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Almond Board of California

Annual Report - 1996

Project Title: **Almond Variety Development**

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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. Continue to 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 self-fertility and production consistency, protection from 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 rapidly eliminate of inferior seedlings from breeding populations and to identify the best parent combinations for breeding goals.

Summary:
Advanced selections from the Almond Variety Development Program are now in production at the Regional Variety Trials with several selections continuing to show promise. A high level of inbreeding has led to a narrow genetic base for future almond improvement. New germplasm has been introduced, including new sources for self-compatibility, and disease and insect resistance. Large-scale crossings between new germplasm and elite California lines is taking place. Techniques have also been developed for the rapid and accurate identification of genetic identity and probable parentage; cross-compatibility grouping; insect resistance; seed lipid and vitamin-E composition; and noninfectious bud-failure potential. Performance of important almond cultivars and breeding selections are presented for each area of investigation.

ALMOND VARIETY DEVELOPMENT

A. **A. Identify promising selections for advanced testing and use as breeding parents.**

Advanced almond breeding selections planted in Regional Variety Trials produced their first commercially harvestable crop in 1996. Line 13-1 continued to show good nut size and quality with kernel appearance similar to *Carmel.* It showed excellent bloom overlap with *Nonpareil* at all three Regional Variety Trials though the density of bloom was poor in the Kern plot possibly due to the low winter chill conditions. Yields averaged 824 lbs/acre, down from the very high crops of 1995 though still significantly higher than the *Nonpareil* trees of the same age (3rd leaf in 1996) and consistent with a bearing habit transition towards greater spur production in the coming years. Hull-split was mid-season similar to *Price.* Line 13-1 suffered higher than normal worm damage in the Chico plot but was one of the most resistant selections in the Kern plot *Alternaria* ratings done by Beth Teviotdale and Mario Viveros.

Breeding selections 2-43W and 2-19E showed good production in 1996 Regional Variety Trials, with nut and tree characteristic continuing to be favorable. These lines were selected by Dr. Dale Kester to be interplanted; both lines are late flowering with good bloom overlap and cross-compatibility, and similar hull split times. Nuts are *Mission* type and size but with shelling per-centages closer to 60%. Line 2-19E suffered from considerable kernel shrivel as well as a somewhat weepy branching habit at the Kern plot in 1996, but was also rated as one of the most resistant to *Alternaria.*

Line 25-75 bloomed very late (early March) as is its habit, yet produced a moderate crop despite the lack of good pollinizer trees supporting a good self-compatibility and self-pollinating tendency of this selection. Tree structure appears to be too spreading for normal management practices, however. The lower sets also contributed to higher levels of blank kernels and worm damage at the Delta College plot.

The remaining lines: 1-87 and 1-102W are late-blooming and early-mid season harvest selections showing only mediocre yields during the first years of production. The low crop also contributed to high nut shriveling at Delta. *Alternaria* resistance was very good to good, respectively.

Self-compatibility was confirmed through controlled crossing studies for three additional UC/Davis advanced selections: UCD36-52, UCD34-26, and UCD45-8 planted at the Nickels Research orchards at Arbuckle, CA. Similar controlled crossing studies at the Wolfskill Experiment Orchards, Winters, CA supports the presence of self-compatibility in 16 other almond lines selected as sources of new germplasm possessing good tree and/or nut characteristics. Self-compatibility and self-fruitfulness have been further analyzed in February, 1997 pollination studies.

Limits to the use of traditional California germplasm in new almond variety development

The genetic similarities or uniformity for almond germplasm used in the breeding program was surveyed by evaluating the similarity of a large number of randomly selected DNA (RAPD) markers. A similarity matrix was generated by the NTSYS-pc version 1.7, based on the simple matching algorithm of Sokal and Sneath. Cluster analysis and dendogram construction was performed using the unweighted pair group method with arithmetic averages (UPGMA) of the same program. 'Nemared' peach was used as an out-group for comparison. A total of 37 informative RAPD bands were scored and used in the UGPMA cluster analysis. The similarity values ranged from 1.00 for bud-sport mutations to 0.484 for cultivars 'Ne Plus Ultra' and 'Padre' (Table 1). A dendogram constructed from similarity data shows separate groupings with affinities to 'Nonpareil' and 'Mission', reflecting their historical importance to almond cultivar development in California (Fig.1). 'Nonpareil', along with 'Ne Plus Ultra', 'I.X.L.' and 'La Prima' originated from a single seedling orchard planted by A.T. Hatch of Suisun, CA in 1879 and are known as the "Hatch" cultivars. Mission originated in Houston, Texas, sometimes being referred to as 'Texas' or "Texas Prolific, and was first introduced into California about 1900. 'Nonpareil' rapidly became the main cultivar due to its good tree and nut qualities. Because almond is self-incompatible, 'Mission 'and 'Ne Plus Ultra' were used as the main pollinizers for 'Nonpareil'. 'Ne Plus Ultra', though a Hatch origin cultivar like 'Nonpareil' shows greater similarity to 'Mission'. Carmel, though reportedly originating as a bud-sport of 'Nonpareil' is clearly distinct. The similarity indices (Table 1) and dendogram branch points (Fig. 1) of remaining genotypes demonstrates the greater divergence of this material resulting from the

breeding with genotypes outside the traditional California almond gene pool. The 'Nemared' peach is shown to be a distinct out-group. 'Trusito', an old self-fertile and very hard-shelled Italian cultivar not previously used in California, is the most divergent of the almond material with uniformly low similarity indices ranging from 0.529 to 0.666. Similarity indices within this range, however, are common between many of the genotypes tested due to both divergent origins and the normal high heterozygosity common to the self-sterile almonds. Enforced outbreeding within a limited gene pool can lead to calculated similarity values not fully reflecting known parantage as with 'Padre, a 'Mission' x 'Swanson' cross showing greater similarity to 'Nonpareil', and 'Nonpareil' x 'Eureka' backcross 'Sonora' showing greater similarity to 'Mission'.

The important 'take-home' message from this type of study, however, is that the present cultivars in California represent a dangerously narrow gene pool. The narrownwss or smallness of the gene-pool results from most cultivars being derrived from crosses between *Nonpareil* and *Mission* (further evidence of this given in the next section). The danger of this very limited genetic base is that it makes the entire industry vulnerable to weaknesses to insect and disease problems (as with bud-failure and *Monilinia* flower blight). The limited genetic base also may not possess the new tree and nut traits required for the evolving California industry (e.g. self-compatibility, self-fruitfullness, etc.). To inject new genetic vitality into the breding program, efforts have been made to identify and incorporate germplasm from ourside the traditioal California material for crossing to elite California material. A summary of some of the more important genetic additions presently being used in the breeding program is provided in the section below.

Item	Notes
36-52	-Self-fertile; 46%-Seal; 56% Kernel/nut; Marcona Kernel, good flavor, 54-39E F7 5-9,10
45-8	-Self-fertile; very large tree; 11%-Seal; 46% Kernel/nut; productive, large nuts, precocious product. F7 5-11,12
54-39E	-Self-fertile; 1-1,2 medium tree; very high crop; 35%-Seal; 9 F7 1-1,2
56-89	-very large tree; very high crop; 10%-Seal; 2%-Doubles; 45% Kernel/nut, very high quality and yield, F7 1+2-11+12

59-1 -Self-fertile; small tree; very high crop; 25%-Seal; 17%-Doubles; 1%-Worm damage; 31% Kernel/nut; F7 3-11,12

7906-13 -3-18 large tree; very high crop; medium nut quality; blooms ~3 days after *Nonpareil*; 62%-Seal; 0%-Doubles; 49% Kernel/nut; *(Mission x P. fenzliana) x Sonora*, F10D 3-18

7906-22 -5-24 large tree; high crop; blooms ~blooms ~3 days after Nonpareil; days before *Nonpareil*; 78%-Seal; 8%-Doubles; 57% Kernel/nut; F10D 5-24

7906-33 -Self-fertile; medium tree; very high crop; medium nut quality; blooms ~5 days before *Nonpareil*; 100%-Seal; 2%-Doubles; 27% Kernel/nut; *(Mission x P. fenzliana x) x Sonora* F10D 3-1

7914-26 -1-4 large tree; medium to low crop; medium nut quality; blooms ~4 days before Nonpareil; 98%-Seal; 56% Kernel/nut; *(Mission x P. webbii) x Sonora*, productive F10D 1-4

