Production of Seedy Fruit by 'DaisySL' Mandarin
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Brief summary. The University of California, Riverside released 'DaisySL' mandarin, a new low-seeded form of 'Daisy' in June 2009. During the 2009-10 season we discovered that 10 of 83 trial trees produced some fruit with high seed content (10-15 seeds/fruit), comparable to that observed in 'Daisy'. On nearly all trees which had seedy fruit, only a small number of seedy fruit were found. The total percentage of seedy fruit (defined as those with more than 6 seeds per fruit) was about 1 to 2% overall, with most of these occurring on trees near Indio that had previously suffered freeze damage. Growers planning to plant 'DaisySL' should be aware of this potential problem with the variety. A more detailed report on this problem is available at:http://plantbiology.ucr.edu/faculty/roose.html.

During the 2009-2010 season we discovered in our field trials that some trees of 'DaisySL' mandarin (released in June 2009) produced some fruit with higher seed counts than previously seen. This report summarizes the current data on this and possible explanations for this characteristic. Fruit with high seed counts were not found in samples tested before the release of this variety, despite examination of a very large number of fruit on many trial trees over several years.

CVARS trees. We first noted seedy fruit in samples from the trial at CVARS (Coachella Valley Agricultural Research Station, Thermal, near Indio) in December 2009. In that sampling each sample analyzed was a composite of fruit from several trees, so we thought it was possible that only one or two trees were producing seedy fruit, and that these were mislabeled control ('Daisy') trees rather than 'DaisySL'. In February, we cut all remaining fruit on each tree and noted whether seedy fruit occurred in clusters on individual branches or randomly over the tree. We found that 4 trees produced some fruit with high seed counts (10-15 seeds per fruit) although most of the fruit on these trees had few seeds. Seventy percent of the fruit on another tree was seedy. The other 7 trees produced no fruit with more than 6 seeds per fruit, and most fruit on these trees had 1-2 seeds per fruit as is typical for 'DaisySL'. In general, seedy fruit occurred in small clusters of 1-3 fruit per branch. Several of the 7 trees that did not produce high-seed-content fruit did produce some fruit with 5 or 6 seeds, and these need to be evaluated again in 2011. It is important to note that the CVARS trees did not produce much fruit until 2010 and those produced in earlier years did not have high seed counts. The 'DaisySL' trees at CVARS were planted in October 2005, suffered some freeze damage in January 2007 when the trees were quite young, and therefore these trees are smaller than trees at other locations and did not fruit until the 2008-09 season when only a few fruit per tree were available.

Field samples. The discovery of seedy fruit on trees at CVARS led us to evaluate larger samples of fruit from all available locations. For nearly all trees, at least 100 fruit per tree were cut, with samples including fruit from all main branches of the tree. When a seedy fruit was found, additional fruit were sampled from the same branch to determine the size of the affected sector. Branches producing seedy fruit were flagged to facilitate re-sampling in 2011. The results of this sampling are summarized in Table 1. No seedy fruit were found in the trees at SCREC (South Coast Research and Extension Center, Irvine) and Santa Paula. Excluding CVARS, 5 of 71 trees had one or two small branches that produced some seedy fruit, and the percentage of seedy fruit was only 0.25%. Including CVARS trees increased the percentage of seedy fruit to 1.1%. No seedy fruit were found on the 'DaisySL' "mother tree" at UCR or on the two trees propagated from CCPP (Citrus Clonal Protection Program) buds at the Rubidoux

greenhouse and planted at Lindcove which served as the bud source for all field trees except those at UCR.

