

Yield, pollination aspects and kernel qualities of almond (*Prunus dulcis*) selections trialed in the southern San Joaquin Valley

ERIC MERCURE¹, JÚLIA HALÁSZ², ATTILA HEGEDŰS² AND CRAIG LEDBETTER³

Additional index words: alternate bearing, bloom phenology, double kernel, *S*-allele profile, variety trial

Abstract

A field trial was established in the southern San Joaquin Valley to determine yield potential for nine almond selections grown under commercial conditions. Kernel yields were first quantified in 2008 at the end of the third growing season and continued through the 2010 harvest. Harvested tonnage varied significantly, depending on the particular accession, specific field location and harvest year. The almond accessions were genotyped for *S*-locus alleles, characterized phenologically during bloom, and analyzed for kernel dimensions, pellicle color profiles, nut defects and harvest quality parameters. Bloom duration and overlap of the nine experimental accessions generally coincided with that of 'Nonpareil' during the three examined bloom periods. Analysis of *S*-alleles revealed semi- or cross-incompatible pairs with some commercial cultivars, and confirmed the self-compatibility status of four of the trialed selections. Harvest year effects were significant on all examined kernel shape and appearance characters as well as all nut defects and harvest quality parameters except for the level of blank and shriveled kernels. Cumulative kernel yield differences and specific character faults have led to the removal of five accessions from the trial after three harvest seasons.

The trialing of newly bred or introduced plant accessions is common to most crops and growing regions, with the California almond industry being no exception. The almond industry in California continues to expand, and variety trials, both formal and producer-initiated, are aimed at finding those specific cultivar combinations that maximize kernel yields in a specific growth environment. The University of California Regional Variety Trials (RVT) for almond have been used successfully in past decades to examine bloom synchronicity, yield potential and specific nut qualities of new and standard cultivars in a single environment (Lampinen et al., 2002; Micke et al., 1998). Historically, RVTs have been planted in the northern, central and southern regions of California's central valley system because of specific edaphic and

environmental differences within the broad region. Data were collected from RVTs for many years to examine varietal consistency and provide cumulative yield analysis in this occasional alternate bearing tree nut crop. However, RVTs are established infrequently, thus creating bottlenecks for recently developed advanced almond selections in need of validation trialing.

Producer-initiated variety trials can fill the gap between the infrequent RVT plantings, and in some cases provide better estimates of kernel yield in commercial plantings. The trialing of self-compatible almonds is a good example in that pollination isolation is necessary to quantify yield in a single-cultivar situation (Kodad and Socias i Company, 2008; Socias i Company et al., 2005), something untenable in the larger RVT plantings. Self-

¹ Paramount Farming Company, 33141 E. Lerdo Highway, Bakersfield, CA 93308.

² Corvinus University of Budapest, Department of Genetics and Plant Breeding, P.O. Box 53, Budapest, H-1518, Hungary

³ United States Department of Agriculture, Agricultural Research Service, Crop Diseases, Pests & Genetics Research Unit, 9611 S. Riverbend Avenue, Parlier, CA 93648-9757 craig.ledbetter@ars.usda.gov.

compatible selections can be isolated from other potential pollenizers through geographical separation, non-synchronicity of bloom, incompatibility of *S*-alleles and bagging/caging (exclusion of pollination vectors) of self-compatible selections.

Almond selections developed at the Agricultural Research Service (ARS) in Parlier, California need yield evaluations prior to any decision on cultivar introduction. Recent breeding efforts at ARS have emphasized the use of 'Tuono' as a donor parent for self-compatibility, and California-adapted almond accessions for specific kernel quality characteristics. Progeny populations have been rogued of off-type seedlings (self-incompatible, extreme hardshell, bitter or poor quality kernels), leaving a small number of remaining trees. These selections have been evaluated for kernel quality characteristics as well as bloom and harvest phenologies, but yield analysis has not been logistically possible at the Parlier, CA location. Since no new RVTs were scheduled for establishment in the foreseeable future, it was deemed necessary to establish rapidly these selections in a producer-initiated trial to examine potential yield. Hence, a collaborative effort began in 2005 with an independent almond producer to examine the commercial potential of advanced almond selections.

In this research, our objectives were as follows: 1) quantify kernel yields of ARS almond selections under commercial orchard conditions in the southern San Joaquin Valley; 2) determine bloom phenologies and *S*-allele profiles for pollination compatibilities; and 3) compare nut and kernel quality characteristics among the evaluated accessions. All evaluations used 'Nonpareil' almond as a standard for comparison with the work being conducted from 2008 through 2010 growing seasons.

Materials and Methods

Plant materials. Nine unnamed almond accessions evaluated in this study were devel-

oped by the ARS in Parlier, California. Five of the accessions are self-incompatible, being developed in previous efforts, and used as parental germplasm for the development of new self-compatible cultivars (Ledbetter, 2010). The four remaining accessions are self-compatible, each having 'Tuono' as a common parent. Budwood of the nine accessions was collected during May 2004 and provided to Fowler Nursery Inc. (Newcastle, CA) for the development of nursery stock. 'Nonpareil' trees were also propagated by the nursery for use as standards for comparison. All trees were propagated on 'Nemaguard' peach seedling rootstock and delivered to the field site in February 2005 for planting.