7915-88 -3-4 medium tree; 100%-Seal; 16%-Doubles; 0%-Worm damage; 55% Kernel/nut; thin hard shell, *Mission x P. webbii*; F2 F10D 3-4

7916-9 -5-23 large tree; high crop; blooms ~3 days after *Nonpareil*; 84%-Seal; 0%-Doubles; 2%-Worm damage; 55% Kernel/nut; F10D 5-22

7920-45 -Self-fertile; 3-3 small tree; high crop; medium nut quality; blooms ~4 days before *Nonpareil*; 100%-Seal; 53% Kernel/nut; *(Mission x P. argentia) x Sonora* F10D 3-3

7920-61 -7-24, good tree and nut, twins~12%; F10D 7-24

1-100W -medium tree; medium crop; Low BF, productive late bloom; F7 3-2,3

1-102W -medium tree; medium to low crop; Productive, very late bloom; F7 5-6

1-87W -medium to low crop; *Mission x Paxman* late bloom; F7 2-4,5

2-19E -medium tree; medium crop; 49 %-Seal; 50 % Kernel/nut; *Tardy x Arbuckle*, California type, late bloom; F7 4-8,3-10

2-43W -medium tree; medium crop; 44 %-Seal; 10 %-Doubles; 0 %-Worm damage; 55% Kernel/nut; *Tardy x Arbuckle*, Calif type, bright + productive late bloom; F7 3-4,5

25-75 -Self-fertile; Self-pollinating; large tree; high crop; 25%-Seal; 5%-Doubles; 1%-Worm damage; % Kernel/nut; , P. mira; productive; late bloom; F7 5-7,8

28-21 -Self-fertile; late bloom; F7 2-1,4-1

7923-55 -3-7 large tree; very high crop; low nut quality blooms ~4 days before *Nonpareil*; 24%-Seal; ZZBIT, x *Milow* F10D 3-7

7924-5 -1-11 large tree; medium crop; medium nut quality; blooms ~3 days after *Nonpareil*; 60%-Seal; 66% Kernel/nut; , *Milow* cross F10D 1-11

7927-54 -3-13 medium tree; very high crop; good nut quality; blooms ~2 days after *Nonpareil*; 100%-Seal; 2%-Doubles; 51% Kernel/nut; Like *Padre, Padre x (Mission x P. webbii)* F10D 3-13

7934.54 -1-2 medium crop; blooms ~1 days after *Nonpareil; F5,4-6 x Solano* F10D 1-2

7934-54 -large tree; high crop; medium nut quality; blooms ~3 days after *Nonpareil*; 96%-Seal; 0%-Doubles; 4%-Worm damage; 58% Kernel/nut; twins; F10D 1-7

8007-15 -5-26 large tree; high crop; blooms ~2 days after *Nonpareil*; 100%-Seal; 2%-Doubles; 0%-Worm damage; 29% Kernel/nut; FLVR? F10D 5-26

8007-24 -1-26 ZFLAT x *Mission* F10D 1-26

8011-11 -1-22 large tree; very high crop; medium nut quality; blooms ~blooms ~2 days after *Nonpareil*; days before Nonpareil; 90%-Seal; 44% Kernel/nut; California type; F10D 1-22

8011-22 -Self-fertile; 3-25 medium tree; very high crop; good nut quality; blooms ~3 days after *Nonpareil*; 0%-Seal; 0%-Doubles; 0%-Worm damage; 59% Kernel/nut; *(Nonpariel x P. webbii)* x almond) x (peach x almond) F10D 3-25

8021-95 -3-26 very high crop; medium nut quality; blooms ~3 days after *Nonpareil*; 100%-Seal; 0%-Doubles; 0%-Worm damage; 36% Kernel/nut; F10D 3-26

8024-12 -Self-fertile; 3-6 large tree; very high crop; medium nut quality; blooms ~4 days before *Nonpareil*; 8%-Doubles; 60% Kernel/nut; Z F10D 3-6

LeGrOP -F10D 10-3+4; Self-fertile; Compact tree; some doubles.

USDACP05-33 -Possible Self-fertile; OP large tree; very high crop; blooms ~2 days after *Nonpareil*; 100%-Seal; 54% Kernel/nut; F10D 5-4

B. Generate large progeny populations from crosses between breeding parents.

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 acccurately 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. Over 30,000 controlled crosses between selected almond lines possessing self-compatibility and/or good tree and nut characteristics were made in the Spring of 1996 resulting in approximately 9,000 seed. This seed is now being germinated for Spring, 1997 planting. Approximately 5,000 seed were planted in the Spring, 1996 with almost 3,000 of the better seedlings transplanted to Davis, CA evaluation blocks. Over 5,500 individual trees from 1993, 1994, and 1995 were evaluated this season, approximately 1/3 of which had begun to produce nuts. Tree and nut qualities were assessed, with the most promising items selected for self-compatibility studies in 1997.

Developing markers to characterize and tag specific genotypes and genes.

A total of 60 decamer RAPD primers were used of which 21 produced consistently reproducible bands among the 18 accessions used for this study (Table 2). Approximately two-thirds of the primers either did not amplify discrete products (only smears) and/or were not informative in distinguishing the groups tested. All cultivars are distinguishable by unique RAPD profiles generated by multiple primers except the putative bud-sport mutations previously described. These RAPD profiles support three distinct origins for the almonds tested: bud-sport mutations; progeny from the inter-breeding of early California cultivars; and progeny from out-crosses to non-Californian material.

Mutations. No differences in RAPD patterns were found within either the 'Mission' mutation group ('Mission' and 'Shaw Mission'), or the 'Nonpareil' group ('Nonpareil', 'McKenespy Nonpareil', 'BF-Nonpareil', 'Tardy Nonpareil', and 'Weststeyn'). A clear difference in 18 of the 37 markers was observed between these two mutation groups. The 'Shaw Mission' differs from 'Mission' by its expression of a distinctive die-back or failure of terminal buds during the initiation of winter dormancy. BF has been shown to be genetically controlled and heritable. This Bud-

Failure (BF) trait also distinguishes the 'McKenespy Nonpareil' (no BF expression) and 'BF-Nonpareil' (high BF expression) from standard 'Nonpareil' (variable expression). The 'Tardy Nonpareil' and 'Weststeyn' sports have a nut morphology similar to 'Nonpareil' but flower approximately 10 days later and differ also in tree structure and productivity. Crossing studies with 'Tardy Nonpareil' suggest polygenic control of the altered phenotype similar to the changes in tree structure and productivity recently reported distinguishable by RAPD analysis of cherry. The failure to discriminate among these almond clones suggests the mutations are localized and so discernable only in highly saturated genetic maps.

Progeny from interbreeding of early California cultivars. 'Carmel', which is listed by Brooks and Olmo as a bud sport or mutation of 'Nonpareil', has RAPD patterns more consistent with it's origin as a progeny of 'Nonpareil' and 'Mission'. Early California almond cultivars were commonly grafted onto almond seedling rootstocks, with 'Nonpareil' pollinated by 'Mission' being a common seed source. Shoot growth from the seedling rootstock sometimes produced limbs and nuts of good horticultural type. Most current California cultivars are believed to have originated in this manner and so it is probable that some novel phenotypes were mistakenly identified as bud-mutations or sports of the budded cultivar. A 'Nonpareil' x 'Mission' origin of 'Carmel' is further supported by earlier isozyme (H) and S-allele inheritance studies. RAPD profiles also allow placement the cultivars 'Peerless' and 'Thompson' in the 'Nonpareil' x 'Mission' group yet allow their differentiation from each other and from 'Carmel'. The cultivars 'Ne Plus Ultra', 'Padre' and 'Sonora' have similar RAPD band distribution patterns yet can be differentiated from this group by unique banding patterns such as those at C05-740, A14-520, and C11- 984 respectively. Somewhat surprisingly, recent self-incompatibility (S) allele analysis indicates that both 'Mission' and 'Ne Plus Ultra' have the S_5 self-incompatibility allele in common despite their reported origins in different times and places. The S-allele, which controls the gametophytic self incompatibility response has been reported to be highly polymorphic in nature. A common allele for 'Ne Plus Ultra' and 'Mission' thus suggests the possibility of consanguinity for these cultivars and so a risk of significant inbreeding in current California cultivars. Early reports suggest that 'Mission' was a seedling of a local variety known as 'Languedoc'. The seed from which 'Ne Plus Ultra', 'Nonpareil' and the other Hatch cultivars were selected has

