Project Number: 94-M7

Almond Board of California

Annual Report - 1994

Project Title: **Almond Variety Development**

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Location: Department of Pomology, University of California at Davis

Objectives:

Long Range:

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

II. Develop breeding strategies, including the effective use of new biotechnologies, for achieving rapid genetic improvements in almond varieties.

Current:

A. Identify effective parental combinations which result in self-fertility, high quality and yield, and later flowering period. Continue studies to elucidate the underlying physiology and genetic control and inheritance of these traits. Generate and evaluate breeding program candidates for variety release. Characterize selfing potential, nut quality and yield potential of new progeny populations, breeding lines and variety standards.

B. Test genetic strategies for developing self-fertility, improved tree yield, and protection from Bud-failure and other disease and insect problems.

C. Develop methods for the efficient transformation and regeneration of established almond varieties.

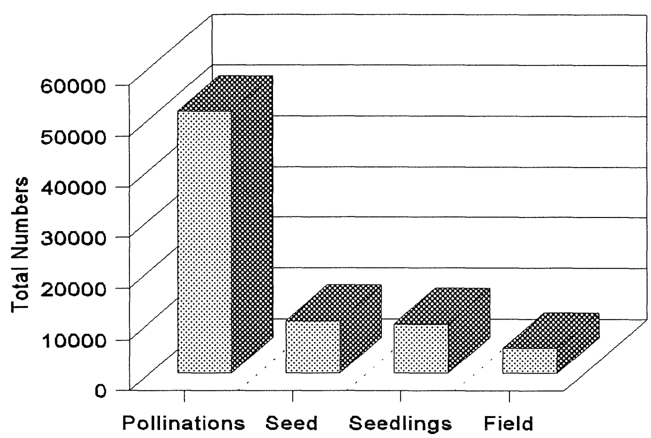
ALMOND VARIETY DEVELOPMENT

The aim of the breeding program 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, good shell-seal with high crack-out ratio, or specific flowering time), thus leading to improved program efficiency.

QUANTITY OF BREEDING MATERIAL

Important limitations on the number of seedlings processed by the breeding program include the number of seedlings which can be produced by controlled crosses during the limited pollination period, and the number of seedling trees which can be evaluated by a technical staff which has been greatly reduced by recent University cut-backs. The target for the breeding program has been 5,000 new seedlings per year from controlled crosses with a 5 year seedling block evaluation period. This goal would result in 25,000 individual seedlings being evaluated over the 5 year turnover period making this program one of the largest tree nut breeding programs in the US and probably the world. The major limitation to progeny population size is crossing success, particularly the weather conditions during flowering. Results for 1994 are shown in Figure 1, where 51,400 controlled pollinations were made using 220 different crossing combinations. A total of 10,200 nuts were harvested yielding 9,627 germinating seed

