollen between varieties. Undesirable consequences of this arrangement include the need for planting often inferior varieties (and the consequent complications to orchard management since different varieties often have to be managed separately), the increasingly high cost of honeybees resulting from the increasing difficulty of beekeepers to maintain healthy hives, and a high vulnerability of the final almond crop to poor weather conditions during the short but crucial flowering/cross-pollination season. Several plant species related to

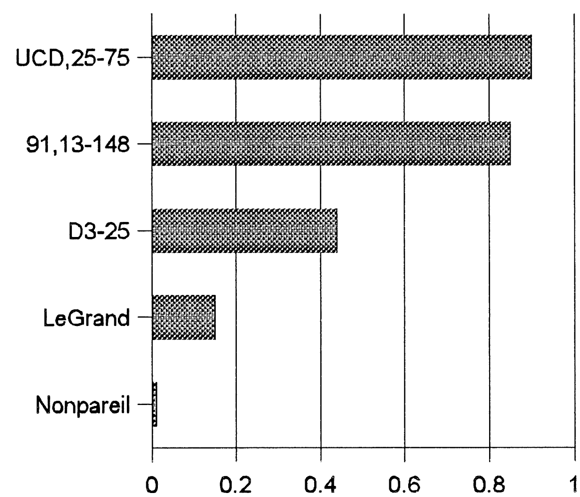

Figure 12. Level of self-fruitfulness of selected self-compatible breeding lines (Bagged seed set relative to unbagged bee cross-pollination).

almond, including peach (*Prunus persica*), are self-fruitful, that is the pollen is compatible on its own flower and the flower structure is such that self-pollination consistently occurs. Self-compatibility is controlled primarily by a single gene, while the capacity for self-pollination (flower structure) appears to be controlled by several unrelated genes. At the present time, we have successfully transferred self-compatibility genes from a range of different species, including *Prunus mira, Prunus persica, Prunus argentia*, and *Prunus webbii*, to cultivated almond types. In 1999, over 300 progeny trees were tested for self-fruitfulness by bagging branches with insect proof mesh and using the air blast from a portable leaf blower to move the pollen from the anthers to the stigma of the same flower. Subsequent flower set was compared to the natural sets on adjacent bee pollinated (unbagged) branches. A range in the level of self fruitfulness was observed in these tests, with several examples presented in Fig. 12. As can be seen, the variety *Le Grand*, while possessing a gene for self-compatibility from peach, fails to show consistently high levels of self-fruitfulness. Related selfing tests, using controlled hand pollinations have confirmed that the level of expression of the self-compatibility gene is affected by other, yet unidentified genes. Molecular markers have now been developed which have allowed the rapid identification of the major California incompatibility groups (see citations at the end of this report). These markers are now being further developed to identify different self-compatibility genes to aid in their selection. Over 40 genotypes have been identified from field tests, which possessed levels of self-fruitfulness and 15 of the most promising selections combining both self-compatibility and improved horticultural quality have been selected and propagated for further testing. While kernel and shell characteristics of several of these selections presently approach commercial requirements (see Fig. 13) further breeding may be required to modify tree architecture to be compatible with California orchard management systems. An example is seen in Selection 25-75 which has been planted to the last two (edge) rows of the Delta college RVT. This genotype represents an earlier generation of the breeding program for self-compatibility, and as such, it retains many of the tree branch

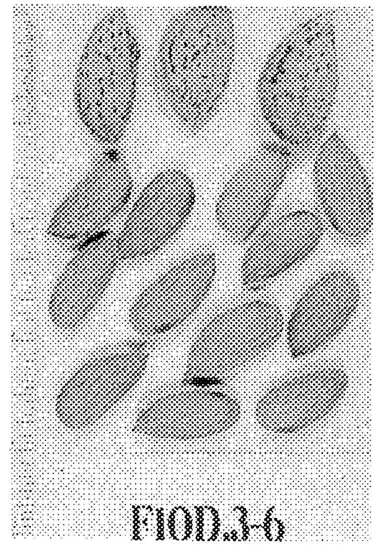

Figure 13. Shell and kernel characteristics of an advanced self-compatible selection F10D,3-6.