independently been reported to have probably originated from the Languedoc region of France. Thus the soft-shelled characteristic for which this region was known may have encouraged independent selection from within the same initial germplasm. 'Ne Plus Ultra' has a similarity index of 0.789 with 'Mission' as compared to 0.666 between 'Ne Plus Ultra' and 'Nonpareil' and 0.555 between 'Nonpareil' and 'Mission' (Table 1). The presence of the S_5 incompatibility allele in California cultivars, such as 'Thompson' had previously been interpreted as having 'Mission as a probable parent. The identification of S_5 in 'Ne Plus Ultra' indicates that some of these cultivars may in-effect be sib-mating between 'Nonpareil' and 'Ne Plus Ultra', thus increasing the risk of both inbreeding depression, as well as an increased probability of passing on the Bud-failure and plum rootstock graft-incompatibility traits associated with the original Hatch cultivars. While the similarity-index (Table 1) and resultant dendogram (Fig. 1) show roughly equal similarities between 'Thompson' and both 'Mission' and 'Ne Plus Ultra' (0.809 and 0.789 respectively), an examination of individual RAPDs, particularly patterns in the primers A01-860, A20-1900, B01-680 and C05-740 virtually rule-out 'Ne Plus Ultra' as a possible parent with 'Nonpareil, while supporting a 'Nonpareil' by 'Mission' origin (Tables 2 and 3).

Progeny from out-crosses to non-Californian material. The remaining genotypes, 'Sonora', 'Padre', 'Trusito', UCD,8011-22, SB13,25-75, and SB6,56-89 demonstrate unique banding patterns which clearly distinguish them from the early California cultivar group. The frequency of unique bands is also in general agreement with their putative genetic origin. Thus 'Sonora' and 'Padre', which are the product of controlled outbreeding of 'Nonpareil' and 'Mission', have 3 band and 5 bands (respectively) which are distinct from the 'Nonpareil' - 'Mission' gene pool. "Trusito', an old, self-compatible Italian selection can be clearly distinguished from the 'Nonpareil' - 'Mission' gene pool by uniqueness at 4 of the 37 bands evaluated. 'Trusito', as well as the other self-compatible selections UCD,8011-22, SB13,25-75, and SB6,56-89, can be clearly differentiated from all cultivars tested by unique banding patterns and often unique individual bands. Examples include C06-320 for 'Trusito' and A04-369 for SB13,25-75. The peach out-group 'Nemared' shows unique bands for 6 of the 37 RAPD bands evaluated, supporting its distinct genetic origin.

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In addition to characterizing the genetic origins and diversity of almond material available for cultivar improvement in California, the use of DNA based markers will allows the ability to determine parentage of established seedlings and trees. This ability can bring efficiency to the breeding efforts by allowing mixed crosses to occur on the same tree (as might occur with caged self-compatible cultivars where bees are used to introduce pollen from another genetic source, - although some selfing would occur, we could identify seedlings trees resulting from such selfing and allow their early rouging). These markers could also be used to determine parentage of other unknown cultivars and to assess the contribution of different pollen sources (either pollinizer trees or enpollination)on subsequent seed set and yield.

C. Test genetic sources and strategies for developing improved varieties.

Self-compatibility

Most year-to-year variation in crop production appears due to differences in initial seed set, which in turn 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 self-compatibility 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, should 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. The use of self-compatible almond varieties, while still necessitating bee pollinators, should result in significant increases in pollinator efficiencies since virtually all bee visits will provide (self-)compatible pollen to the stigma.

The specific protein controlling self-incompatibility in California almond varieties has now been identified and isolated for 13 California almond cultivars (Table 4) which represent the majority of self-incompatibility types used commercially. Stylar proteins from these cultivars were surveyed by IEF and 2D-PAGE combined with immunoblot and N-terminal amino acid sequence analysis to identify *S*-RNases associated with each of the 6 major California incompatibility groups (Fig. 2). All four *S*-proteins involved could be successfully distinquished from each other in the highly basic zone of the gel. While the techniques used are too tedious for use in routine identification of cross-incompatibility groupings for new cultivars, such rapid and accurate groupings might be possible in the future if ELISA-type probes specific to these proteins can be developed. Such a molecular test would be rapid and accurate. Presently, each new cultivar has to be test-crossed with a representative of each known cross-incompatibility group; a process that takes considerable labor and several years to be confident in the results.

This study also confirmed the loss of function for one of the two *Nonpareil* incompatibility genes in the *Jefferies* bud-sport mutation. A more thorough understanding of how the *Jefferies* mutation functions may facilitate the genetic engineering of a self-compatible *Nonpareil*.

Resistance to Navel orangeworm.

Resistance to worm and fungal damage to almond kernels was analyzed for California almond varieties and important breeding lines. X-Ray studies (~~see Fig. 8~~) have shown the importance of an inner, thin but highly lignified endocarp rather than the thicker and more fibrous

outer endocarp or shell to worm resistance, supporting the feasibility of developing a worm resistant shell with high crack-out ratios.

High rates of navel orangeworm (NOW) larval mortality were observed in controlled feeding studies when fresh *Mission* or *Carmel* hulls were added to the diet. This natural insecticidal effect was not observed in most other varieties tested or when *Mission* hulls were dried when added to the insect diet, suggesting the insecticidal compounds may be volatiles. The identification of these hull compounds is being pursued in cooperation with USDA labs in Albany, CA, and if successful could allow the breeding of natural insecticide like compounds into future varieties. Additional 1996 insect feeding studies, where NOW was reared on kernel and hull tissue of approximately 20 almond cultivars and breeding lines continued to show variation in level of resistance, though the initial ranking of cultivar resistance does not appear to be consistent with 1995 results. This was not too surprising given our recent recognition of the importance of volatiles in NOW resistance (which would be partially lost in many of our 1996 samples due to a drying pre-treatment employed to reduce the high levels of mold contamination experienced in 1995). Feeding study evaluations are not yet complete, however, and the final results will not be available for statistical analysis until late June, 1997, (available upon request). Interestingly, the Spanish cultivar *Terragona* continues to show high levels of NOW larval development in hull tissue bur consistently low levels of NOW development when larvae are reared on kernel tissue.

Almond cultivar Fatty-acid and Vitamin E composition

Major varieties and breeding lines were evaluated for fatty-acid and vitamin E (alpha-tocopherol) composition and stability. Total fat content and fatty-acid methyl esters (FAMEs) were determined according to the procedure of Garces and Mancha [detailes available on request]. This method allowed complete oil extraction and fatty acid trans-methylation in the same tube. A 100mg kernel tissue sample and 0.5 ml (10g-l-1 in methanol) of margaic acid (17:0) were boiled at 80C for 2 hr under N2 gas with a reagent mixture containing methanol:heptane:benzene:2,2-dimethoxypropane:H2SO4 (37:36:20:5:2, by volume). After cooling at room temperature two phases were formed. The upper containing FAMEs was collected. Separation of the FAMEs was done by using FID-GC equipped with SP-2330 column

(30 m, 0.25mm I.D.,0.2um film thickness) (Supelco, Bellfonte, PA). The FAMEs were identified based on Rf of known standards (Sigma). The presence of 17:0 as an internal standard allowed the calculation of total lipids based on the area of the standard.

Results using this techniques were identical to results obtained from the much more tedious cold-press extraction technique, and appear to offer improved product resolution in addition to ease of analysis. Oil content of almond kernels varied significantly among cultivars between the 1995 and 1996 season samples. (Table 5 and Fig. 3) with oil content in 1995 being higher than 1996. Breeding lines incorporating non-traditional almond germplasm demonstrate higher total oil contents, greater year-to-year uniformity and a more desireable fatty-acid composition. Almond oil consists of 5 fatty-acids: palmetic (16:0), palmetoleic (16:1), stearic (18:0), oleic (18:1), and linoleic (18:2). Linolenic acid (18:3) was found in trace amounts in a few samples (<0.02%, data not shown). Minor differences were found in the proportions of 16:0, 16:1 and 18:0 fatty acids. Palmetic acid was found to be the major saturated fatty acid in all cultivars and breeding lines (ranging from 5-7.3%), which did not vary greatly between cultivars. Palmetoleic acid was found in minute concentrations (0.26-0.72%). Stearic acid is also a minor saturated fatty-acid ranging from 1.1-3.1%.