Fig. 1. 1994 Controlled-Crosses



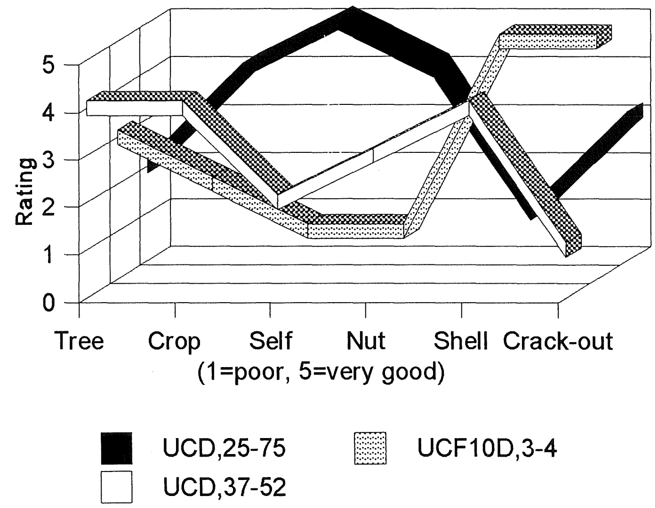
following stratification (chilling the seed to overcome seed dormancy). Seedling selections resulted in 4,840 progeny plants for Spring, 1995 field transplanting. We are working to further improve the efficiency of this critical stage of the breeding program through several strategies. The observed set of 20% is considered good for controlled crosses in almond. The need to emasculate flowers from self-compatible breeding lines before controlled pollination results in some damage to the pistil leading to higher rates of abortion. Emasculation removes both the pollen bearing anthers as well as the petals at the pre-popcorn bud stage leaving the still developing pistil vulnerable to weather related stresses such as excessive moisture, heat, desiccation, and frosts. To overcome these problems we utilize self-incompatible breeding lines as the seed parent when possible (thus eliminating the need for emasculation). We have also improved the techniques for emasculation and pollen application, closely organize our efforts to allow massive numbers of crosses, have work around inclement weather by concentrating our efforts to bagging limbs and bud emasculation during rainy periods (high-humidity facilitates emasculation success) then using a large number of student volunteers to make the large number of controlled crosses when the weather breaks, and utilizing enforced (caged or bagged) crosses and selfs of individual branches and entire (caged) trees. In addition, field and lab studies from 1994 have confirmed that almond anthers will not dehisce (release pollen) if maintained under high humidities and that honey bees can be used under these conditions to transfer a selected donor pollen to flower pistils with low risk of unwanted cross- or self-pollination. To exploit this finding, we are setting-up a large bee-cage facility where high relative-humidities can be maintained even during dry periods to allow bee vectored controlled-pollination (using dry and dehisced donor pollen supplied to mini (4 frame) bee hives. We are also developing a population of parent trees in containers for placement in the high-humidity bee cage during flowering. Our experience with bee-vectored controlled cross-pollination of normally self-unfruitful almond trees, indicates this approach will not only lead to considerably less labor requirements during the critical flowering period since bees rather than people are making these crosses, but an overall increase in the per-cent set due to multiple pollination events and the absence of damage when emasculations are performed. Possible problems with this approach include difficulties in establishing vigorous trees in small (10-20 gal.) containers, and difficulties with pistil stigma receptivity under high-humidities. The establishment of container-grown trees would also allow a 1-to-2 week extension of the now limiting pollination period, allowing both a greater number of pollinations and a decreased vulnerability to weather patterns.

Improvements implemented in 1993 and 1994 in field management and evaluation methods, (including closely managed drip irrigation on high density, central-leader trained trees, early rouging of inferior seedlings based on early performance and genetic markers, and numerically codified scoring using small and inexpensive palmtop computers), have allowed consistent and reliable evaluation of the large progeny population sizes. Improvements are needed in our understanding and rating of early tree performance, (particularly cropping potential), and our ability to maintain good seedling vigor and survival during the stressful greenhouse growth and subsequent field transplanting. (We are now experimenting with the direct field planting of progeny seed using herbicides to control winter and spring weeds, though initial (Winter, 1994) populations suffered very high losses due to adverse weather conditions).

QUALITY OF BREEDING MATERIAL

Analysis of data trends from the large progeny populations now being produced and evaluated by the breeding program are leading to improvements in the selection of individual parents as well as specific parental combinations for specific targeted traits, thus leading to improved overall quality of the progeny from these crosses. Specific quality characteristics analyzed in progeny populations include: flowering period; seed, shell, and hull size; shell seal and crack-out; nut quality; and disease and insect damage; harvest date; and general crop load. Figure 2 shows breeding line performance for three parents used in 1994 and 1995 crosses. (Relative rankings are used here with '1' indicating poor performance to '5' indicating very favorable performance). The solid line represents UCD,25-75, a breeding line favored for its high degree of self-compatibility and

Fig. 2. Breeding Line Performance



ability to self-pollinate, (see Fig. 3) and a good nut quality yet with serious deficiencies in tree (too willowy) and shell (too open) characteristics. Breeding strategies attempted to match crossing parents with complementary lines such as UCD,37-52 or UCF10D,3-4. This strategy is complicated by the high number (> 20) of primary characteristics bred for, and a general lack of knowledge on the genetic control and so inheritance of individual traits. (For example, flowering period control is now known to be very complex and so not readily predictable).

In addition to identifying promising parental combinations for specific goals, such population analysis has also identified traits for which the present California germplasm is inadequate, leading to the identification and utilization of outside germplasm to improve progeny performance. Examples of such introduced germplasm include the introgression or incorporation of genes for: self-compatibility and self-pollination from *Prunus mira* from eastern U.S.; self-compatibility, high-cropping potential, compact tree type, late bloom and well sealed shell from *P. webbii* from both Western and Eastern Europe; high cropping-potential, high tree vigor with good shell seal from *P. fenzliana* from Turkey; and good shell-seal with high crack-out ratios from *P. argentia* from Iran and Iraq.