A total of 120 trees of each accession were planted using a randomized complete block design, with 30 trees planted per accession per block. Land utilized in the study had been planted previously with almonds from 1998 through 2001. Tree density was 212 trees per hectare (86 per acre), with 7.3 m spacing between rows and 6.4 m between trees in the row. Trees were cultured using standard orchard management techniques and drip irrigation, and remained un-pruned for the duration of the study.

Bloom phenology and S-allele profiling. Bloom interval was evaluated in the research orchard of the ARS San Joaquin Valley Agricultural Sciences Center in Parlier, CA. Dates of first bloom (< 1 % of flowers open) and full bloom (> 90 % open flowers) were obtained for each accession through daily orchard observations. Data were collected for each bloom period from 2008 through 2010.

Apical meristems (<1.5 cm) were harvested from each accession in July 2011 and promptly lyophilized for *S*-allele analyses. Genomic DNA was then extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Polymerase Chain Reaction (PCR) was conducted according to Sutherland et al. (2004) using the degenerate primers EM-PC2consFD and EM-PC3consRD

for the amplification of the second intron region of the *S-RNase* gene. To amplify the first intron, the fluorescently labeled (_{JOE}) Pa-ConsI-F primer (Sonneveld et al. 2003) was used in combination with the EM-PC1consRD primer (Ortega et al., 2005). PCR was carried out in a PTC 200 thermocycler (MJ Research, Budapest, Hungary). The PCR products were separated on 2% TAE agarose gels for 3 h at 100 V and DNA bands were stained with ethidium bromide. Fragment sizes were estimated by comparison with the 1-kb + DNA ladder (Promega, Madison, Wis, USA). To determine the exact size of the *S-RNase* first intron region fragments under 500 bp, the fluorescently labeled products were run in an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary). To identify the *S*-allele in hybrid 23.5-16, PCR was conducted according to Ortega et al. (2006) using the degenerate primers PaConsI-F and EM-PC2consRD, reverse complement sequence of EM-PC2consFD (Sutherland et al., 2004). Some PCR products were cloned and sequenced in an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary). DNA and deduced amino acid sequences were compared using BLASTN or TBLASTX at NCBI (Altschul et al., 1990) and CLUSTAL W program (Thompson et al., 1994).

Kernel dimensional and color analyses. Kernel shape and appearance characteristics were evaluated using almond samples harvested from the ARS research orchard in Parlier, CA. Nuts from all accessions were initially dried on polypropylene tarps on the orchard floor before sample collection. Almond fruit were randomly selected from tarps for each accession in each harvest year (2008 through 2010). Prior to analysis, samples were oven-dried for approximately 18 h at 45°C. Dimensional measurements of kernel length, width and thickness were collected using a digital caliper (Starrett,

Athol, MA) accurate to 0.01 mm from 15 kernels of each accession in each harvest year. These values were used to construct kernel dimensional ratios length:width (LW), length:thickness (LT) and width:thickness (WT) on an individual kernel basis. A Minolta Chroma Meter CR-200 (Minolta Camera Co. Ltd., Osaka, Japan) equipped with an 8 mm measurement aperture was used to obtain kernel pellicle color coordinates luminosity, chroma and hue. All double kernel and insect-damaged nuts were excluded from the shape and appearance evaluations.

Field harvest procedures. Trees were harvested when hull split was evident on approximately 80% of almonds in a given accession. Nuts were shaken by machine onto the orchard floor, and allowed to dry to 5% kernel moisture and hull moisture of 13% (average). This typically required five to seven days, depending on ambient conditions. When sufficiently dry, nuts were machine swept into a single row (windrow) between the rows of trees. Approximately 4 kg of windrow nuts were sampled and evaluated per accession per block to provide estimates of kernel defects and harvest quality parameters. Nuts were then recovered into a scale-equipped shuttle to obtain a total nut weight. Total nut weight per hectare was then determined by calculating individual tree harvest weights from windrow data and multiplying by 212 trees/hectare.

Data analyses. Data presented in this study were analyzed and tabulated using JMP stats (Cary, NC, SAS, Version 7). Data were subjected to Levene's test to ensure variance homogeneity prior to ANOVA.

Separate ANOVAs were performed for each of the nine kernel dimensional and color parameters using almond accession and harvest year as main effects. Kernel dimensions, dimensional ratios and pellicle color coordinates were analyzed in each harvest year using 15 undamaged kernels per accession. When *F*-ratios were identified as significant

($P < 0.05$), Tukey's Honestly Significant Difference (HSD) test was used to separate differences among means.

For almond kernel yield analysis, main effects in the ANOVA for dependent variable kernel yield were almond accession, field block (rep) and harvest year. There were ten almond accessions evaluated in four field blocks for three harvest years during this study. Kernel yields were also quantified from each harvest year in a separate ANOVA (almond accession and field block as main effects) to examine cumulative kernel yield from 2008 through 2010 harvest years. Mean separations on cumulative yield were performed using a Tukey's HSD test at the $P < 0.05$ level.