The major difference between cultivars and breeding lines was found in the proportions of the mono-unsaturated fatty-acid (MUFA), *Oleic* 18:1, and the poly-unsaturated fatty-acid (PUFA) *Linoleic* 18:2. The proportion of PUFA was the highest and ranged from ~62 to 76%. All cultivars and breeding lines contained a similar proportion of MUFA except *Rosetta, Price, Ne Plus Ultra, Padre,* and *Sonora*. It is noticeable that all breeding lines contained very high proportions of MUFA in both 1995 and 1996, which are desirable as they appear to lower the unhealthy low-density-lipoprotein cholesterol and so total cholesterol without altering the beneficial high-density-lipoprotein-cholesterol levels. The proportion of PUFA followed almost exactly the opposite trend in MUFA. A high correlation was found between MUFA and PUFA levels (Fig. 4). Results indicate that California almonds contain very low (<10%) saturated fatty-acids, high MUFA and low PUFA. As in other oil-seeds, there is a strong negative correlation between oleic (18:1 or MUFA), and linoleic acids (18:2 or PUFA). The pool of oleic acid appears to be controlled by its conversion to linoleic acid possibly by oleic desaturase.

Oil content varied according to orchard location (Fig. 5) though differences do not appear to be due to sample moisture content which ranged between 2-3% for all locations. Surprisingly, in a separate set of experiments using almond samples of selected cultivars held in cold storage for up to 12 years, no significant differences were observed over time for both total oil content and Linoleic/Oleic composition regardless of whether samples were stored shelled or in-shell (Table 6, Fig. 6). Clear differences in the inhibition of lipid oxidation was observed in the samples stored for longer than 10 years even though alpha-tocopherol (vitamin E) levels remain fairly constant for all samples tested (Fig. 7).

In addition to the possible health benefits of the very low linolenic levels and lower linoleic-to-oleic ratios identified, such ratios are typically associated with reduced susceptibility to oxidative deterioration and so rancidity of nut-meats and/or almond oil resulting in a longer storage life and possibly greater mold resistance.

Synthesis of almond chimeras.

Chimeric *Nonpareil* trees possessing an epidermal layer from *Nemared* peach have been developed through a new technique of meristem micro-grafting. This approach may lead to more rapid strategies for improving insect and disease resistance and for transferring self compatibility to established almond varieties without the risk of changing their kernel qualities. If this approach is found to be broadly applicable to different almond varieties and epidermal donor species, it could greatly complement or even replace genetic-engineering approaches which can result in some adulteration of kernel quality. *Nonpareil* periclinal chimeras have now been field planted for production evaluation beginning about 1999. Present success at synthesizing these chimeras is very low and work continues on improving overall efficiencies.

D. D. Develop evaluation guidelines for characterizing nut and tree and nut quality.

Preliminary guidelines for characterizing nut and tree quality have been developed and summarized in the 1995 Annual Report. Evaluation criteria have now been given numeric codes to allow rapid and accurate field data collection onto small hand-held computers. These criteria have been slightly modified to facilitate field use but remain essentially the same as previously reported. Several years of data collection are needed to assess year-to-year and location

consistency of specific tree and nut criteria, and to identify seedling criteria useful in predicting mature tree performance. Digital images of seedling growth habit of selected progeny planted in 1997 will also be recorded for comparison to later digital images of mature tree structure. Such digitizing allows the use or powerful image analysis and statistical procedures for identifying seedling predictors of mature architecture.

Shell-seal evaluation

As previously discusses, a modification of an X-Ray nut monitoring technique developed by Tom Schatzki at the USDA/ARS Albany, CA lab (the technique was developed partly in response to an ABC funded project) allows the nondestructive evaluation of almond shell seal discontinuities leading to insect infestation. Using this technique, tens of thousands of nuts can easily be screened for worm damage, and where identified, the point of entry can be precisely determined. (A sample image of in-shell *Mission* is shown in ~~Fig.~~ 8). Even greater efficiencies are being pursued through the development of techniques to capture digitized X-Ray images rather than the X-Ray transparency film presently used.

Noninfectious Bud-Failure identification

The potential for developing Noninfectious Bud-Failure in new cultivar sources and candidates for cultivar release is presently determined by a test-crossing technique requiring several hundred crosses and up to 7 years evaluation of subsequent seedling performance. A new technique using flower symmetry (the uniformity of flower petal size) to assess shoot growing point fitness has allowed accurate prediction of high bud-failure potential in clonal sources which had not yet shown any outward signs of bud-failure. Additional tests of are planned for 1997-98. If successful, this approach might not only allow more efficient bud-failure screening, but could lead to a better understanding of the epidemiology and genetic nature of noninfectious-bud-failure and so the possibility of ELISA-type molecular screening opportunities.

Table 1 - Similarity matrix for almond genotypes based on the proportion of shared fragments generated using the NTSYS Program.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Padre	1.000																	
2. Sonora	0.555	1.000																
3. Peerless	0.631	0.857	1.000															
4. Thompson	0.594	0.829	0.883	1.000														
5. Trusito	0.529	0.578	0.650	0.666	1.000													
6. Ne Plus Ultra	0.484	0.756	0.820	0.789	0.571	1.000												
7. Carmell	0.742	0.717	0.780	0.700	0.594	0.666	1.000											
8. UCD,8011-22	0.666	0.647	0.666	0.628	0.562	0.645	0.727	1.000										
9. SB6,56-89	0.666	0.648	0.615	0.526	0.628	0.588	0.555	0.709	1.000									
10. SB13,25-75	0.685	0.615	0.731	0.650	0.648	0.500	0.684	0.545	0.555	1.000								
11. Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000							
12. Mckenespy Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000						
13. BF-Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000	1.000					
14. Tardy Nonpareil	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000	1.000	1.000				
15. Weststyn	0.742	0.615	0.731	0.700	0.648	0.666	0.789	0.848	0.666	0.684	1.000	1.000	1.000	1.000	1.000			
16. Mission	0.486	0.780	0.790	0.809	0.564	0.789	0.700	0.514	0.578	0.550	0.550	0.550	0.550	0.550	0.550	1.000		
17. Shaw Mission	0.486	0.780	0.790	0.809	0.564	0.789	0.700	0.514	0.578	0.550	0.550	0.550	0.550	0.550	0.550	1.000	1.000	
18. Nemared	0.413	0.363	0.457	0.470	0.451	0.333	0.500	0.370	0.266	0.500	0.500	0.500	0.500	0.500	0.500	0.294	0.294	1.000

Table 2. Amplification fragments analyzed, sorted by size.

Primer	C06	C06	A04	A09	A04	A14	B02	A08	B13	C10	A14	A05	A04	C06	B01	B10	A14	A09	C05	A10	A01	B10	A11	A14	A18	C11	A19	B04	A14	B10	B05	C11	C05	A01	A20	B10	B05
Size	260	320	369	400	400	440	450	480	500	500	520	630	640	680	680	680	700	738	740	850	860	860	870	960	970	984	1230	1230	1290	1400	1476	1500	1500	1800	1900	1900	1960
PADRE	0	0	1	0	1	1	0	1	0	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1	1	0	0	1	0	0	1	0	1	0	0	1	0
SONORA	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	1	1	1	0	0	1	0	0	1	1	0	0	1	0	1	1	1	1	1	1	1
THOMPSON	0	0	1	0	1	1	0	0	0	1	0	0	0	1	1	0	1	1	1	1	1	1	1	0	0	1	1	0	0	1	0	1	1	1	1	1	1
PEERLESS	0	0	1	0	1	1	0	0	0	1	1	0	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	1	1
NE PLUS ULTRA	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	0	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1
CARMEL	0	0	1	0	1	1	0	0	1	1	0	0	0	1	0	0	1	1	0	1	0	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1
NONPAREIL	0	0	1	0	1	1	0	1	0	0	0	0	1	1	0	1	1	1	1	0	0	1	1	1	1	0	1	1	0	1	1	0	1	0	0	1	1
McEN NONPAREI	0	0	1	0	1	1	0	1	0	0	0	0	1	1	0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	1	1	0	1	1	0	1	1
BF NONPAREIL	0	0	1	0	1	1	0	1	0	0	0	0	1	1	0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	1	1	0	1	1	0	1	1
TARDY NONPAR	0	0	1	0	1	1	0	1	0	0	0	0	1	1	0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	1	1	0	1	1	0	1	1
WESTSTEYN	0	0	1	0	1	1	0	1	0	0	0	0	1	1	0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	1	1	0	1	1	0	1	1
MISSION	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	1	1	1	1	0	1	1	1	0	0	0	1	0	1	1	0	1	1	0	1	1
SHAW MISSION	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	1	0	1	1	1	1	1	0	1	0	1	0	1	1	1	1	0	0	1	1	1
TRUSITO	0	1	1	1	1	1	0	0	1	1	0	0	0	1	1	0	1	0	1	1	1	1	1	0	1	0	1	0	1	1	1	1	0	0	1	1	1
SB13, 25-75	1	0	0	1	1	1	0	0	1	0	1	0	0	1	0	1	1	1	1	0	1	0	1	1	1	0	1	0	1	1	1	1	0	0	1	1	1
SB6, 56-89	0	0	1	1	1	1	0	1	1	0	1	0	0	1	0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1
F5, 19-13	0	0	1	0	1	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	1	0	1	1	0
NEMARED	0	0	1	1	0	0	1	0	0	1	0	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0

Table 3 - Possible origins for almond cultivars on the basis of RAPD analysis.

