Project Number: 98-TG-o0

Almond Board of California

Annual Report - 1998

Project Title:	**Almond Variety Development**
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Cooperating Personnel:	W. Micke, 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.

Abstract:

Advanced breeding selections planted in 1993 in the Kern County Regional Almond Variety Trial (RVT) continue to show good performance in terms of yield and nut quality. Reduced yields were observed from Manteca and Butte County RVTs as a result of pollination and disease problems. U.C. Davis selection 13-1, showed a bloom period consistently covering the critical early bloom of *Nonpareil,* and, as in 1997, outproduced both *Carmel* and *Nonpareil* at the 1998 Kern RVT. Selection 13-1 has shown a relatively high susceptibility to anthracnose and alternaria leaf spot at the Butte County RVT. A generally poor tree vigor was also observed at the Manteca RVT, but the poor performance of adjacent *Nonpareil* trees indicates a soil or rootstock disease problem. U.C. Davis selections 2-19E and 2-43W also yielded well in Kern RVT with lower yields from the Manteca and Butte County RVTs. Despite the high levels of anthracnose, alternaria, and scab present at the Butte RVT in 1998, selection 2-19E showed freedom from disease symptoms and so may have useful resistance to these diseases. Crop on these trees was well below RVT average, however. Performance of selections 1-87, which also demonstrated elevated disease resistance, was also below RVT average. Selection 13-1 remains the most promising candidate for additional grower trials in the almond production areas where anthracnose is not a problem. Selections 2-19E and 1-87 may have commercial value in high disease areas despite their mediocre performance if their use allows a significant reduction in pesticide applications.

Considerable insect damage was observed in breeding and RVT plots with late season ant foraging accounting for a surprising proportion of the total damage. Breeding lines developed for resistance to worm damage through thin yet well sealed shells also showed good resistance to ant damage. Thicker, *Mission* type shells showed the best resistance though the need to divert

nut resources to developing such thicker and highly lignified shells has been thought to result in reduced tree yield potential. Chemical analysis of the lignin content of shells and hulls from important California almond breeding lines and varieties now indicate that thicker, well sealed shells are possible without associated losses in total tree yields.

Nut deformities, particularly partially filled and/or gummy nuts, and nuts with multiple embryos (resulting in a fragmented or shattered appearance of the peeled or blanched nut) were exceptionally severe in 1998. These problems can be traced to development failures during early embryo growth. Breeding lines resistant to this problem have been identified and the genetic control and inheritance are now to being studied.

Approximately 5,000 new seedlings from controlled crosses were planted in 1998 as part of breeding programs to further improve field performance, particularly disease and insect resistance, and self-compatibility. Self-compatible selections with larger *Nonpareil* type kernels have now been developed and these are being used as parents in controlled crosses to combine good kernel quality with consistent and high yields. Self-compatible selections in regional grower trials have yielded as well in 1998 (with its poor pollination conditions) as the more favorable 1997 season. Such year-to-year yield consistency is seen as an important advantage of self-compatible varieties (in addition to the field management advantages resulting from single variety orchards).

Summary of Progress: 1998.

<u>Development of a high quality pollinizer for the early *Nonpareil* bloom period.</u>

The primary breeding objectives include:
1) the development of improved pollenizers, particularly for the early *'Nonpareil'* bloom, and,
2) the development of the next generation of California almond variety possessing improved disease and insect resistance, and self-compatibility. Attainment of these goals would reduce grower inputs and so costs, reduce pesticide levels in orchards and nut products, and reduce the current year-to-year fluctuations in California production (Fig. 1). Despite recent increases in almond acreage, year-to-year production continues to fluctuate widely. Cross-pollination failures at flowering are widely believed to be a major cause of yield fluctuations. A comparison of statewide production shows good agreement with fluctuations in Regional Variety Trials (Fig 1), supporting the view that these differences are not the result of localized problems but rather reflect inherent limitations within the varieties, particularly their need for cross-pollination in the