Kernel defects (double kernels, shriveled/blank kernels, insect damaged kernels) and harvest quality parameters (kernel weight, harvest turnout) were estimated from windrow samples obtained during each of the three harvests. Harvest turnout was defined as the percentage of windrow material that was actual edible kernels. Kernel defect data required log transformations to stabilize variances prior to ANOVA. Separate ANOVAs were performed on each defect or quality parameter and mean separations were performed using a Tukey's HSD test at the $P < 0.05$ level.

Results and Discussion

Kernel dimensional and color evaluations. The effects of harvest year, almond accession and their interaction were evaluated for kernel dimensions, kernel dimensional ratios and pellicle color coordinate values. Main and interaction effects were highly significant ($P < 0.01$) for all measured variables. Across almond accessions, kernel length was influenced significantly by harvest year (F -ratio = 72.95, $df = 2,472$, $P < 0.01$), being significantly higher in 2009 and 2010 as compared with 2008. Harvest year also affected significantly kernel thickness (F -ratio = 12.60, df

= 2,472, $P < 0.01$) and kernel width (F -ratio = 110.64, $df = 2,472$, $P < 0.01$), with thicker kernels being harvested in 2010 as compared to other years and significantly wider kernels being harvested in 2009 (Table 1). Noted significant differences in kernel dimensions are indicative of kernel weight differences, as highly significant correlations have been demonstrated between kernel dimensions and kernel weight (Kester, 1965). Harvest year also affected significantly ($P < 0.01$) all three kernel dimensional ratios. Pellicle luminosity, chroma and hue were all significantly ($P < 0.01$) higher in 2010 as compared with when almond accessions were averaged across other harvest years (Table 1).

Specific differences among the ten accessions for kernel dimensions, kernel dimensional ratios and pellicle color coordinate values averaged across harvest years are also presented in Table 1. Averaged across harvest years, almond accessions varied significantly ($P < 0.01$) for all nine evaluated variables. Accession 5627-6 had the longest kernels (26.9 mm), not differing significantly from those of Y121-1 (26.1 mm), whereas the widest kernels were observed in Y121-1 (14.0 mm) and Y113-125 (13.8 mm), which did not differ significantly from kernels of Y114-165 (13.4 mm). Kernels of nine accessions were all significantly longer than those of 23.5-16 (21.1 mm). Kernel thickness varied significantly (F -ratio = 14.74, $df = 9,472$, $P < 0.01$) from 7.3 mm (82-73) to 8.5 mm (Y113-125).

Kernel dimensional ratio LW was affected significantly (F -ratio = 73.59, $df = 9,472$, $P < 0.01$) by almond accession, with LW ratio of 'Nonpareil' (1.86) not differing significantly from accessions Y121-1 and Y121-61 (Table 1). Accession effects were also significant (F -ratio = 30.29, $df = 9,472$, $P < 0.01$) for LT ratio, which ranged from 2.71 (23.5-16) to 3.47 (82-73 and Y121-1). Almond accessions also varied significantly (F -ratio = 23.99, $df = 9,472$, $P < 0.01$) for dimensional

Table 1. Main effects of harvest year and accession on kernel dimensions, kernel dimensional ratios and pellicle color coordinate values for 10 almond accessions evaluated during three growing seasons, 2008-2010, in Parlier, CA.

	Kernel dimensions (mm)			Kernel dimensional ratios			Pellicle color coordinate values		
	Length	Width	Thickness	LW	LT	WT	Luminosity	Chroma	Hue
Harvest year									
2008	23.9 b ^a	12.7 c	7.9 b	1.88 a	3.04 c	1.62 c	51.3 b	38.0 b	70.0 c
2009	25.1 a	13.6 a	7.9 b	1.84 b	3.19 a	1.74 a	51.7 b	38.4 b	71.1 b
2010	25.2 a	13.4 b	8.1 a	1.89 a	3.13 b	1.66 b	54.9 a	43.1 a	71.6 a
Accession									
23-122	23.5 cd	11.5 de	8.1 abc	2.04 a	2.91 cd	1.43 d	53.6 bc	41.0 ab	71.0 bc
23.5-16	21.1 e	12.6 c	7.8 cde	1.68 c	2.71 d	1.61 bc	48.4 ef	36.2 e	70.3 bc
5627-6	26.9 a	12.8 bc	8.3 ab	2.10 a	3.23 b	1.54 cd	51.7 cd	36.2 e	71.5 abc
82-73	25.4 b	12.2 cd	7.3 f	2.08 a	3.47 a	1.66 b	56.8 a	41.7 a	73.3 a
K10-26	23.3 cd	11.4 e	8.1 abc	2.03 a	2.87 cd	1.42 d	49.6 de	38.3 cd	69.8 cd
Nonpareil	23.4 cd	12.6 c	7.6 def	1.86 b	3.08 bc	1.66 b	56.5 a	42.0 a	71.8 ab
Y113-125	23.1 cd	13.8 a	8.5 a	1.67 c	2.72 d	1.63 bc	46.6 f	32.7 f	67.0 e
Y114-165	22.4 d	13.4 ab	8.0 bcd	1.67 c	2.79 d	1.67 b	46.4 f	35.4 e	68.1 de
Y121-1	26.1 ab	14.0 a	7.5 ef	1.86 b	3.47 a	1.86 a	47.7 ef	36.5 de	66.9 e
Y121-61	23.7 c	12.8 bc	7.4 ef	1.86 b	3.18 b	1.72 b	55.4 ab	39.5 bc	70.3 bc

^a Means followed by the same letter within a main effect grouping in a column do not differ significantly according to a Tukey's HSD test at the $P \leq 0.05$ level.

ratio WT, which was highest in accession Y121-1 (1.86) and lowest in K10-26 (1.42). All 'Nonpareil' kernel dimensional ratio values calculated in this study were observed to be within the annual mean range for this cultivar as reported previously (Ledbetter and Sisterson, 2010).