	Thompson	Carmel	Padre	Sonora	Trusito
Non Pareil x Mission	YES	YES	YES	NO	NO
Non Pareil x Ne Plus Ultra	NO	NO	NO	NO	NO
Non Pareil x Peerless	NO	NO	NO	NO	NO
Mission x Ne Plus Ultra	NO	NO	NO	NO	NO
Mission x Peerless	YES	NO	NO	NO	NO
Ne Plus Ultra x Peerless	NO	NO	NO	NO	NO

Table4 . Almond Cultivars used and their *S*-genotypes.

CIG	Genotype	Cultivar
I	*ScSd*	IXL, Nonpareil, Tardy Nonpareil
II	*SaSb*	Languedoc, Mission
III	*SaSc*	Sauret no.2, Wood Colony
IV	*SbSc*	Merced, Rosetta
V	*SaSd*	Carmel, Sauret no.1
VI	*SbSd*	Monterey
None	*Sc^{m}Sd*	Jeffries

Table 5

	Oil %		C 16:0		C 16:1		C 18:0		C 18:1		C 18:2	
Cultivars	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
LeGrand	51	40	6.1	5.8	0.43	0.43	2.0	1.5	71.9	76.0	19.5	16.3
Rosetta	42	42	6.6	5.4	0.41	0.37	1.3	1.6	67.0	73.4	24.7	19.2
Price	38	39	6.3	5.0	0.42	0.41	1.5	1.5	65.6	72.8	26.3	20.3
Mission	52	48	5.6	5.3	0.30	0.31	2.0	1.8	70.4	71.9	21.7	20.6
Thompson	44	42	6.0	5.6	0.31	0.41	2.0	1.6	72.6	71.5	19.1	20.8
Ne Plus Ultra	50	46	6.3	6.1	0.33	0.49	1.9	1.3	65.5	70.7	26.0	21.4
Padre	52	51	6.1	5.3	0.26	0.33	2.3	1.6	64.8	70.9	26.5	21.8
Aldrich	52	49	6.2	5.5	0.35	0.31	1.4	1.4	68.9	71.0	23.1	21.8
Fritz	49	39	6.5	5.8	0.49	0.44	1.3	1.3	68.0	70.6	23.7	21.8
Wood Colony	44	47	6.2	5.6	0.44	0.42	1.3	1.1	72.0	70.5	20.1	22.4
Sauret	43	39	6.5	5.8	0.54	0.48	1.5	1.2	69.8	70.2	21.5	22.3
Sonora	41	44	7.3	5.8	0.47	0.36	1.6	1.6	61.7	69.3	28.9	22.9
Monterey	48	37	6.7	6.3	0.41	0.51	2.2	1.4	68.2	67.5	22.5	24.2
Nonpareil	48	40	6.8	6.4	0.54	0.49	1.6	1.2	68.1	66.8	23.0	25.0
Carmel	49	41	6.4	6.0	0.38	0.48	1.3	1.4	62.4	66.6	29.5	25.5
Butte	53	48	6.6	6.2	0.72	0.51	1.8	1.6	63.9	64.7	27.0	26.9
Peerless	44	39	6.5	5.8	0.41	0.45	1.4	1.3	65.8	64.6	25.8	27.7
Breeding Lines												
F7,5-9+10	47	45	5.7	5.9	0.52	0.55	1.6	1.5	76.8	73.0	15.4	19.1
F7,1-1+2	46	46	6.2	5.8	0.47	0.46	1.6	1.4	74.1	72.8	17.6	19.5
F7,5-11+12	47	46	5.6	6.3	0.40	0.42	1.6	1.3	75.7	71.3	16.6	20.6
F7,5-7+8	42	47	6.6	6.7	0.43	0.44	2.3	1.9	64.0	61.8	26.7	29.2
F7,5-10	-	51	-	6.1	-	0.60	-	1.4	-	75.0	-	17.0
F10D 3+4-3	-	55	-	5.8	-	0.41	-	3.1	-	73.4	-	17.3
F7,5-12	-	53	-	6.2	-	0.41	-	1.3	-	73.9	-	18.1
F10D 4 25	-	48	-	6.1	-	0.64	-	1.5	-	72.4	-	19.3
F7,1-11	-	48	-	5.8	-	0.52	-	1.9	-	70.8	-	21.0
F7,3-12	-	53	-	6.3	-	0.42	-	1.8	-	70.3	-	21.2
F7,7-12	-	51	-	7.0	-	0.72	-	1.3	-	68.9	-	22.1
F7,6-12	-	50	-	7.1	-	0.63	-	1.2	-	68.5	-	22.6
F7,2-9	-	50	-	6.2	-	0.62	-	1.2	-	67.9	-	24.0
LSD (5%)	5	3	0.7	0.6	0.22	0.12	0.3	0.3	3.0	3.2	2.7	2.8

Table 6 Comparison of almond oil content and quality as influenced by the presence of the shell during storage at 5°C. Almonds have been in storage since 1984.

Cultivar	Oil %	16:0	16:1	18:0	18:1	18:2
Carmel						
In-shell	50 ±1	5.6 ±0.2	0.34 ±0.01	1.9 ±0.3	69.1 ±3.4	23.0 ±3.2
Kernel	48 ±0.3	5.8 ±0.2	0.41 ±0.01	1.6 ±0.3	68.2 ±0.01	23.9 ±0.2
Nonpareil						
In-shell	48 ±2	5.3 ±0.3	0.50 ±0.13	1.5 ±0.1	76.3 ±1.7	16.4 ±1.3
Kernel	49 ±4	5.4 ±0.2	0.50 ±0.02	1.6 ±0.3	75.4 ±1.7	17.1 ±1.5
Sonora						
In-shell	52 ±2	5.3 ±0.5	0.36 ±0.04	1.6 ±0.1	70.1 ±4.3	22.7 ±3.9
Kernel	53 ±1	5.7 ±0.4	0.37 ±0.04	1.6 ±0.5	69.3 ±1.9	23.0 ±2.1
Mission						
In-shell	45 ±3	5.1 ±0.2	0.32 ±0.06	2.2 ±0.3	73.3 ±5.2	19.1 ±5.0
Kernel	48 ±3	5.2 ±0.3	0.43 ±0.05	1.9 ±0.2	76.9 ±0.5	15.5 ±0.6