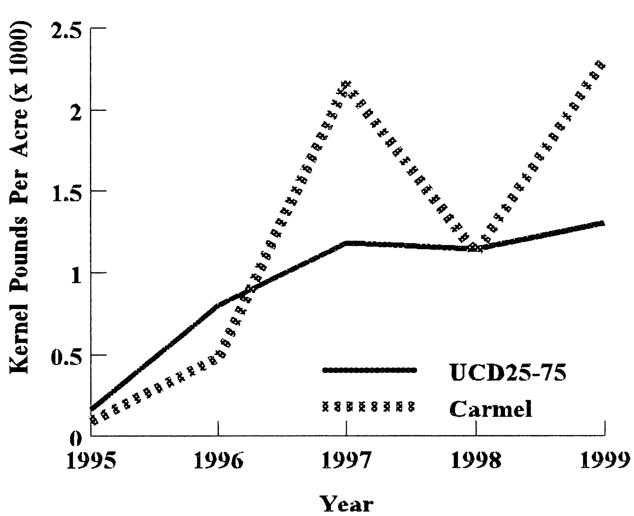

Figure 14. Production pattern for self-compatible UCD25-75 relative to self-incompatible cv. *Carmel*, showing evidence of year-to-year yield buffering by self-compatibility in UCD25-75.

and bearing habits of the wild species (Prunus mira) from which self-compatibility was transferred. While the resultant small tree size and willowy branching habit limit crop potential, the goal of this planting was to evaluate year-to-year crop consistency for this self-compatible and partially self-fruitful selection. Results, presented in Fig. 14 using the production of the similarly maturing variety *Carmel* for comparison, confirmed the hoped-for year-to-year cropping consistency for 25-75 which supports the reduced vulnerability of this and similar self-compatible varieties to the adverse weather conditions at flowering which have had such a dramatic affected commercial varieties.

Based on our current knowledge the breeding program is developing along a 2-stage strategy. Stage-1 will develop self-compatible almonds for grower testing within five years. These selections could be planted in either solid or mixed blocks, and will still need honey-bee pollinators but probably at significantly lower numbers. Advanced self-compatible lines will then serve as the basis for subsequent selections for capacity to self-pollinate with the resultant self-fruitfulness. The self-compatible trait would also assist in the development of disease resistant varieties as resistance genes are more easily characterized in selfed populations. Key questions to be resolved include the level of improved honeybee pollination efficiency that self compatibility would confer, and the level of variability in field performance for both self compatible and self pollinating varieties under different environmental and genetic backgrounds.

Research Publications in 1998-99.

1. Bartolozzi, F., M.L. Warburton, S. Arulsekar, and T.M. Gradziel. 1998. Genetic characterization and relatedness among California almond cultivars and breeding lines detected by randomly amplified polymorphic DNA (RAPD) analysis. J. Amer. Soc. Hort. Sci. 123(3):381-387.
2. Abdallah, A., M.H. Ahumada and T.M. Gradziel. 1998. Oil content and fatty acid composition of almond kernels from different genotypes and from different California production regions. J Amer Soc Hort Sci 123:633-637.
3. Ushijima, K., H. Sassa, R. Tao,H. Yamane,A.M. Dandekar, T.M. Gradziel and H. Hirano. 1998. Cloning and characterization of cDNAs encoding the Rnases in almond (*Prunus dulcis*):primary structural features and sequence diversity of the S-RNases in Rosaceous. Mol Gen Genet 260:261-268.
4. Tamura, M., K., T.M. Gradziel and A.M. Dandekar. 1999. Cloning of genomic DNA sequences encoding almond (Prunus dulcis) S-RNase genes (Accession No. AF148465, AF148466, AF148467, AF148468). (PGR99-117) Plant Physiol 120:1206.
5. Gradziel, T., N. Mahoney and A. Abdallah. (in press). Aflatoxin production among almond genotypes is unrelated to either kernel oil composition or *Aspergillus flavus* growth rate. HortScience 35:xxx-xxx.
6. Tamura, M., K. Ushijima, H. Sassa, H. Hirano, R. Tao, T.M. Gradziel and A.M. Dandekar. (In Press). Identification of self-incompatibility genotypes of almond by allele specific PCR analysis. Theor Appl Gen.
7. Boskovic, R., K.R. Torbutt, I. Batlle, H. Duval and T.M. Gradziel. (Submitted TAG). Stylar ribonucleases in almond: correlation with and prediction of incompatibility genotypes.