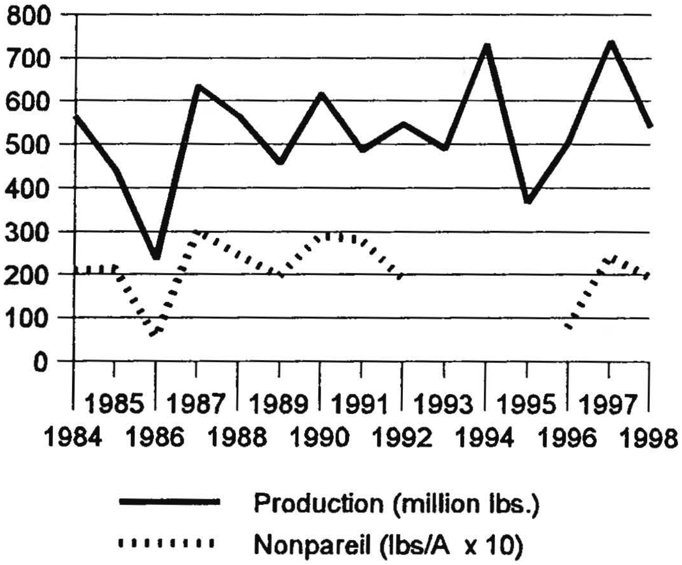

Figure 1. Fluctuations in California almond production from 1984 to present, with average yields for *Nonpareil* at the Manteca College RVT (1984-1992) and younger RVT at Kern Co. (1996-1998) provided for comparison.

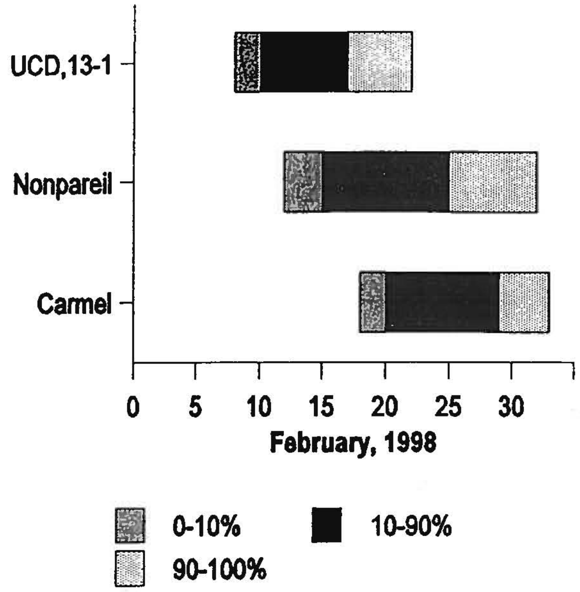

Figure 2. Bloom overlap of UCD,13-1 and *Carmel* with *Nonpareil*.

winter season when weather and honeybee foraging patterns are erratic. Particularly vulnerable is the early-to-mid bloom of the major California variety *Nonpareil*. *Carmel*, which is planted as a pollinizer for this period, has often flowered after *Nonpareil*, leaving the most critical early to mid-bloom incompletely cross-pollinated. UCD selection 13-1 was bred to provide a high production, high nut quality pollinizer for this early *Nonpareil* bloom period, and was planted in 1993 in the new RVTs to evaluate its state-wide performance. Since initial production in 1996, UCD13-1 has consistently covered the targeted early *Nonpareil* bloom despite the occurrence of unusual patterns in Winter chill and Spring heat units which have often pushed *Carmel* bloom concurrent to or later than *Nonpareil* (1998 results shown in Fig. 2). In the Kern RVT, where good management and disease control allow a clearer assessment of optimal yield potentials, 13-1 has consistently been one of the top performers. Tree production and nut quality have been comparable or superior to both *Carmel* and *Nonpareil* (Fig. 3). While 13-1 had originally shown some resistance to Alternaria leaf spot at the Kern RVT in 1996-97, this disease has become a problem in 1998 at both the Kern and Butte RVT. In addition, damage from scab and anthracnose have been observed on this selection in the Butte RVT following the cool, wet Spring of 1998. The thin shell, while contributing to a high crack-out, has also led to higher levels of ant and Navel orangeworm damage at the Butte and Manteca RVT when compared to *Nonpareil* and *Carmel*. Kernel quality is good, with low numbers of doubles, twins, or other nut distortions. Early processing evaluations (i.e. blanching, dicing, etc.) have also been favorable and larger scale industry evaluations are planned for 1999. While 13-1 produced very well in Kern RVT, final yields in both the Butte and Manteca RVT have been well below the *Carmel* standard

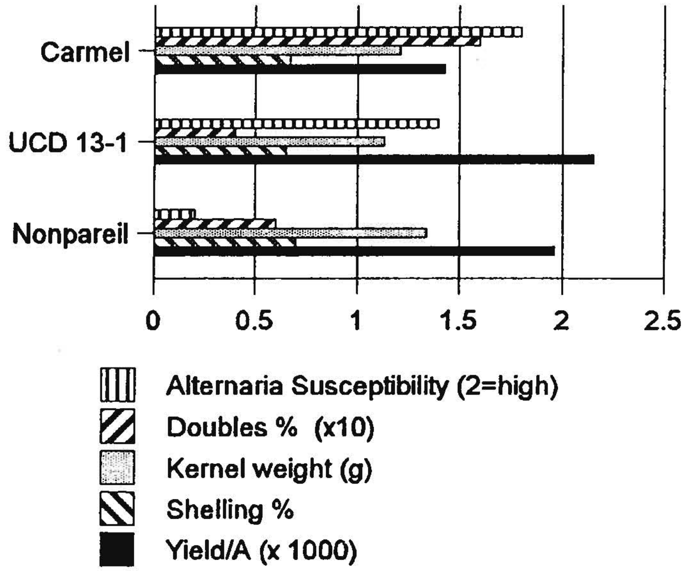

Figure 3. Kern RVT performance of 13-1 relative to *Carmel* and *Nonpareil* in 1998.