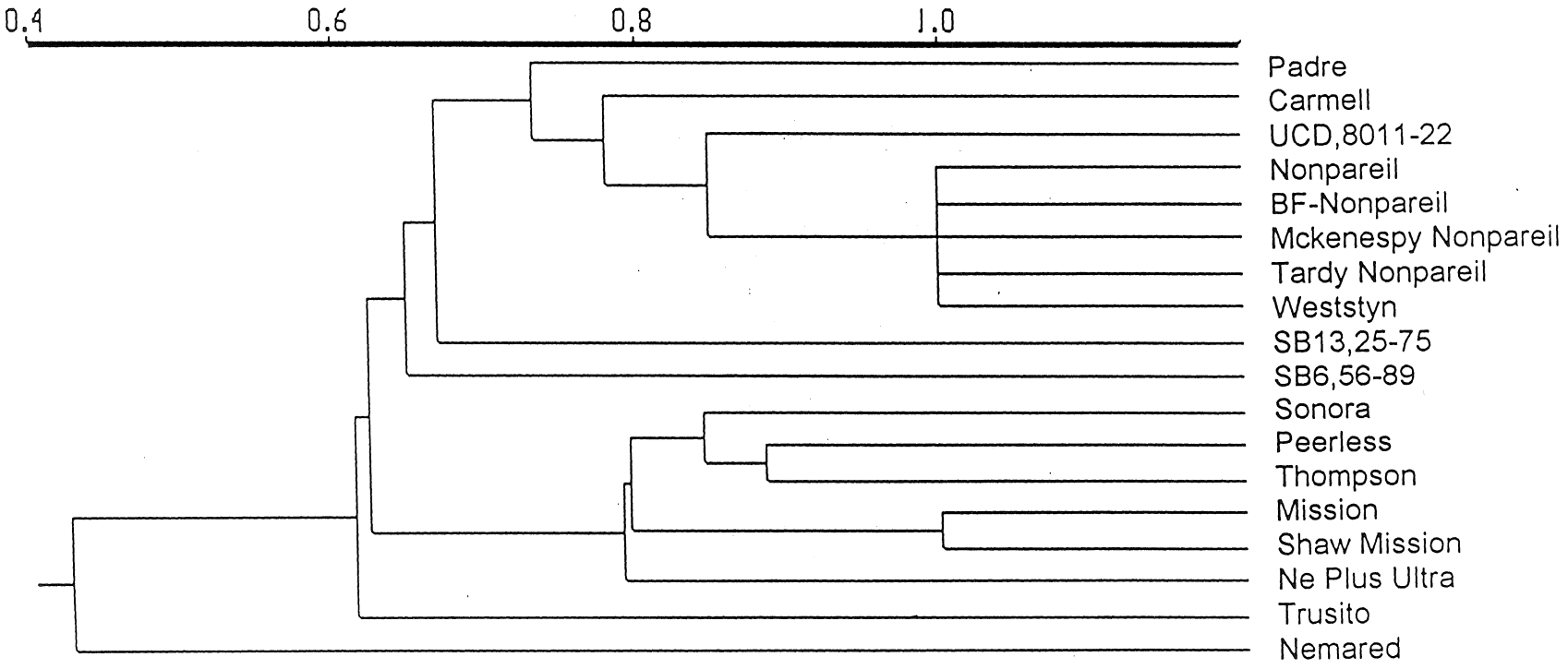

Fig. 1 - Dendrogram based on the similarity index data for 18 almond genotypes.

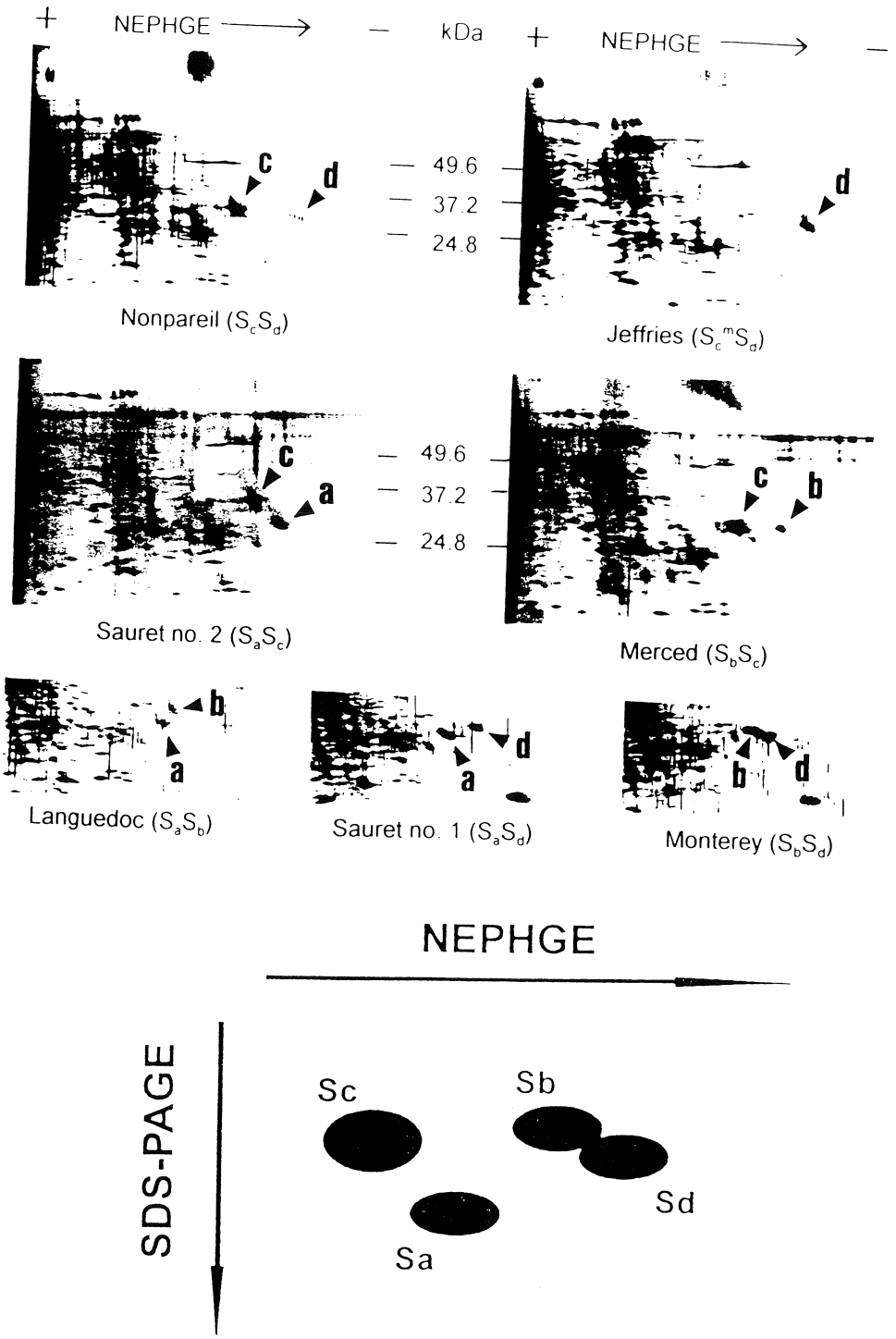

Fig. 2 Spacial distribution and relative intensities of almond *S*-proteins in 2D-PAGE profiles.