Significant differences were observed among almond accessions averaged across harvest years for pellicle luminosity (F -ratio = 50.77, $df = 9,472$, $P < 0.01$), chroma (F -ratio = 44.25, $df = 9,472$, $P < 0.01$) and hue (F -ratio = 27.41, $df = 9,472$, $P < 0.01$). Luminosity ranges from 0 (black, no reflectance) to 100 (white, complete reflectance), and represents the degree of darkness or lightness associated with pure chromatic colors. It is thus an important character whose value generally corresponds with perceived object brightness (Wallach, 1963). Luminosity of accession 82-73 almonds was numerically highest (56.8) and not significantly different from 'Nonpareil' (56.5) or Y121-61 (55.4). Differences in pellicle chroma val-

ues were insignificant between 'Nonpareil' (42.0) and 82-73 (41.7), and these two accessions similarly did not differ in chroma value with accession 23-122 (41.0). Accession 82-73 demonstrated the highest average pellicle hue value (73.3), not differing significantly from 'Nonpareil' (71.8). Pellicle hue values were lowest in accessions Y113-125 (67.0) and Y121-1 (66.9).

Bloom phenology and S-allele analysis. The specific dates and duration of the bloom period are important factors affecting the potential for pollination and nut set (Ortega et al., 2004). Inclement weather during the period of bloom can limit bee flights, thus limiting pollination opportunities. Allelic profiles of almond accessions at the floral compatibility locus are also important (Table 2), particularly among self-incompatible accessions, as sharing an allele among pollination partner accessions reduces by 50% the amount of pollen useful for functional fertilization and nut set.

Accession 23.5-16 was confirmed to carry

the S_5 -allele. However, its complete genotype remained unresolved since first intron PCR amplified a fragment of 304 bp, while PCR analysis of the second intron failed. Therefore, a portion of the *S-RNase* gene (from signal peptide to the C2 region) was amplified, cloned and sequenced. This sequence turned out to be different from all known *P. dulcis* *S-RNase* alleles and shared the greatest similarity with *P. tenella* S_7 -*RNase*. Hence this allele has been labeled as S_{17} -*RNase* and its DNA sequence was submitted to the NCBI GenBank database (accession number: ID 1614335). From a practical perspective it means that 23.5-16 is expected to be at least semi-compatible with all commercial cultivars that have been *S*-genotyped to date, and is fully compatible with those not carrying the S_5 -allele. However, a detailed analysis of the S_{17} -allele and its presence in other species may also enrich our knowledge of *S*-allele evolution in *Prunus*.

Three of the five self-incompatible accessions (23-122, 5627-6 and K10-26) and two of the self-compatible accessions (Y113-125

and Y121-1) evaluated in this test share a single allele (S_8) with 'Nonpareil'. Complete pollination incompatibility occurs when pollination partner accessions do not differ in their allelic status, as is the case with 82-73 and 'Nonpareil' (both being S_7S_8). In addition, other self-incompatible accessions will be cross-incompatible with commercial almond cultivars besides 'Nonpareil' (Kodad and Socias i Company, 2009). Examples include 23-122 with 'Thompson' or 'Wood Colony' (all S_5S_7) and K10-26 with 'Carmel' or 'Livingston' (all S_5S_8). These combinations must be avoided in commercial orchards to ensure successful pollination and fruit set. Further, accession 5627-6 forms a novel cross-incompatibility group (CIG) with late-blooming 'Titan' (S_8S_{14}). We propose to name this CIG XXVIII to continue labels after Ortega et al. (2006) and Kodad and Socias i Company (2009).

First and full bloom dates of the ten almond accessions during the 2008 through 2010 harvest years are presented in Table 2. Dates and duration of bloom vary slightly

Table 2. *S*-locus allelic status and bloom interval of ten almond accessions during three successive bloom periods, 2008 through 2010, grown in Parlier, CA.