While these results increase the confidence of the breeding program for meeting and even exceeding commercial requirements for the traits evaluated, a major challenge to the breeding program remains the accurate prediction of actual crop production potential of a particular selection. Continued progress is needed in our ability to characterizing the critical components responsible for consistent crop production (for example, tree and branch architecture, bearing habit, fruit sizing potential, etc.).

BREEDING OBJECTIVES:

The primary objectives of the breeding program are to improve production stability for both grower and processor, and to improve market quality.

PRODUCTION STABILITY -GROWER

Grower needs in future almond varieties include consistent production with reduced inputs. Projects for reducing grower inputs include reducing orchard management requirements, and breeding genetic resistance into almond varieties for navelorange worm and aflatoxin causing *aspergilllus* infections, blossom and twig blight, and non-infectious bud-failure.

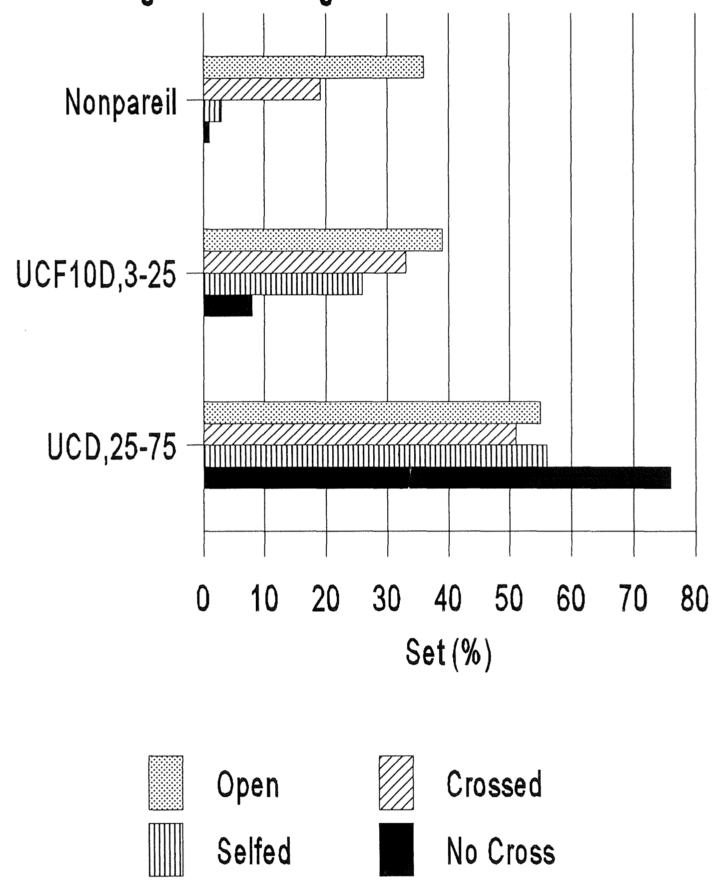
REDUCING ORCHARD MANAGEMENT REQUIREMENTS

Reduced orchard management is being pursued through the development of a self-compatible and self-pollinating almond, and through the improvement of cropping-potential consistency of new varieties.

Self-fruitfulness

Self-compatibility in a high quality, Nonpareil-type almond would allow solid block plantings eliminating present management difficulties with orchard maintenance (due to need for separate spraying, harvest, etc.). The further incorporation of self-pollination could reduce the present dependency on timely honey-bee cross-pollination to achieve good orchard yields. Progress in developing self-compatible and self-pollinating almonds of commercial quality are demonstrated in Figure 3 where self-fruitfulness is compared for three breeding lines including Nonpareil as a standard. 'No Cross' (branch is bagged to eliminate bee pollinators and no cross pollen is applied) or self-pollinating ('Selfed') on Nonpareil results in an almost complete loss of crop. Controlled hand-pollination ('Crossed') results in roughly 20% set, while 'Open' (open pollination where the branch is not bagged but allowed to cross-pollinate naturally) results in approximately 36% set. The larger open-pollinated to controlled-cross set is probably due to flower damage during hand cross-pollination and an underestimation of the number of flowers on the 'Open' branch section due to later opening flower buds. In contrast the breeding line UCF10D,3-25, which approaches Nonpareil in nut quality and surpasses it in yield potential (Fig. 4) shows similar 'open-pollination' sets but much higher 'no-cross', 'selfed' and 'crossed' sets (latter probably partly due to later blooming, uncounted selfs). This behavior indicated the presence of self-compatibility but only partially self-pollination. Both high self-compatibility as well as high self-pollination is seen in line UCD,25-75 which has good kernel type but an undesirable (willowy) tree type. Continued backcrosses of these and related breeding lined to high tree and nut quality lines have been made in 1993, 1994 and 1995 with resulting second-generation progeny to begin tests in 1996. The initial objective of these crosses is to incorporate a high degree of self-compatibility in a productive, Nonpareil-type almond. Self-pollination would be subsequently incorporated into such commercial quality varieties. The initial emphasis on self-compatibility reflects the greater knowledge and heritability of this trait, and the probable inadequacy of presently known self-pollinating germplasm sources to completely eliminate the need for honey-bee pollinators in the orchard at flowering.