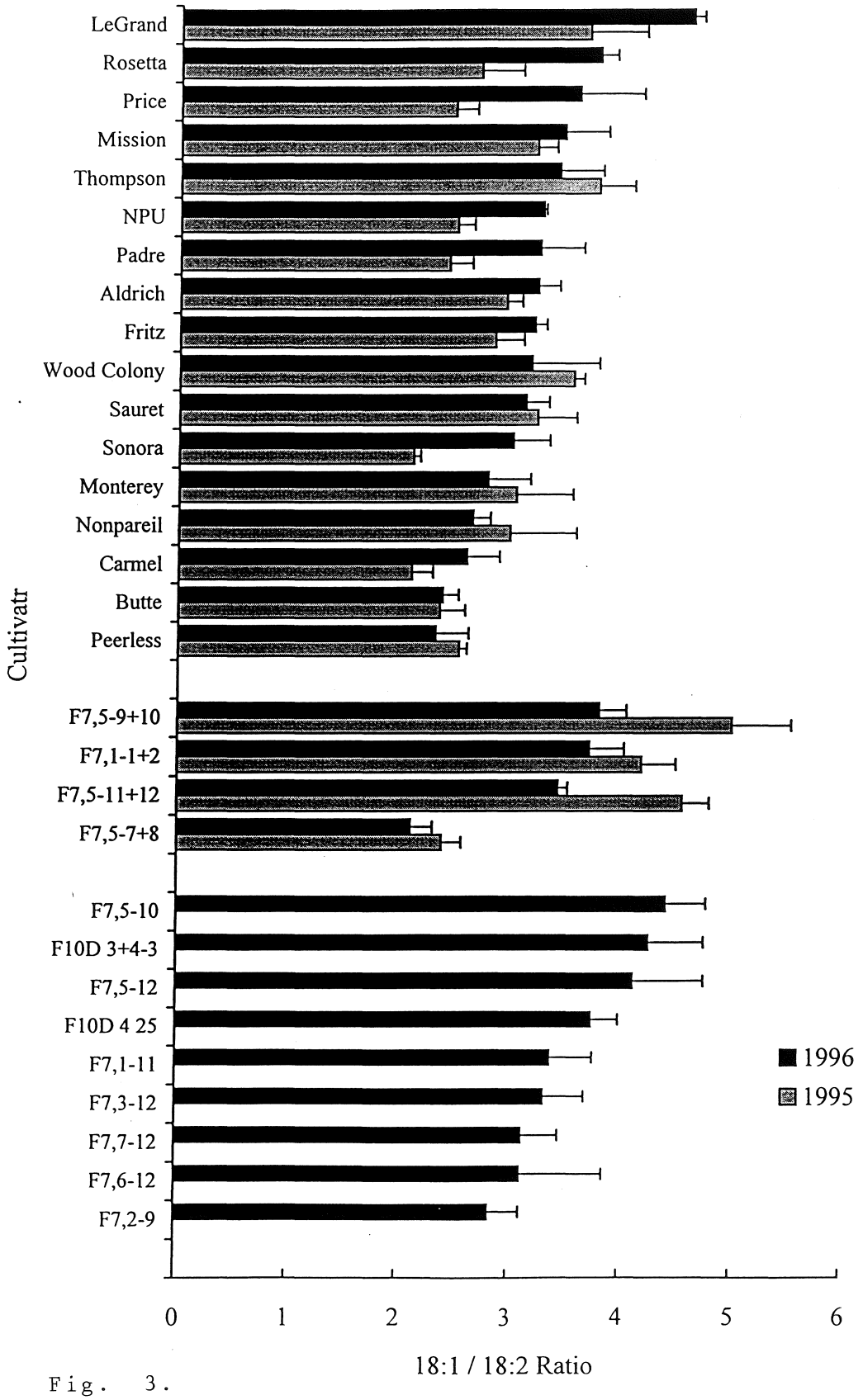
LeGrand
Rosetta
Price
Mission
Thompson
NPU
Padre
Aldrich
Fritz
Wood Colony
Sauret
Sonora
Monterey
Nonpareil
Carmel
Butte
Peerless
F7,5-9+10
F7,1-1+2
F7,5-11+12
F7,5-7+8
F7,5-10
F10D 3+4-3
F7,5-12
F10D 4 25
F7,1-11
F7,3-12
F7,7-12
F7,6-12
F7,2-9
Cultivatr
1996
1995
0
1
2
3
4
5
6
18:1 / 18:2 Ratio

Fig. 3.

(4)

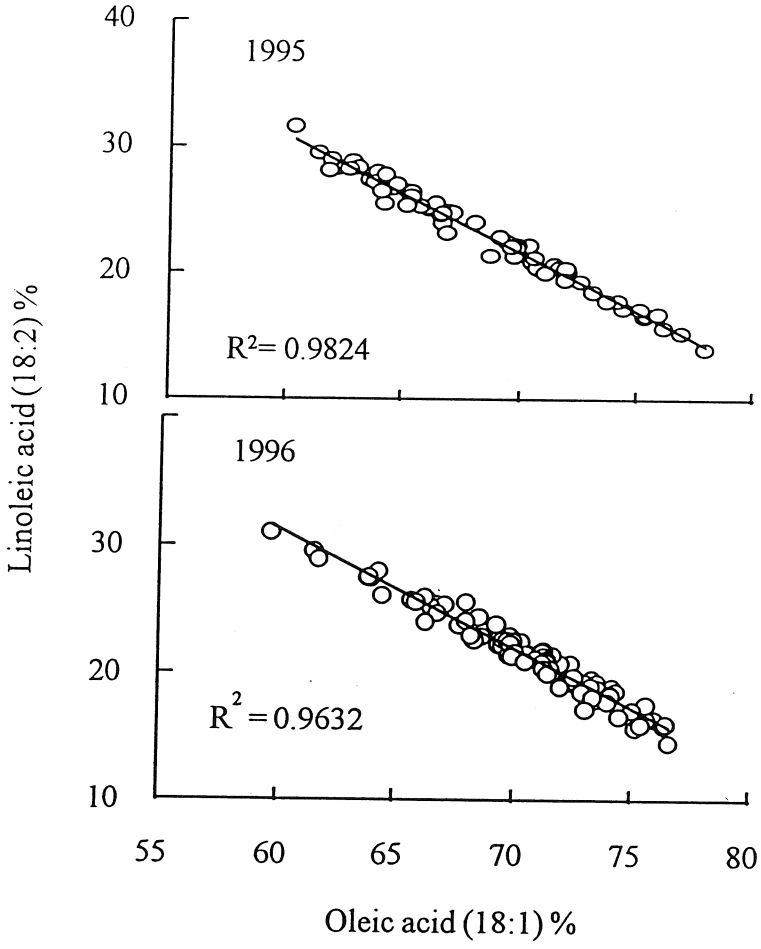
40
30
20
10
1995
R²= 0.9824
1996
30
20
10
R² = 0.9632
55
60
65
70
75
80
Linoleic acid (18:2) %
Oleic acid (18:1) %

Fig. 4

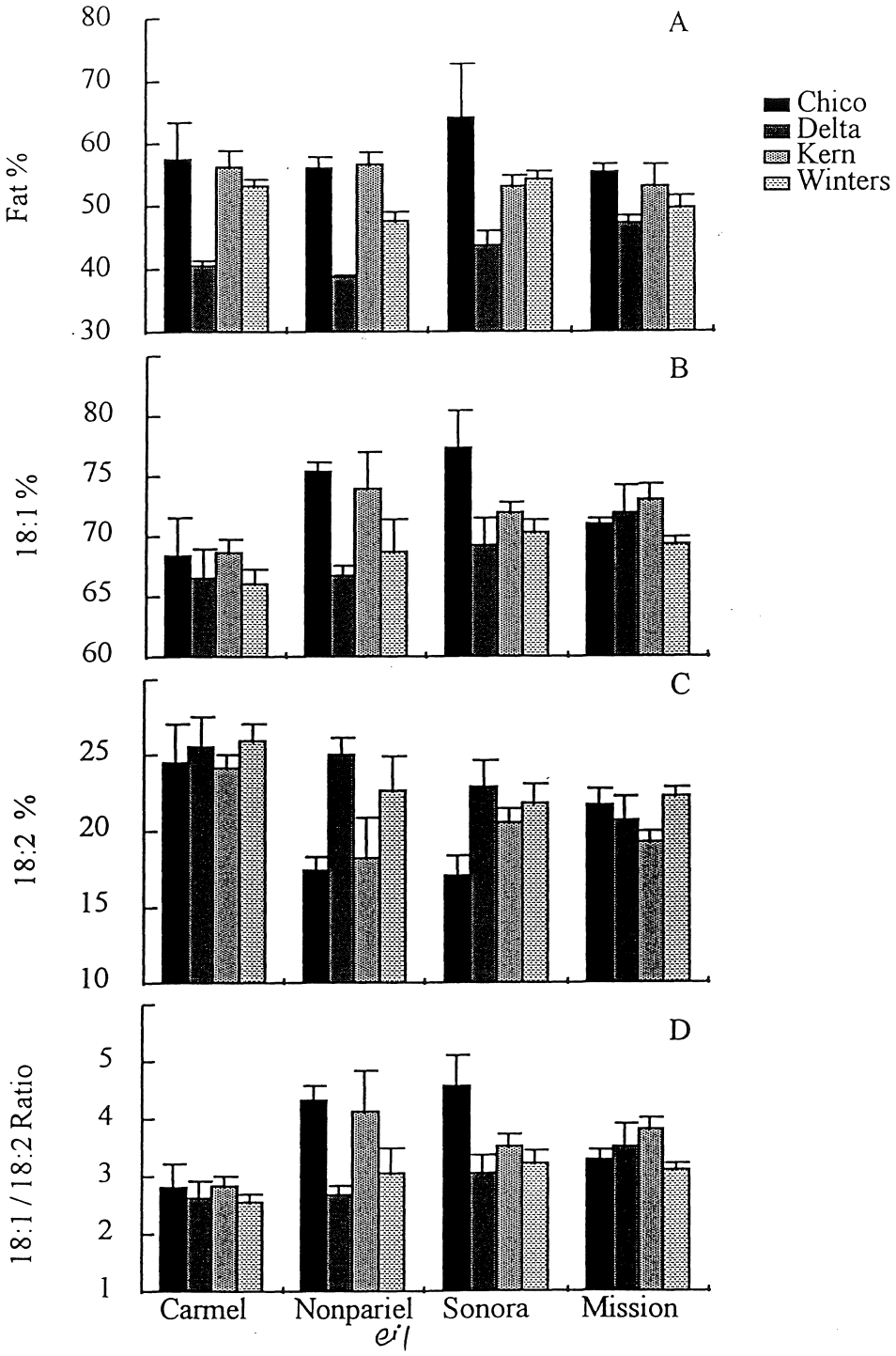
A
B
C
D
Fat %
18:1 %
18:2 %
18:1 / 18:2 Ratio
80
70
60
50
40
30
75
65
25
20
15
10
5
4
3
2
1
Chico
Delta
Kern
Winters
Carmel
Nonpariel
Sonora
Mission
oil

Fig. 5.