(Figures 4-6). The same was true for the other UCD selections in these trials with the exception of selection 25-75 which, due to its small tree size and peach-like growth habit produced consistent, though low yields at all trials. Low yields for the other varieties and selections at the Butte RVT can be attributed to the cool, wet Spring of 1998 with associated disease outbreaks of anthracnose, alternaria leaf spot, leaf blight, rhizopus hull rot, and scab. Field evaluations at Butte identified a general resistance (or at least low susceptibility) to disease in selection 2-19E and 25-75, and to a lesser degree, 1-87. [Both 1-87 and 13-1 showed symptoms of alternaria leaf spot in 1998]. Anthracnose and scab damage was noted on selections 13-1, 2-43W, 1-102W as well as several varieties. High numbers of double nuts were also observed in 2-43W, 1-87 and several varieties. Nut deformities, particularly partially filled and/or gummy nuts, and nuts with multiple embryos (resulting in a fragmented or shattered appearance of the peeled or blanched nut) were exceptionally severe in 1998. These problems can be traced to development failures during early embryo growth. Breeding lines resistant to this problem have been identified and the genetic control and inheritance are now to being studied.

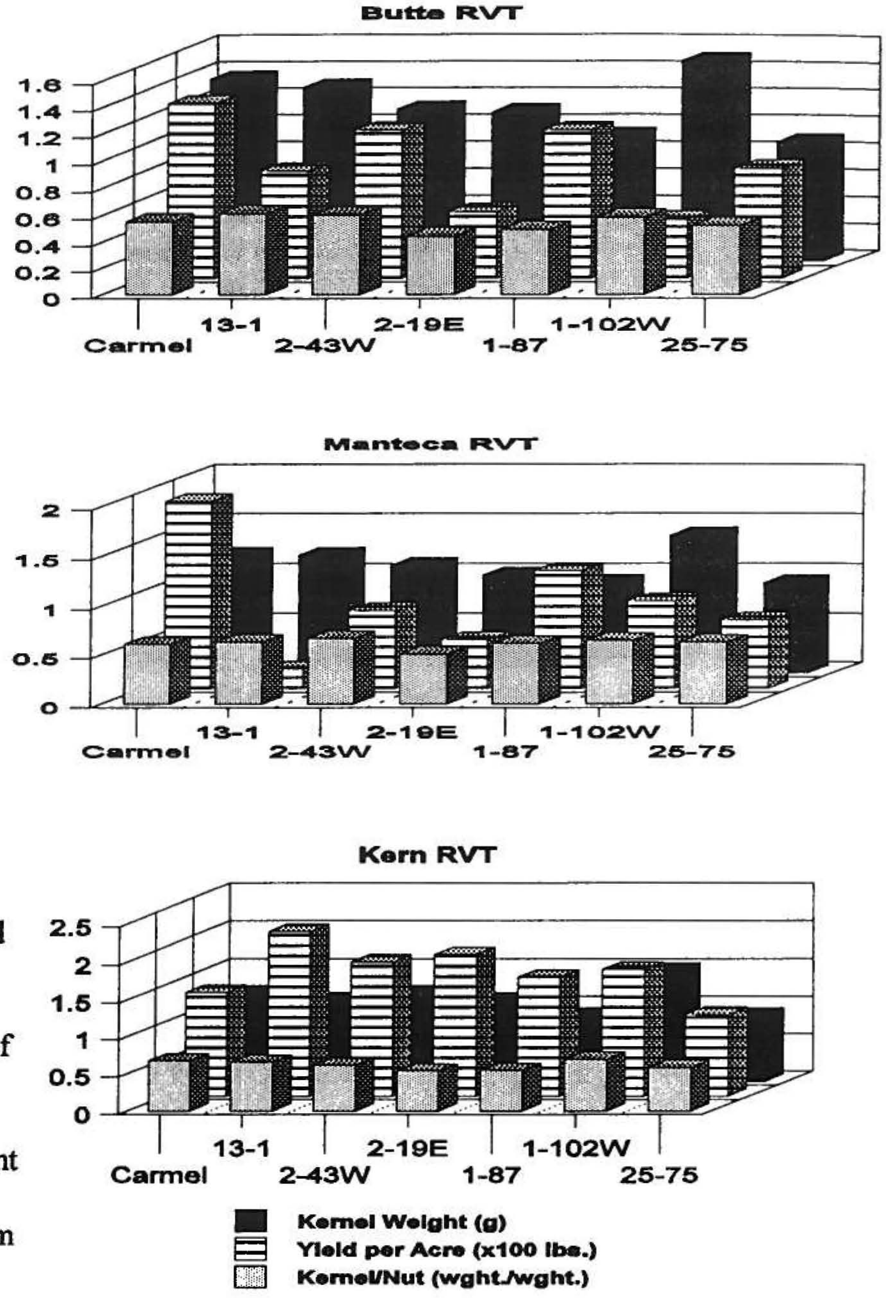

Figure 4, 5, 6. 1998 performance of advanced breeding selections at Regional Variety Trials (RVT)

Interestingly, while selection 2-19E yielded well at Kern, it had low yields at Butte despite the absence of any observable disease symptoms. Yields for 13-1 and 2-19E at the Manteca RVT were also low, though the incidence and damage from disease was not as obvious as in Butte. Management changes at this plot in 1998 have led to some concerns about the care given these trees, however. In Fig. 8, individual row yields are plotted to identify possible location (soil factors, irrigation, etc.) effects at this site. This is possible by comparing yield for the alternating (odd numbers in Fig. 7) rows which are *Nonpareils* interplanted as standards throughout the plot. As can be seen, dramatic yield reductions are apparent from rows 20 to 37, with the lowest yielding *Nonpareil* (at less than one-third of the maximum *Nonpareil* row yield) being adjacent to the 13-1 row (rows 33 and 32, respectively). Thus, external factors appear to be involved here, and yield assessments need to be done with caution. A second lesson from this analysis results from the large fluctuations observed among the *Nonpareil* rows. Long term evaluations at the previous RVTs have shown that while *Nonpareil* was often not the top yielder in a particular year, its average over the years was among the best. This apparent production consistency, however, may only be a consequence of our determining *Nonpareil* yields as an average from all rows, thus buffering against isolated failures as demonstrated in Fig. 8. The importance of pollinizer trees on both sides of the almond trees is also seen in the low production 'edge effect' observed on the first (*Nonpareil*) and last two (25-75) rows. Two rows of selection 25-75 was deliberately placed at this low-yield edge boundary to minimize cross-pollination in order to assess its capacity for self-fertilization. Interestingly, both rows (64 & 65) show similar yields, as would be expected if self-pollination rather than cross-pollination was predominant for this selection.

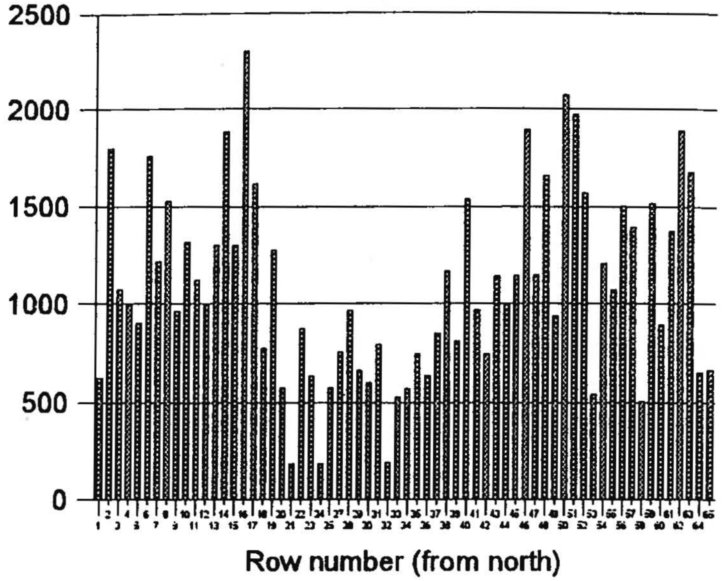

Figure 7. Variations in yields (lbs./A) of almond selections in Manteca RVT at different east-west cross-section of field. Odd numbered rows are controls; *Nonpareil* to R49 with remainder being *Mission.*

<u>Developing lower input varieties: Pest resistance and Self-compatibility.</u>
<u>Disease resistance.</u>