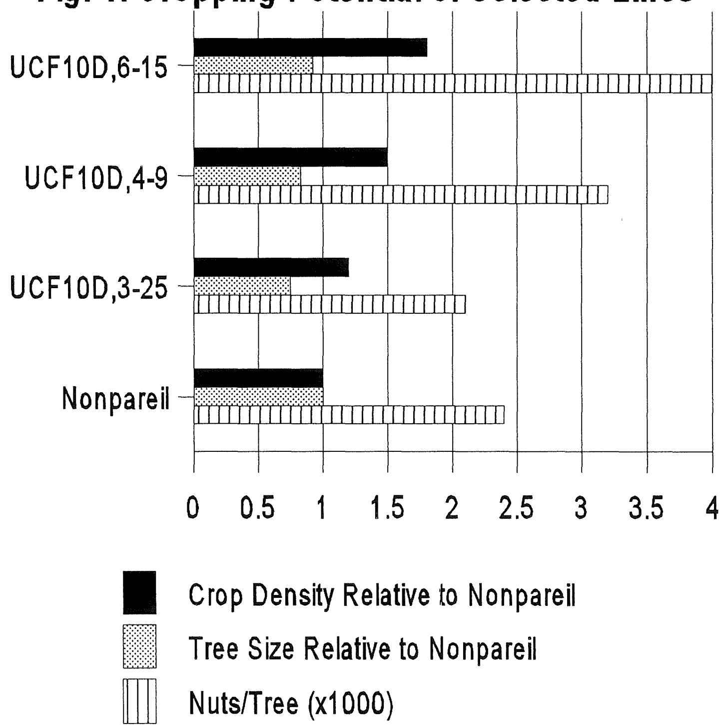
Fig. 3. Breeding Line Self-fruitfullness



CROPPING POTENTIAL

Production stability is being further pursued through the development of more uniform and consistent cropping potential within the almond tree. Opportunities for significant improvements are suggested by cropping data from selected breeding lines over the last two years as demonstrated in Fig. 4. Selected lines show a higher crop density than Nonpareil even though tree size may be smaller. (While UCF10D,3-25 approaches Nonpareil in nut quality both other lines suffer from low crack-outs and nut defects, for example doubles, etc.). While the mechanism for this improvement varies by breeding line, it appears to be mainly the result of a more uniformly high distribution of fruit-wood through-out the tree canopy, as with the greater formation of flower-bearing current season lateral branches throughout the canopy. The resulting tree-architecture for some of these lines is distinctly different than current standards, yet appear fully amenable to present orchard practices including shaking. As with the self-compatible lines, further crosses to improve commercial quality are taking place as are further studies to determine the long-term stability (alternate-bearing habit, etc.) of these cropping potentials. In addition, physical models of shaker force transfer in different tree architectures are being pursued using computer models originally developed to assess building architecture vulnerability to earthquake damage.