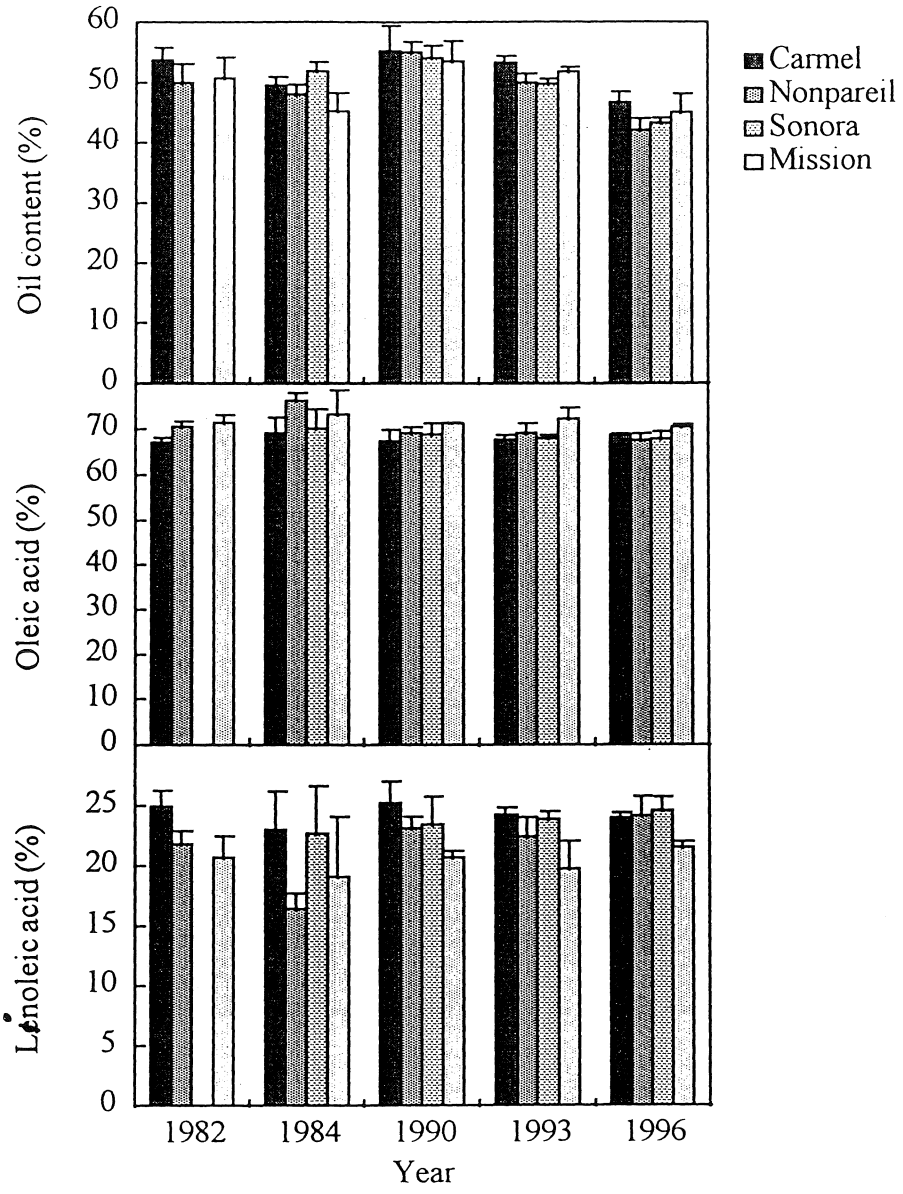
Oil content (%)
Oleic acid (%)
Linoleic acid (%)
Carmel
Nonpareil
Sonora
Mission
1982
1984
1990
1993
1996
Year

Fig. 6.

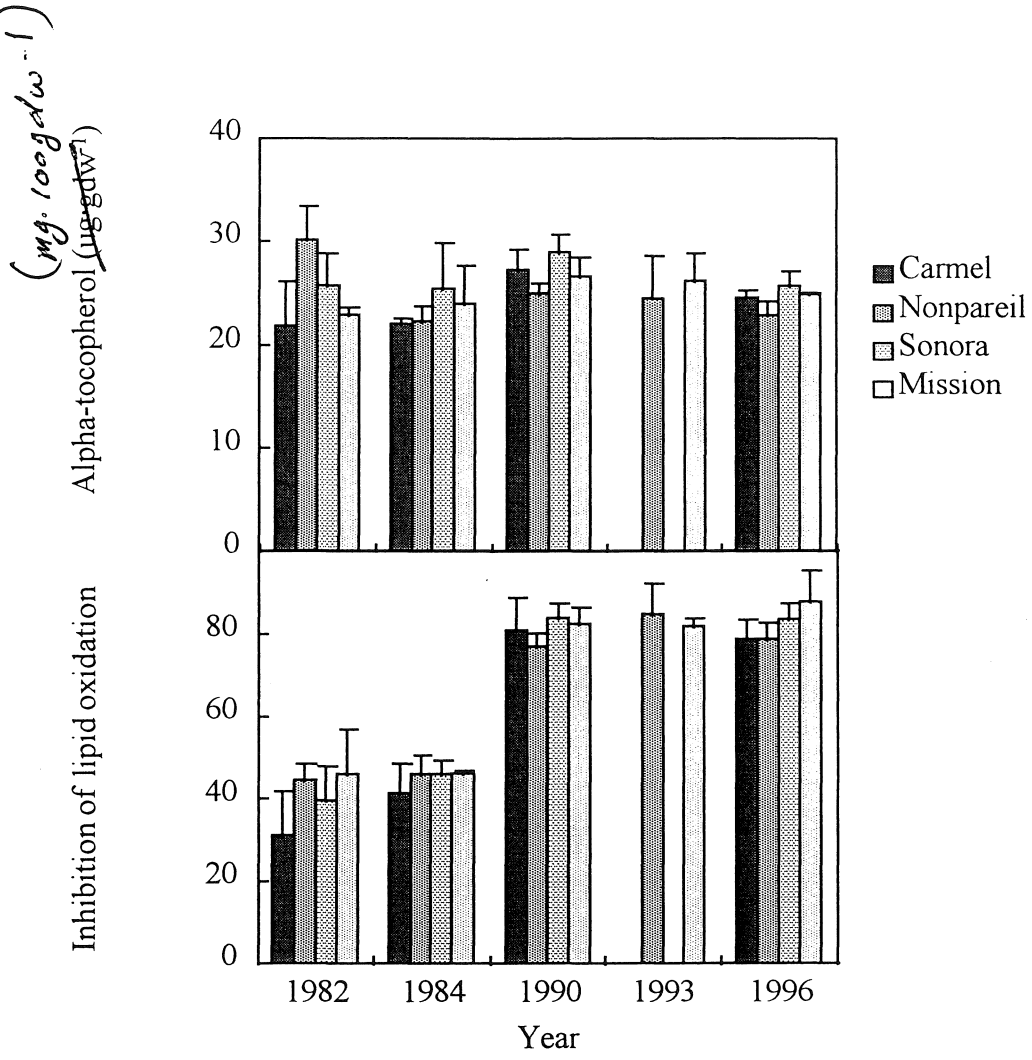
Alpha-tocopherol (μg gdw⁻¹)
Inhibition of lipid oxidation
40
30
20
10
0
80
60
40
20
0
1982
1984
1990
1993
1996
Year
Carmel
Nonpareil
Sonora
Mission

Fig. 7.