While the 13-1 yield discrepancy between Kern and Manteca RVTs may be at least partly due to non-genetic factors, the apparent susceptibility of 13-1 to anthracnose and possibly Anternaria leaf spot under the high disease pressures at Butte in 1998, creates a breeder's dilemma. While 13-1 appears to meet the initial breeding goal of a consistent and high quality pollinizer for *Nonpareil* early bloom, it also appears deficient in our second-generation goal of pest resistant varieties. Since these diseases are not presently major problems in most growing areas, (particularly the San Joaquin Valley), we are moving forward with the probable release of selection 13-1 by as early as 2000. The need for pest resistance in the next generation of UCD varieties has, however, been given added impetus by both the increasing threats from both old and new diseases at a time when access to effective pesticides is becoming increasingly restricted, and the decreasing farm-gate value of the crop. Preliminary disease screenings at UC/Davis has identified potential resistance sources for several important diseases including anthracnose (Fig. 8). Selections 3-18, and to a lesser extent, 2-19E showed no or only minimal damage following controlled inoculation of intact almond fruit. Selection 2-19E also demonstrated resistance to Anternaria leaf spot at the Kern RVT screenings performed by Jim Adaskaveg (UCR). Selection 3-18 is one of the new breeding lines derived from wild, Central Asian almond populations (as described in the 1997 report). While still deficient in both kernel and tree (production) quality, it represents the wealth of new and potentially very useful germplasm being incorporated into the breeding program. Selection 2-19E, which was bred as a possible pollinizer for the later *Nonpareil* bloom, has performed very well at the Kern RVT, though not as well at Manteca and Butte (as previously discussed with figures 4-6). Selection 2-19E remains a candidate for possible release as a new variety. Both 2-19E and 3-18, as well as several other promising breeding lines have been crossed with tester lines in 1998-99 to evaluate the genetic control and heritability of resistance. In addition to the diseases already discussed, preliminary field evaluations are also underway for shot-hole and blossom blight diseases. Resistance is also being pursued for aflatoxin causing Aspergillus infection of almond kernels. While several potential sources of aflatoxin resistance have been identified (as summarized in Fig. 9 as well as the 1997 Annual Report), the most promising appears to be the control of the insect pests, particularly navel orangeworm (NOW) which predispose the nuts to infection.

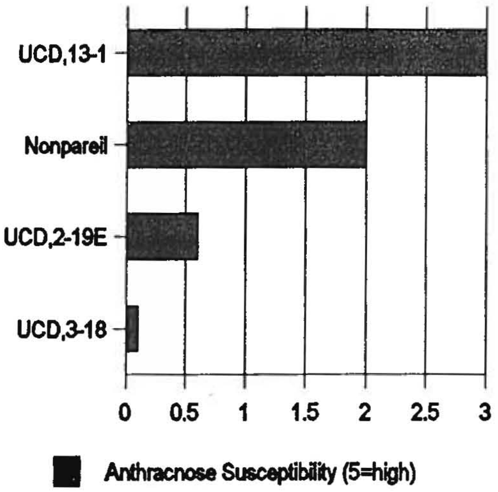

Figure 9. Differences in the resistance to Anthracnose in almond breeding lines in 1998.

Insect resistance.

Pest targeted are those damaging the almond kernel, and include navel orangeworm (NOW), peach twig borer (PTB), and more recently ants. Opportunities for NOW control have been identified in genotypes which either prove toxic or repellant to initial NOW feeding on almond hulls and/or nuts, or which are prevented from damaging the kernel by an impregnable shell barrier (Fig. 9). The development of an impregnable shell barrier has the added benefits of not involving (potentially) toxic plant chemicals, and since it is a physical barrier, should be effective for a broad range of

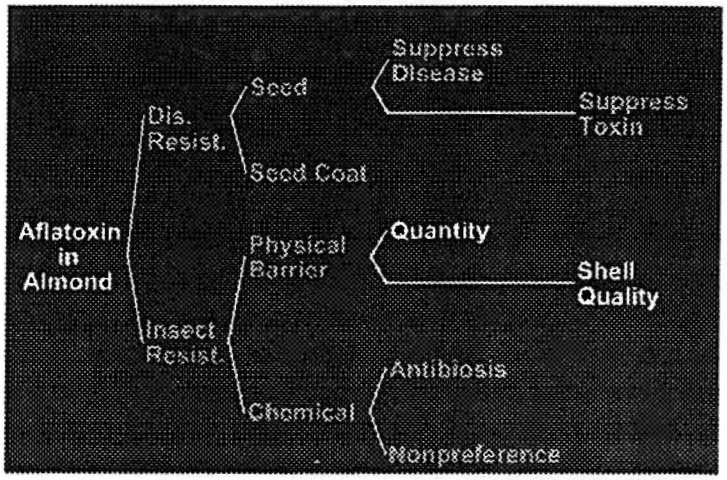

Figure 8. Flow chart summarizing strategies being employed to develop resistance to NOW and subsequent aflatoxin contamination.