Fig. 4. Cropping Potential of Selected Lines



NAVELORANGE WORM (NOW) AND, AFLATOXIN CAUSING *ASPERGILLLUS* INFECTIONS

Previous work has shown a close association between aflatoxin contamination and NOW infestation, and has implicated NOW damage to the seed and seed-coat as promoting *aspergillus spp.* infection and subsequent aflatoxin contamination (see 1993 report and 1994 publications). A strong correlation between shell-seal and NOW infestation continues to be supported by present evaluations (Fig. 5), particularly the finding that a completely sealed shell confers resistance to both NOW infestation and *aflatoxin* contamination. Relatively minor cracks in the shell which are easily missed visually may, however, allow and even promote NOW infestation and survival. A modified gas-flowmeter has been found to be a consistent and accurate test for shell seal integrity (Fig. 6, where intact nuts were first gas-pressurized and resulting gas leakage rate recorded as cc/min) and it is now being used both to evaluate new material, and to better understand the nature and location of critical shell fractures. For example, in the samples shown both IXL and line 1-69 visually appeared to have completely sealed shells though fractures were detected following gas-flowmeter analysis. The gas-flowmeter

Figure 5

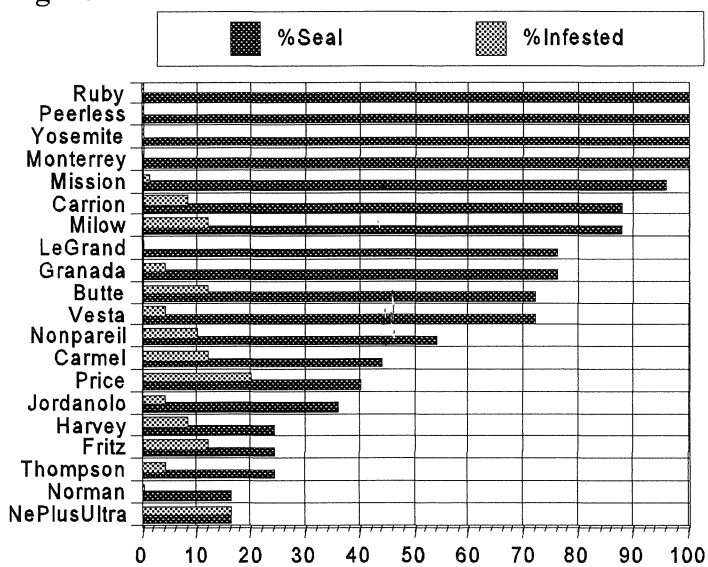
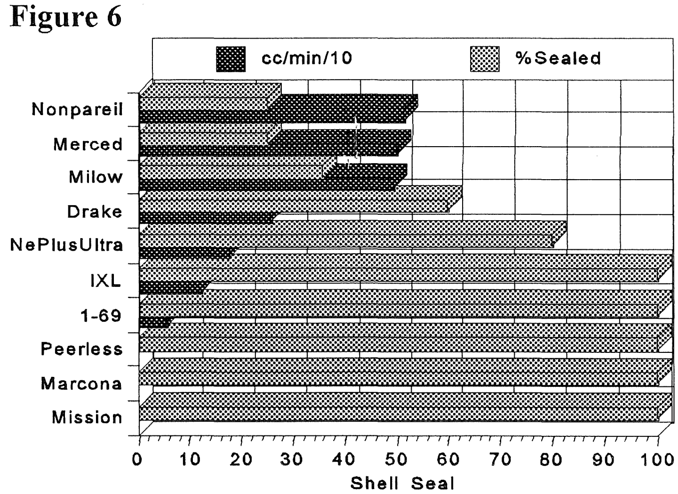