Almond accession	<i>S</i> -locus allelic status	2008		2009		2010	
		First	Full	First	Full	First	Full
23-122	S_5S_7	21 Feb	1 Mar	12 Feb	27 Feb	15 Feb	25 Feb
23.5-16	S_5S_{17}	21 Feb	1 Mar	14 Feb	26 Feb	14 Feb	25 Feb
5627-6	S_8S_{14}	19 Feb	27 Feb	13 Feb	25 Feb	12 Feb	21 Feb
82-73	S_7S_8	24 Feb	3 Mar	18 Feb	2 Mar	17 Feb	1 Mar
K10-26	S_5S_8	24 Feb	4 Mar	18 Feb	1 Mar	17 Feb	27 Feb
Nonpareil	S_7S_8	21 Feb	1 Mar	12 Feb	26 Feb	15 Feb	26 Feb
Y113-125	S_7S_8	19 Feb	28 Feb	12 Feb	27 Feb	12 Feb	21 Feb
Y114-165	S_7S_{18}	25 Feb	3 Mar	17 Feb	4 Mar	17 Feb	26 Feb
Y121-1	S_7S_8	25 Feb	7 Mar	19 Feb	3 Mar	17 Feb	28 Feb
Y121-61	S_7S_{27}	21 Feb	29 Feb	18 Feb	1 Mar	17 Feb	27 Feb

among years as trees bloom in response to both annual chill hour and heat unit accumulation (Egea et al., 2003). Average bloom duration was 9.7 d in 2008, less than in either 2009 (12.6 d) or 2010 (11.2 d). Across harvest years, no single accession had bloom duration consistently longer or shorter than the other accessions. Accession Y113-125 was the earliest blooming accession, consistently overlapping with the economically important early 'Nonpareil' bloom (Asai et al., 1996), and Y121-1 generally bloomed the latest. Bloom overlap of all accessions with cultivar 'Nonpareil' is evident in Table 2, with accessions 23-122 and 23.5-16 matching the 'Nonpareil' bloom particularly well.

Almond yield analysis. Almond kernel yields varied significantly ($P < 0.01$) with almond accession, harvest year and field block. The interaction of almond accession by harvest year was significant (F -ratio = 6.33, $df = 18, 120$, $P < 0.01$), but all other interactions were not. Averaged across harvest year, accession Y121-1 yielded the most almond kernels ($1917.2 \text{ kg} \cdot \text{ha}^{-1}$) and accession 23.5-16 yielded the least ($864.0 \text{ kg} \cdot \text{ha}^{-1}$). Almond yield in 2010 was highest ($2098.5 \text{ kg} \cdot \text{ha}^{-1}$) as averaged across almond accessions and lowest in 2009 ($1189.3 \text{ kg} \cdot \text{ha}^{-1}$), providing a general indication of alternate bearing tendencies. *A priori* information on field fertility suggested a nutritional gradient across the blocks. The gradient was confirmed with a significant block effect, and yields decreased sequentially, from the northernmost block ($1392.0 \text{ kg} \cdot \text{ha}^{-1}$) through the block most southern ($946.9 \text{ kg} \cdot \text{ha}^{-1}$). Almond yields, as influenced by main effects accession, harvest year and block, are presented in Fig. 1.

Profitability for an almond producer depends on both kernel yield and kernel quality of the harvested and processed nuts. In this study, the significant Accession $\times$ Year interaction is important information on an accession's potential profitability, as accession performance varied with harvest year. Almond

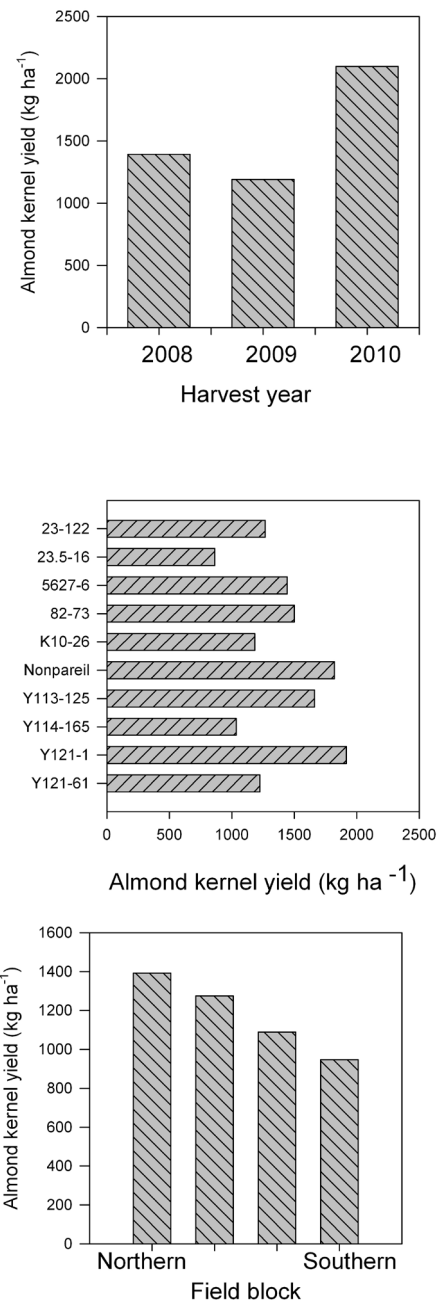


Fig. 1. Least square means for main effects of harvest year, almond accession and field block on almond kernel yield for ten almond accessions trialed in the southern San Joaquin Valley.

Table 3. Least square means for annual and cumulative almond kernel yields, and index of biennial bearing for 'Nonpareil' and nine almond accessions harvested during three consecutive years from the southern San Joaquin Valley.