insects and diseases which damage the kernel. Our research has identified two key components of a well-sealed shell: a strong and continuous inner shell, and a well-formed and completely sealed suture such as that found in the variety *Mission*. Both good shell structure and complete suture seal require higher levels of the high density plant biochemical *lignin*, which, in turn, has been associated with lower yield potentials since it has been believed that the plant must divert energy away from kernel to lignin production. Our recent findings, however, refute these assumption by showing that no clear relation exists between yield of a variety and the amount of lignin produced in its hulls and/or shell (Fig. 10). These findings clear the way in theory for the development of new varieties with a *Nonpareil*-type kernel and *Mission* type shell with its resistance to NOW, PTB, ants, and *Aspergillus* infection. We are currently developing a fairly complete understanding of the critical components of shell structure and integrity. However, the process of suture sealing and cementing (lignification) appears to respond to several independent environmental and genetic variables, and so defies an easy and consistent predictive model.

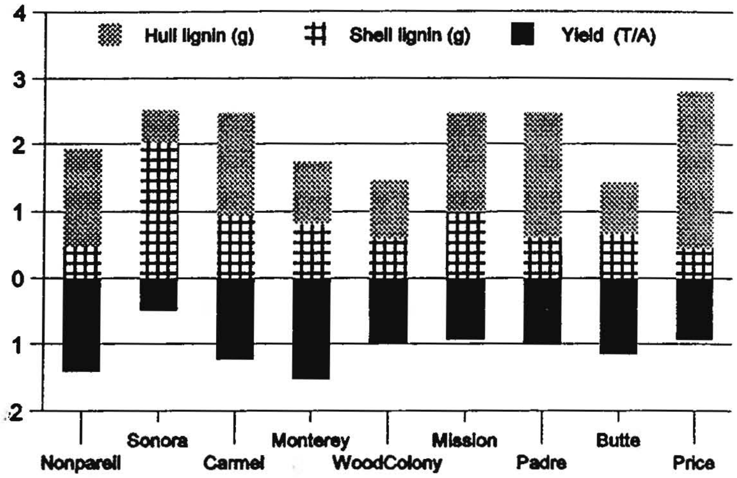

Figure 10. Comparison of hull and shell lignin contents and yields/A (1997) for selected varieties showing an absence of any penalty of high lignin (shell hardness) on yield potential in almond.

Related breeding approaches targeting the control of kernel pests by modifying kernel composition (particularly kernel fatty acids as summarized in the 1997 Annual Report), have also created opportunities for improving the nutritional, (phytonutrient) value of almond to the consumer. Continued studies in this area have identified additional breeding lines having lower linoleic/oleic fatty acid ratios. This is desirable as it improves both the nutritional value and storability of the almonds and products made from them (Fig. 11). In addition, these studies have verified the linear relation between linoleic and oleic acid reported in the 1997 Annual Report, supporting their close biochemical relationship (Fig. 12). Further research in this area, particularly concerning the genetic control of this putative biochemical pathway, may offer opportunities in the future to fine tune this pathway and the resultant fatty-acid composition of the kernel. These opportunities are currently being pursued through both genetic

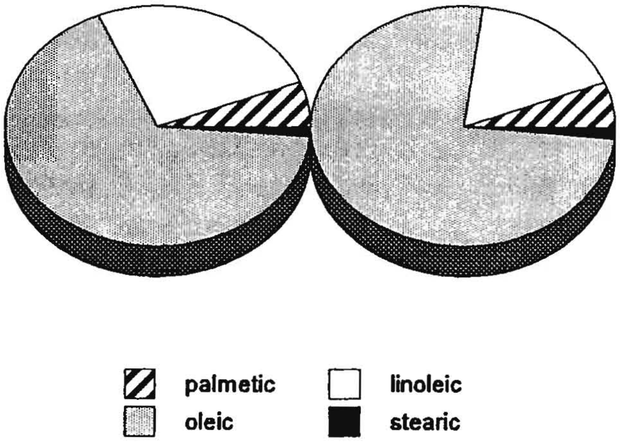

Figure 11. Improved oleic/ linoleic acid ratio in an advanced UCD breeding line (right) compared to *Nonpareil* (left).

engineering and more traditional controlled genetic recombination strategies.