Bulk samples. In addition to the single tree samples summarized above, between December 1, 2009 and February 24, 2010 a total of 1089 fruit were sampled from 12 trees at each of 6 locations (72 trees total) for fruit quality analyses. Each sample was composed of 15 fruit, 5 sampled from each of three trees on Carrizo or C35 rootstock (fruit from individual trees were not tracked separately). In these samples, we counted total seeds in each 15 fruit sample, and noted the number of fruit that had more than 5 seeds/fruit and the number of seeds in such fruit. This allows us to examine the frequency distribution of fruit with 0-5 seeds vs more than 5 seeds. This data (Figure 1) shows that about 3.0% of all fruit had more than 5 seeds, and if the samples from CVARS are excluded, about 1.7% had more than 5 seeds. The mean number of seeds per fruit in this dataset was 1.69. If fruit with more than 5 seeds are excluded, the mean seed count was 1.42. The frequency distribution of seeds per fruit in this data (Figure 2) shows that few samples averaged more than 2.0 seeds per fruit except at CVARS where 5 of the 8 fruit samples averaged above 2.0 seeds per fruit.

Figure 1. Number of 'DaisySL' fruit with various seed counts during 2009-2010 season.

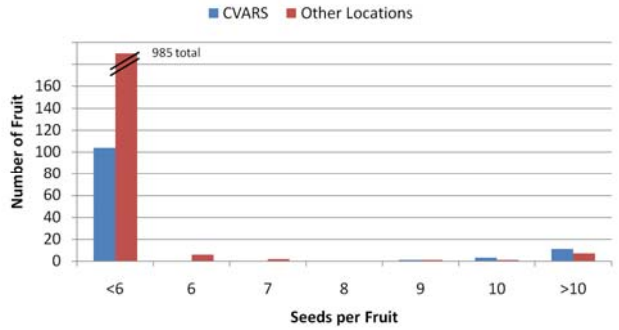
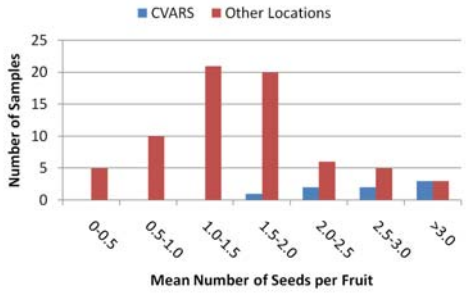
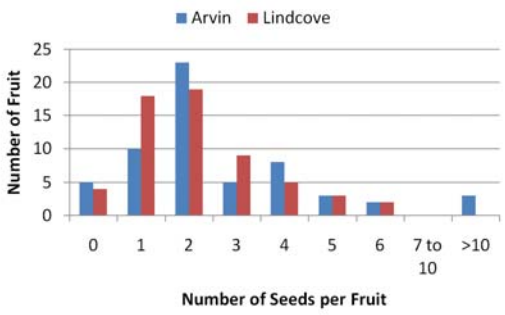


Figure 2. Frequency of samples with different seed counts at CVARS and other locations in 2009-10 season



In two samples of 59 and 60 fruit, the number of seeds in each fruit was recorded. This shows (Figure 3) that most fruit had 0 to 4 seeds. Three fruit had more than 10 seeds and none had 7-10 seeds. In other words, fruit fell into a class with a mean of about 2 seeds per fruit (similar to the larger samples discussed above) or they had more than 10 seeds/fruit, similar to counts in fruit from the parent variety ('Daisy').

Figure 3. Seed counts in samples of 'DaisySL' fruit from Arvin and Lindcove, sampled in February 2010.



Seedy fruit were often produced on branches which grew from a bud just below a stub from a previously terminal fruit (Figure 4). Seedy fruit usually appeared larger than low-seeded fruit (Figure 5), and often had coarser rind texture (Figure 6). Seedy and low seeded fruit from a single tree at CVARS are shown in Figure 7 which illustrates the often larger size of the seedy fruit. It should be noted that seedy fruit of 'Daisy' are not typically larger than the low seeded fruit of 'DaisySL'.

Figure 4. Origin of branch producing seedy fruit.

Figure 5. Seedy fruit of 'DaisySL' from Lindcove.

Figure 6. Low seeded (left) and seedy (right) fruit of 'DaisySL' showing coarser rind of seedy fruit.