Accession	Biennial bearing index ^a	Almond kernel yield (kg·ha ⁻¹)			Cumulative yield 2008 through 2010
		2008	2009	2010	
23-122	0.16	1403.7	1706.6	2435.8	5471.4 bc ^y
23.5-16	0.07	878.6	819.3	996.4	2662.0 e
5627-6	0.38	1259.6	789.5	2010.6	4291.4 d
82-73	0.05	1380.3	1459.3	1604.4	4778.2 cd
K10-26	0.16	1190.5	1254.4	2032.7	4564.3 cd
Nonpareil	0.17	1706.7	1525.4	2681.5	6296.7 ab
Y113-125	0.28	1593.0	1115.5	2224.3	5175.1 cd
Y114-165	0.16	1200.5	1393.5	2076.0	4416.2 cd
Y121-1	0.40	1994.0	1203.1	3551.4	6578.5 a
Y121-61	0.44	1312.6	626.7	1371.2	2929.9 e
Std. Error		126.0	140.8	161.5	232.3

^a See Hoblyn et al. (1936) for details.

^y Means followed by the same letter within a column are not significantly different at the $P < 0.05$ level according to a Tukey's HSD test.

kernel yields should increase as trees grow larger (Hill et al., 1987), but average kernel yield in 2009 (1189.3 kg·ha⁻¹) was reduced when averaged across accessions as compared with yield from 2008 (1241.9 kg·ha⁻¹). Hence, almond accessions evaluated during these harvests demonstrated alternate bearing, the degree expressed being dependent on the specific almond accession (Table 3). The index of biennial bearing (I) as defined by Hoblyn et al. (1936) is also included to provide a relative value in order to compare accessions in their propensity to alternate bear. Kernel yield in the first year of production (2008) varied between 878 kg·ha⁻¹ (23.5-16) and 1994 kg·ha⁻¹ (Y121-1). Accession Y121-61 produced only 626 kg·ha⁻¹ in the second production year, less than half its yield in 2008 (1312 kg·ha⁻¹). Yield of accession 23-122 averaged 1706 kg·ha⁻¹ in 2009, slightly higher than that of 'Nonpareil,' (1525 kg·ha⁻¹), and both accessions had similar indices of biennial bearing (0.16 and 0.17, respectively). The heaviest yields were achieved during 2010, ranging between 996 kg·ha⁻¹ (23.5-16) and 3551 kg·ha⁻¹ (Y121-

1). Based on the index of biennial bearing, the tendency to alternate bear was highest in accession Y121-61 (0.44) and lowest in accession 82-73 (0.05). It should be noted that the index of biennial bearing calculated for 'Nonpareil' in this study differed little from the index calculated for 'Nonpareil' in a Kern county (southern San Joaquin Valley) orchard evaluated over a ten-year period (Tombesi et al., 2012).

Cumulative kernel yield from 2008 through 2010 was influenced significantly by accession (Table 3, F -ratio = 29.76, df = 9,79, $P < 0.01$), field block (F -ratio = 66.50, df = 3,79, $P < 0.01$) and their interaction (F -ratio = 1.97, df = 27,79, $P = 0.025$). Kernel yield was highest in the northernmost field block (4716 kg·ha⁻¹), and decreased sequentially to the lowest yielding southern field block (3486 kg·ha⁻¹). The significant Accession x Block interaction was influenced by the varied cumulative accession performance and compounded by their respective biennial bearing indices. Cumulative kernel yield of Y121-1 was highest (6578 kg·ha⁻¹), and not different significantly from cumulative yield

of ‘Nonpareil’ (6296 kg·ha⁻¹). On the low end of yield, accessions 23.5-16 and Y121-61 did not differ significantly (2662 and 2929 kg·ha⁻¹, respectively), but were significantly lower in yield than all the rest.

Kernel weight varied significantly as affected by both harvest year (Table 4, *F*-ratio = 2.06, *df* = 2,119, *P* < 0.01) and almond accession (*F*-ratio = 6.52, *df* = 9,119, *P* < 0.01), ranging from 1.46 g (2010) to 1.16 g (2008) when averaged across accessions. Accession Y121-1 had the highest 3-year average kernel weight (1.42 g), not differing significantly from accessions 23-122, 82-73, K10-26, ‘Nonpareil’ and Y113-125. Harvest turnout was affected significantly by both harvest year (Table 5, *F*-ratio = 62.32, *df* = 2,119, *P* < 0.01) and almond accession (*F*-ratio = 0.14, *df* = 9,119, *P* < 0.01). Harvest turnout increased significantly in each successive harvest year, beginning at 22.4% in 2008 and averaging 27.9% across almond accessions in 2010. Across harvest years, harvest turnout ranged from 18.1% (Y114-165) to 26.4

% (Y121-1), with no significant differences among accessions 23-122, 23.5-16, 5627-6, 82-73, ‘Nonpareil’, Y113-125 and Y121-1 (Table 4). The effect of harvest year was significant for the level of double kernel almonds (*F*-ratio = 4.10, *df* = 2,119, *P* = 0.02) and insect damaged kernels (*F*-ratio = 64.00, *df* = 2,119, *P* < 0.01), but not for the level of blank and shriveled kernels (Table 4, *F*-ratio = 0.14, *df* = 2,119, *P* = 0.87). Levels of blank and shriveled kernels varied from 2.92% (2010) to 3.45% (2008), with these means being affected by an elevated (19.5%) level of blank and shriveled kernels in accession Y114-165. Across accessions, insect damaged kernels were significantly higher in 2008 (8.68%) compared to other harvest years. Insect damaged kernels decreased significantly each successive harvest year with only 0.73% noted across accessions in the 2010 harvest. Double kernel almonds were most frequent during the 2010 harvest (12.5%), perhaps influenced by specific environmental conditions during that year (Egea

Table 4. Least square means by main effects harvest year and accession, for average kernel weight, nut defects and harvest turnout of 10 almond accessions grown in the southern San Joaquin Valley, 2008-2010.