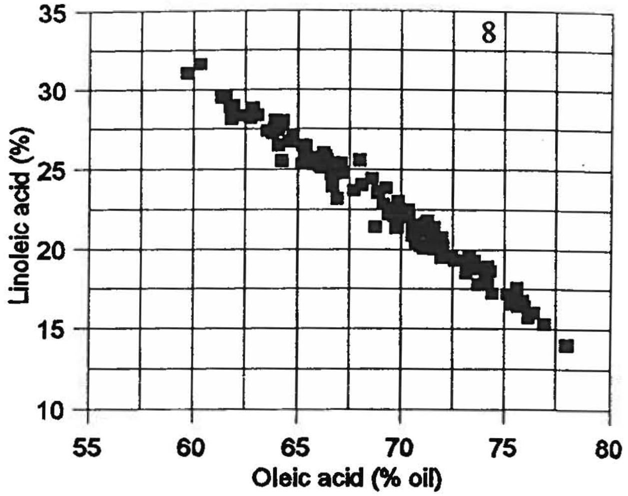

Figure 12. Relationship between oleic and linoleic acid showing high correlation (R2=0.98) suggesting linoleic acid is direct precursor to oleic acid.

Developing Self-compatible almond varieties.

Self-compatibility would allow the self-pollination of almond, thus increasing honeybee pollination efficiency (and so reducing the hive requirements, and so rental costs) and also reducing year-to-year production fluctuations. Honey-bees would still be needed to insure movement of the pollen from the anther to the stigma since natural self-pollination is rare in almond Highly self-compatible varieties might also allow single variety orchards with their inherent savings in orchard management costs. The self-compatible and partially self-pollinating breeding line 25-75 was placed in the new RVTs (despite its having too small and bushy a tree for commercial release), to test for this hoped-for buffering against year-to-year production fluctuations. Results from the last three years of production show an increase from the first to second year (as would be expected with increased tree size) followed by an apparent stabilization of production between 1997 and 1998 (Fig. 13). In contrast to the good pollination conditions of 1997, pollination weather in 1998 was relatively poor due to frequent rains and cold temperatures, and most varieties at the RVTs showed a decrease in yields in 1998. While a promising start, several additional years of yield data are necessary before trends can be accurately characterized. The consistent production between the two end rows (both 25-75) in the Manteca plot, however, also support a relative independence of this self-compatible selection from the proximity of potential cross-pollinizer varieties (As discussed previously with Fig. 7).

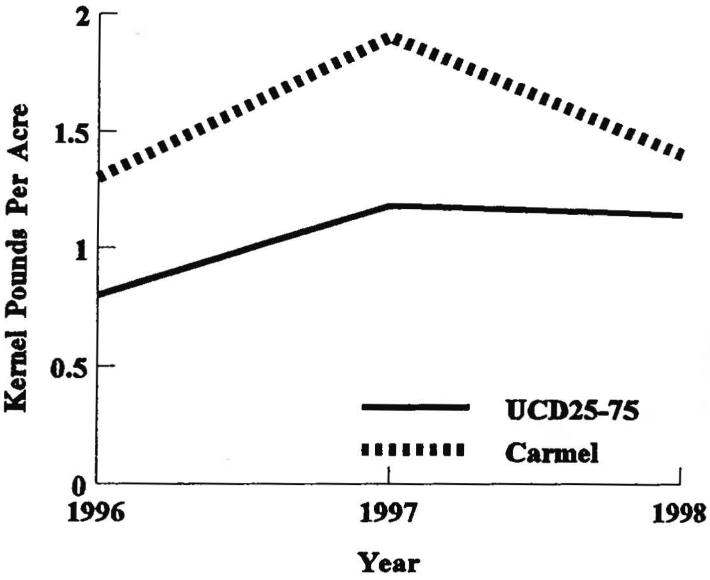

Figure 13. Production pattern for self-compatible UCD25-75 relative to self-incompatible cv. *Carmel*, showing some initial evidence of year-to-year yield buffering by self-compatibility in UCD25-75 (as opposed to the absence of such buffering in the only partially self-compatible cv. LeGrand reported in 1997 Annual Report).

Selection 25-75, along with the other first generation self-compatible selections (described in the 1997 Annual Report), have been used as parents to generate the second generation of breeding lines combining self-compatibility with improved kernel quality and tree yields. This work involved the making of large numbers of controlled

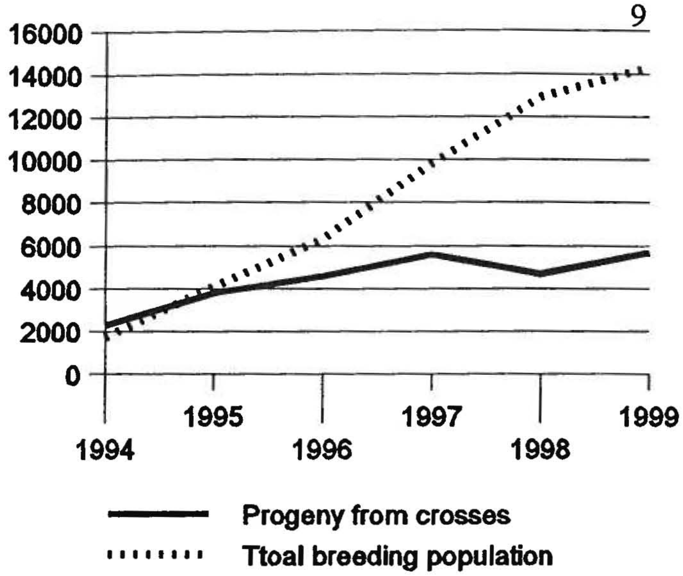

Figure 14. Summary of number of progeny from crosses by year and consequent total number of seedling progeny in the almond breeding program .