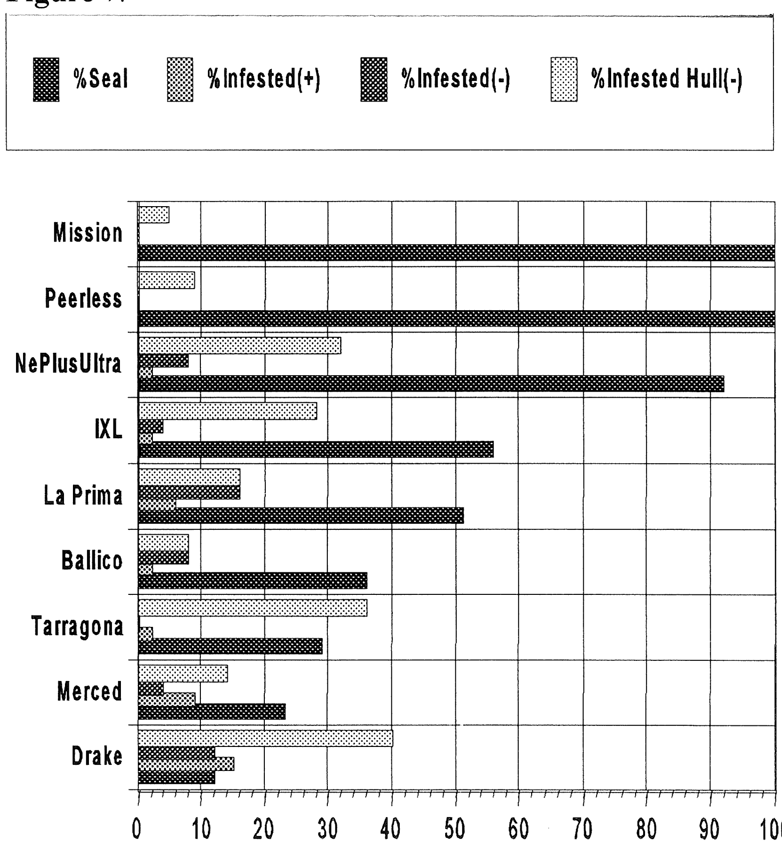
Figure 6



technique has been used to help identify the important sites of NOW entry which appear to include both the shell suture as well as shell fractures at the ventral vascular bundle located parallel to the suture seal. Both the degree of shell lignification as well as the timing of lignin deposition relative to stage of fruit development now appear to be important determinants of shell seal integrity. A more rapid field assay using specially developed 'feeler gauges' has been developed to test for fractures at these sites and will be further tested in 1995. Breeding lines have been identified which appear to possess a thin, highly sealed shell conferring both worm resistance and high crack-out ratios (for example, line UCF10D,3-4 in Fig. 2). Progeny from controlled crosses to these lines have been made to further incorporate this trait into commercial lines as well as to determine its inheritance.

The relation between shell-seal and NOW infestation is supported by separate experiment summarized in Fig. 7. In this work NOW eggs were placed on the outside maturing kernels under laboratory conditions and subsequent nut infestation recorded (designated by [+]) Lab results were compared with both kernel and hull infestation in the field using only natural infestations (designated by [-]). Results indicate fairly good agreement between controlled lab results with field infestations with observed differences probably due

Figure 7.



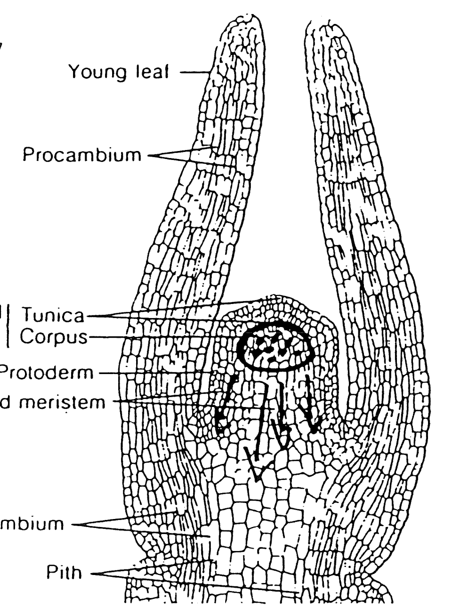
differences in harvest time and specific variety location. This data also suggests that NOW shows a preference to certain hull genotypes for egg deposition or subsequent success of larval development with a distinct non-preference and possibly even toxicity (antibiosis) found for Mission and Ballico hulls. If these results are verified, they offer another strategy for NOW and *aspergillus* resistance in the development of lines where specific hull constituents discourage NOW oviposition and larval development. (We are presently working with entomologists and biochemists from UCD and USDA/Albany, CA in pursuing these resistance strategies and have secured USDA funds to augment ABC funds for this work).

BLOSSOM AND TWIG BLIGHT

In 1994, we have begun a collaboration with Dr. Jim Adaskaveg, UCD Plant Pathology, to develop resistance to blossom and twig blight in almond as caused by *Monilinia* spp. The relative resistance of important breeding lines is being assessed as well as the site and mechanism of resistance when identified. Promising lines were identified in 1994, with further evaluation to take place in 1995. A similar collaboration has been very successful in developing promising strategies for resistance to this disease in clingstone peach over the last 4 years. While both the almond and peach projects are continuing despite Dr. Adaskaveg's departure from UCD, the loss of his Internationally recognized expertise with *Monilinia* pathogenesis are expected to slow progress in this area.