	Kernel wt. (g)	Nut Defects (%)			Harvest turnout (%)
		Blank and shriveled	Doubles	Insect damaged	
Harvest Year					
2008	1.16 b ^z	3.45	5.58 b	8.68 a	22.4 c
2009	1.41 a	3.40	7.98 ab	4.63 b	25.6 b
2010	1.46 a	2.92	12.50 a	0.73 c	27.9 a
Std. Error	0.02	0.44	1.35	0.62	0.35
Accession					
23-122	1.19 abcd	1.00 b	13.25 ab	2.75 d	23.0 abc
23.5-16	0.98 d	2.00 b	0.12 c	2.50 d	22.4 abc
5627-6	1.12 bcd	1.75 b	0.75 c	5.25 bcd	22.5 abc
82-73	1.26 abc	1.25 b	1.25 c	4.25 cd	23.6 ab
K10-26	1.17 abcd	1.25 b	9.25 ab	8.75 abcd	21.3 bc
Nonpareil	1.16 abcd	2.75 b	0.75 c	13.50 abc	21.6 abc
Y113-125	1.32 ab	2.25 b	3.75 bc	20.50 a	24.3 ab
Y114-165	1.03 cd	19.50 a	0.25 c	4.75 bcd	18.1 c
Y121-1	1.42 a	2.75 b	24.75 a	14.25 ab	26.4 a
Y121-61	0.95 d	0.25 b	1.75 c	10.25 abcd	20.9 bc
Std. Error	0.06	1.40	4.26	1.96	1.10

^z Means followed by the same letter within a main effect grouping in a column, or without letter designation, do not differ significantly according to a Tukey's HSD test at the *P* ≤ 0.05 level.

and Burgos, 1995). Averaged across harvest years, significant differences existed in levels of blank and shriveled kernels (F -ratio = 9.04, df = 9,119, P < 0.01), double kernels (F -ratio = 13.19, df = 9,119, P < 0.01) and insect damaged kernels (F -ratio = 7.30, df = 9,119, P < 0.01) among almond accessions. Levels of blank and shriveled kernels were generally low, varying between 0.25% (Y121-61) and 2.75% ('Nonpareil', Y121-1), except for accession Y114-165 (19.50%), which was significantly higher than all the rest. Insect damaged kernels varied between 2.50% (23.5-16) and 20.50% (Y113-125) with significant differences among accessions. Elevated insect damage in Y113-125 was attributed to an extremely early hull split, allowing insects more time to damage the developing kernels. Double kernel percentage was highest in Y121-1 (24.75%), not differing significantly from either 23-122 (13.25%) or K10-26 (9.2%) when averaged across harvest years (Table 4).

The high percentage of double kernel fruit in accession Y121-1 presents challenges in post-harvest handling and kernel marketing, since even the leniently scored 'US Standard Sheller Run' grade allows only 25% doubles (Anon., 1997). Hence, almonds from accession Y121-1 are limited to a less prestigious grade when sold unsorted as whole kernels, thus providing lower returns per harvested tonne. Profitability with this accession could be increased through specific manufacturing applications that yield a higher value product than whole natural almonds, and would not be limited by the level of double kernels. Sorting and removal of doubles to a level below that necessary to achieve more prestigious USDA grades on the remaining tonnage would be the simplest solution (Novak et al., 1999), but sorting requires either extra fees from almond handlers or integrated production/handling resources. Interestingly, both natural almond flour and natural diced almond pieces, both of which can be man-

ufactured exclusively from double kernel almonds, can be found at wholesale prices matching or exceeding those of whole natural almonds (http://www.justalmonds.com/Just_Almonds_Almond_Products_s/20.htm, <http://www.newurbanfarms.com/wholesale-bulk/almonds.html>).

Taken as a whole, results from the kernel quality, nut defects and harvest turnout present an intricate picture, that when included with yield data, demonstrate the complexity of choosing appropriate cultivars that will produce the highest profit margins. Almond Marketing Groups in which the experimental accessions would be sold must also be considered, although there are presently no objective standards available to determine into which Marketing Group(s) the accessions would best fit. Five accessions (23.5-16, 5627-6, Y113-125, Y114-165 and Y121-61) were removed from the trial after the 2010 harvest, based primarily on low cumulative yield and specific nut defects. New accessions have been planted in their place, but comparisons within the trial are now restricted by the large differences in tree ages. Further complications arise relative to orchard replant issues that may develop (Browne et al., 2006), and its effect on potential kernel yield. Valid comparisons between the new and formerly planted accessions would require a new planting altogether, something that may occur when a new Regional Variety Trial is established.