Figure 7. Fruit of 'DaisySL' from a single tree at CVARS showing normal low-seeded fruit and several seedy fruit from a single branch on the left.

Interpretation. The most likely cause of particular branches on 'DaisySL' trees producing seedy fruit is that 'DaisySL' is a chimera. Like other plants, citrus trees have three usually separate cell layers (called Layer I, Layer II, and Layer III) with different sets of active genes. The plant develops by division and expansion of cells in each layer, but normally cells in a given layer give rise to cells of the same layer type. Other citrus varieties are known to be chimeras, including Shamouti orange, Thompson grapefruit, and others. The mutation breeding method that we have used alters the genetic composition of one or more cells. Cells from all three layers divide to produce the shoot, so we cannot be certain that all cell layers of the new variety carry the mutation. The original mutation event that causes low seed content probably occurred in Layer II because this layer produces the eggs, pollen and nucellus that determine seed content. Occasionally, perhaps after freeze or pruning damage, a "layer conversion" might occur in which a cell or cells of Layer I (most likely) or III converts to Layer II. We are uncertain whether all Layer II cells in each affected branch result from this conversion event. If the original tissue of 'DaisySL' were (N,M,M) in layers I to III respectively, where N is the normal fertile genotype and M is the sterile mutant genotype, then after conversion of LI to LII, the aberrant branches would be (N,N,M) and therefore fertile. Cells of type N and M might be distinguishable either by studies of chromosome structure or by DNA sequence, but we do not currently have such information.

Solution. If this interpretation is correct, it may also be possible to stimulate or find the alternative conversion event (N,M,M) to (M,M,M) where the original LI is replaced by the mutant cell layer. Such plants would be stable for the mutant type and not produce seedy fruit. The genotype and role of Layer III in this is not clear. Layer III normally produces vascular (conductive) tissue (xylem and phloem). It may or may not be involved in the layer conversion events. It is also possible that 'DaisySL' is (N,M,N) in which case the role of Layer III in conversion events is more important. Understanding which cell layers of 'DaisySL' carry mutations causing low seed counts is therefore important in developing a stable form of this promising variety.

Table 1. Evaluation of 'DaisySL' fruit for seed content in field during 2009-2010 season. When sufficient fruit were available, 100 fruit per were cut in the field and evaluated for seed content. Seedy fruit were those with more than 6 seeds/fruit.

Site	Field	Trees w/o Seedy Fruit	Trees With Seedy Fruit	Total Fruit Cut in 2010	Total Seedy Fruit	Percent Seedy Fruit	Seedy Fruit Source		Per Tree in 2010		No. of Branches Affected	Branch No.	No. of Seedy Fruit per Branch	# Seeds			
							Row	Tree	Total	Seedy				Fruit #1	Fruit #2	Fruit #3	Fruit #4
UCR	1B	18	2	~2000	5	0.25	3	10	~100	4	1	1	4	13	4	15	5
							3	13	~100	1	1	1	1	15			
UCR	15F	1	0	~100	0	0.00											
LREC	63	10	2	~1200	11	0.55	1	19	~100	3	1	1	3	14	11	13	
							27	2	~100	8	2	1	4	11	10	14	13
												2	4	8	13	13	10
LREC	92	2	0	~200	0	0.00											
Arvin		11	1	~1200	2	0.17	25	22	~100	2	1	1	1	14			
CVARS	4A	7	5	599	70	11.69	4	2	66	10	7	nd	1	>10			
							4	3	85	9	3	nd	3	>10			
							4	5	115	13	5	nd	3	>10			
							4	6	128	17	7	nd	2	>10			
							1	9	30	21	9	nd	2	>10			
SCREC	43	12	0	~1200	0	0.00											
Santa Paula		12	0	~1200	0	0.00											
TOTAL		73	10	7699	88	1.14											
W/O CVARS		66	5	7100	18	0.25											

(CVARS=Coachella Valley Agricultural Research Station, LREC=Lindcove Research and Extension Center, SCREC = South Coast Research and Extension Center)