In addition, exploratory work is continuing on the development of periclinal chimeras of almond which possess a resistant outer 'tunica' or skin (epidermis) layer from a different

Fig. 7



resistant breeding line or species. The strategy we are using involves micro grafting the desired almond variety (speckled area in shoot-tip cross-section shown in Fig. 7) into intact and viable resistant meristems. Since this central tissue gives rise to all leaf and fruit tissue except for the skin and all seed tissue, the processing integrity of the variety is maintained. The resistant skin, however, can provide protection to both fruit and leaf tissue from a combination of disease and pests which would be removed at hulling. As this approach does not involve 'genetically engineered' products, it may prove more acceptable to an increasingly safety-conscious public. We have test micro grafted several hundred individuals so far and are now pruning back the apical shoot growth to push axillary buds in which micro-grafted chimeras would be most probable. Sample micro-grafted meristems have also been fixed, sectioned and stained, and are presently being studied to better understand the survivability and viability of donor tissue.

ALMOND TRANSFORMATION AND REGENERATION

While we have been successful in transforming Nonpareil callus tissue, our lab has been unable to induce plantlet regeneration. In 1994 we started a collaborative project with Dr. A. Dandekar, UCD Pomology and J. Driver of Dry Creek Labs, Modesto, CA to exploit their expertise in the areas of genetic engineering and plant regeneration respectively. Plat regeneration from leaf discs now appears to have been achieved at Dry Creek Labs from an almond variety and we are now attempting to repeat and verify this work for Nonpareil in a separate ABC 1995 project. The almond meristem also continues to be the target for genetic transformation attempts as it obviates the need for plantlet regeneration from unorganized almond callus (as detailed in 1993 report). We are now making modifications in our particle-gun system for foreign gene insertion into almond meristems which may allow the transformation of meristems from bud chips and/or intact meristems on complete trees, thus eliminating the need and high costs of tissue culture.

NON-INFECTIOUS BUD-FAILURE

As described in the 1993 Reports, test are now being utilized by our program to determine the vulnerability to bud failure of important breeding lines and candidates for variety release (using peach and almond tester lines developed by Dr. Dale Kester). Approximately 15 breeding lines are now being tested with initial results indicating low bud-failure potential of important lines. Additional test crosses were made in 1995 though initial sets appear to be very low due to adverse weather during and after flowering. Approximately three additional years of assessment need to be made on current items before final judgement can be made. If successful, this program should ensure against grower and nursery loses due to field expression of latent non-infectious bud-failure in new UCD varieties.

PRODUCTION STABILITY -PROCESSOR

Although still at an early stage of development the breeding program is gaining competence in generating and evaluating promising candidates for variety release. As previously mentioned, an evaluation area needing improvement is the prediction of cropping potential based on early seedling tree development. A second major evaluation area needing further development is the assessment of processing quality, including ease of blanching, roasted appearance and flavor, ease of slivering and slicing, salt/flavor adherence, and special use groups such as uniform, small nuts for chocolate candy manufacturers. Feedback and recommendations on processing quality will be solicited directly from the industry once adequate samples become available through regional test plantings. Regional test plantings are now in place for six advanced selections: '2-43W', '2-19E', UC36-52, UCD25-75, UCD56-89, and UCF10D3-25 (Descriptions in 1993 Annual Report). Crop sizes allowing adequate Handler and Processor evaluation should be available by the 1996 season. In response to Processor requests, a project has begun on increasing the post-harvest storage life of almond through the modification of the Linoleic-to- Oleic acid balance. While genetic differences have been shown to exist within the present California varieties, the relatively low range of differences combined with the high variances within samples suggest poor opportunities for genetic improvement with this germplasm. A range of new germplasm will be tested in 1995 for opportunities for improving storage life using traditional techniques. In addition, successful transformation and regeneration of almond in 1995 or 1996 would open up opportunities for manipulating this trait using emerging biotechnologies.

RELEVANT PUBLICATIONS IN 1994

Kester, D.E., T.M. Gradziel, and W.C. Micke. 1994. Identifying pollen incompatibility groups in California almond cultivars. Journal of the American Society for Horticultural Science 119(1):106-109.

Gradziel, Thomas M. and Dechun Wang. 1994. Susceptibility of California almond cultivars to aflatoxigenic *Aspergillus flavus*. HortScience 29(1):33-35.

Gradziel, T.M. and D.E. Kester. 1994. Breeding for resistance to Aspergillus flavus in almond. Acta Hort. 373:111-117.