Acknowledgments

This work was funded by the ARS project, 'Improvement of *Prunus* and *Vitis* scions for fruit quality and pest resistance' (5302-21220-005-00D). Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Literature cited

- Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Anonymous. 1997. United States Standards for Grades of Shelled Almonds. USDA. Agricultural Marketing Service, Fruit and Vegetable Division, Fresh Products Branch. Effective March 24, 1997.
- Asai, W.K., W.C. Micke, D.E. Kester, and D. Rough. 1996. The evaluation and selection of current varieties, p. 52-60. In: W.C. Micke (ed.) *Almond Production Manual*. Publication 3364, University of California, Division of Agriculture and Natural Resources, Oakland, CA.
- Browne, G.T., J.H. Connell, and S.M. Schneider. 2006. Almond replant disease and its management with alternative pre-plant soil fumigation treatments and rootstocks. *Pl. Dis.* 90:869-876.
- Egea, J. and L. Burgos. 1995. Double kernelled fruits in almond (*Prunus dulcis*, Mill.) as related to pre-blossom temperatures. *Ann. Appl. Biol.* 126:163-168.
- Egea, J., E. Ortega, P. Martínez-Gómez, and F. Dicenta. 2003. Chilling and heat requirements of almond cultivars for flowering. *Enviro. Expt. Bot.* 50:79-85.
- Halász, J., A. Pedryc, and A. Hegedüs. 2007. Origin and dissemination of the pollen-part mutated S_C -haplotype that confers self-compatibility in apricot (*Prunus armeniaca*). *New Phytol.* 176:793-803.
- Hill, S.J., D.W. Stephenson, and B.K. Taylor. 1987. Almond yield in relation to tree size. *Sci. Hort.* 33:97-111.
- Hoblyn, T.N., N.H. Grubb, A.C. Painter, and B.L. Wates. 1936. Studies in biennial bearing. *J. Pomol. Hort. Sci.* 14:39-76.
- Kester, D.E. 1965. Size, shape and weight relationships in almond kernels. *Proc. Am. Soc. Hort. Sci.* 87: 204-213.
- Kodad, O. and R. Socias I Company. 2008. Fruit set evaluation for self-compatibility selection in almond. *Sci. Hort.* 118:260-265.
- Kodad, O. and R. Socias I Company. 2009. Review and update of self-incompatibility alleles in almond. *Acta Hort.* 814:421-424.
- Lampinen, B.D., T.M. Gradziel, J.T. Yeager, M.A. Thorpe, and W.C. Micke. 2002. Regional almond variety trials for cultivar evaluation in California. *Acta Hort.* 591:457-464.
- Ledbetter, C.A. 2010. Almond breeding and evaluation activities in Central California: Past and future. *Opt. Médit.* 94:255-260.
- Ledbetter, C.A. and M. S. Sisterson. 2010. Carpo-logical variability of almond [*Prunus dulcis* (Mill.) D.A. Webb cv. Nonpareil] in a single orchard during seven consecutive harvests. *HortScience* 45(12): 1788-1792.
- Micke, W.C., D.E. Kester, T.M. Gradziel, J.T. Yeager, M.A. Thorpe, J.G. Connell, P.S. Verdegaal, and M. Viveros. 1998. Almond cultivar evaluation using regional trails. *Acta Hort.* 470:91-94.
- Novak, T.J., K.H. Colombowala, R.O. Brandt, and R.A. Peets. 1999. Method and apparatus for sorting product. United States Patent No. 5,986,230. 16 November 1999.
- Ortega, E., R.I. Bošković, D.J. Sargent, and K.R. Tobutt. 2006. Analysis of *S*-RNase alleles of almond (*Prunus dulcis*): characterization of new sequences, resolution of synonyms and evidence of intragenic recombination. *Mol. Gen. Genom.* 276:413-426.
- Ortega, E., B.G. Sutherland, F. Dicenta, R. Bošković, and K.R. Tobutt, K.R. 2005. Determination of incompatibility genotypes in almond using first and second intron consensus primers: detection of new *S*-alleles and correlation of reported *S* genotypes. *Pl. Breed.* 124:188-196.
- Ortega, E., J. Egea, and F. Dicenta. 2004. Effective pollination period in almond cultivars. *HortScience* 39(1):19-22.
- Socias i Company, R., J. Gómez Aparisi, and J.M. Alonso. 2005. Year and enclosure effects on fruit set in an autogamous almond. *Sci. Hort.* 104:369-377.
- Sonneveld, T., K.R. Tobutt, and T.P. Robbins. 2003. Allele-specific PCR detection of sweet cherry self-incompatibility (*S*) alleles *S*1 to *S*16 using consensus and allele-specific primers. *Theor. Appl. Gen.* 107:1059-1070.
- Sutherland, B.G., T.P. Robbins, and K.R. Tobutt. 2004. Primers amplifying a range of *Prunus S*-alleles. *Pl. Breed.* 123:582-584.
- Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22:4673-4680.
- Tombesi, S., B.D. Lampinen, S. Metcalf, and T. De-jong. 2012. Relationships between spur- and orchard-level fruit bearing in almond (*Prunus dulcis*). *Tree Physiol.* 31:1413-1421.
- Wallach, H. 1963. The perception of neutral colors. *Sci. Americ.* 208:107-116.