crosses between parents selected to complement their individual traits, and the planting and evaluation of the resulting progeny. Very large numbers of crosses are required to generate the large progeny populations, which, in turn, are needed to insure recovery of the rare genetic recombinant containing the full complement of desired traits (for kernel quality, yield, resistance, tree structure, etc.). In 1998 approximately 5,000 seed were harvested from 1997 crosses and planted, and roughly 20,000 crosses were made in over 300 different crossing combinations, with approximately 6,000 seed harvested, stratified and, germinated for transplanting to the field in 1999. Approximately 2000 of these seedlings will be rouged out before or within the first year of transplanting based on inferior plant structure. Four years of vegetative growth are then required before flowering, fruiting and nut evaluation are possible. The first crosses in this project were made in 1993 with approximately 2,000 seedlings being field transplanted in 1994. We have harvested an average of approximately 5,000 seed from controlled crosses in each of the subsequent 4 years resulting in a rapid growth of the breeding program size (Fig. 14). In 1998, nut characteristics, including self-compatibility, were evaluated from seedling trees from the 1994 planting. Approximately 40 individuals were selected for further evaluation with the remainder discarded. An analysis of the lineage of selected items revealed that most resulted from crosses with only 7 parents, even though over 20 different parents were used in the initial crosses. Even within these elite parents, the proper choice of the specific crossing combination was crucial to success. For example, of the pollen parents used, only D3-13 produced selectable progeny when either 80,11-22 or 25-75 was used as the seed parent (Fig. 15). The successful recovery of 'selectable' progeny from the other pollen parents only occurred when crossed with one but not the other 'elite' seed parent. Information developed from these evaluations was used to develop crossing plans for 1999. Thus, a cycle of genetic improvement has begun, which involves the generation and evaluation of a large population of seedling progeny leading to the

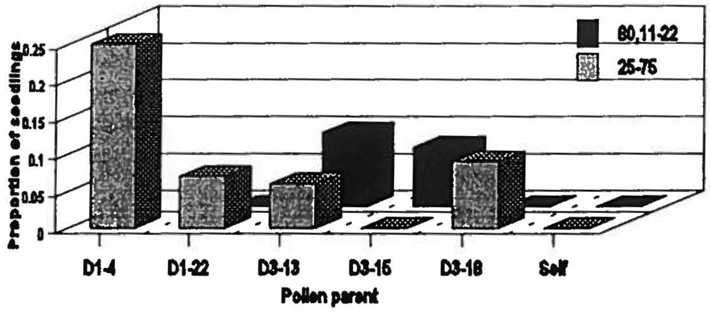

Figure 15. Early field evaluation results showing the importance of proper parent selection when breeding for both self-compatibility with good kernel size.

identification of elite progeny, and, in parallel, elite parental combinations. This information, in turn, leads to improvements in the quality of the parents utilized, which, in turn, leads to improvements in both the overall quality of the progeny populations ,and also our understanding of the inherent strengths and weaknesses (and genetics) of the parental germplasm used.

The size and day-to-day demands of this aspect of the breeding program are such that most of the breeding program resources are dedicated to it. The relatively long period of vegetative growth before fruiting can occur means that the 'fruits' of these efforts are only starting to be realized. The number of progeny selected from this 1994 planting is particularly satisfying since many of the sources of self-compatibility and pest resistance utilized in those crosses were still fairly wild in their characteristics, some of which is commonly transmitted to many, though not all of the progeny. The relative speed with which this very rich and extensive wild germplasm has been transferred to a commercial almond background is partly the consequence of the greater variability in plant and seed characteristics tolerated in tree crops as compared to vegetable and grain crops. Thus, despite the emerging challenges in almond production, pest management and marketing, the genetic materials and breeding methods to meet these challenges appear available. The ultimate challenge, perhaps, is to be able to recognize and prioritize the most important needs of California almonds into the next Century.

[Note: Due to the size and breadth of the almond breeding program, only summaries of 1998 progress is presented in this report. More detailed data on any aspect of the breeding program are available upon request.]

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