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Mono- and Polyembryony among Tetraploid *Citrus* Hybrids[1]

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***Abstract.* The percents of polyembryonic seeds of 65 tetraploid *Citrus* hybrids and 33 related, probable tetraploids were scored. These plants arose from crosses of 2 diploid, sexual, monoembryonic cultivars by a total of 6 tetraploid, partly asexual, polyembryonic pollen parents. Plants classed as strictly monoembryonic were recovered in progenies from 3 of the tetraploid parents. Individuals with low percentages of polyembryonic seeds were obtained in all progenies. The likelihood that 1 or 2 genes are responsible for polyembryony is discussed.**

Mono- and polyembryony in *Citrus* can be largely equated to sexual and nucellar embryony respectively, but in borderline cases classification is uncertain. Instances of as high as 7% polyembryonic seeds have been recorded in cultivars which are not known to produce nucellar embryos (8). In those cases surviving seedlings were shown to be sexual twins or triplets. In contrast, certain cultivars which can produce nucellar progeny sometimes have very low percents of polyembryonic seeds, varying from year to year.

Despite these problems, several workers have proposed inheritance patterns for nucellar embryony in diploid *Citrus* hybrids, and in crosses with the near relative, *Poncirus* (3, 6, 7, 8, 9). Inheritance appears fairly simple and could depend on 1 or 2 genes, although the factors in *Poncirus* may be somewhat different from those of *Citrus*. Tetraploid asexual (nucellar) seedlings have often arisen by spontaneous chromosome doubling in mother plants of *Citrus* taxa which reproduce at least partly by nucellar embryony. These tetraploids can also reproduce by nucellar embryony, indicating that such autotetraploidy does not markedly change the expression of this character. In previous studies, autotetraploids from highly nucellar cultivars have themselves been highly nucellar in reproduction, while those from partly sexual parents have remained partly sexual. Numbers of embryos per seed can vary greatly among individuals of all of these types.

We produced hybrid tetraploid progenies from crosses between sexual diploid seed parents and some of the tetraploids referred to above. These hybrids were in part described in 1969 (2). Such tetraploids evidently receive 2 sets of chromosomes from each parent, due to doubling in the female gametophyte (4, 5). The gene complements of the 4 sets are not expected to be genetically identical, since the parents are distinct taxa. We report here the numbers of hybrids having mono- or polyembryonic seeds, which can be indicative of sexual or nucellar embryony.

The parents of the hybrids were nearly all produced during the early work of H.B. Frost (1). Tetraploid parents obtained by nucellar embryony arose from the following diploid cultivars: 'Paper Rind', 'Ruby', and a seedy sweet orange [*Citrus sinensis* (L.) Osbeck]; 'Dancy' tangerine (*C. reticulata* Blanco); 'Royal' grapefruit (*C. paradisi* Macf.); and 'King' (*C. nobilis* Lour.). The diploid seed parent was 'Sukega' grapefruit (*C. paradisi* × *C. sinesis*?) except in cross number 6 where it was 'Temple' (*C. sinensis* × *C. reticulata*?). All trees were growing at the Citrus Research Center, University of California, Riverside, or at a field station near Tustin. Chromosome counts were made from smears of young shoot tips (2). Embryo counts were usually made repeatedly for each hybrid in 2 or 3 different years (3). For plants showing 0 to 30% multiple embryos, at least 50 seeds per year were examined. For those with >30% multiples, 30 seeds per year were usually examined.

Table 1 shows the number of trees with different percentages of multiple embryo seeds, among 65 tetraploid hybrids from 6 crosses. Thirty-three additional hybrids without chromosome counts, from crosses 3 and 4, are also included. These latter hybrids were also certainly tetraploids, based on their growth habit and relatively high seed numbers in their fruits. Diploids very seldom arise from these crosses, and triploids are regularly nearly seedless. The percentage classes for polyembryony (Table 1) were selected on the basis of known behavior of sexual and partly sexual diploids.

Among all 98 trees, 12 had 0% multiple embryos, 18 had 1-3%, and 12 had 4-7%. If only the 0% class is strictly sexual, the asexual to sexual ratio is about 7:1. However, at least some trees in the 1-3% class, and even the 4-7% class are probably strictly sexual; the inclusion of parts of these classes as sexual results in much lower individual and total ratios. Previous studies in diploids (6, 9) indicated that polyembryony (i.e. asexual embryony) may be determined by a single dominant gene. If a gene *P* is responsible, then asexual, nucellar tetrapolids would be *PPpp* or *PPPP* because of their origin from diploids by somatic doubling. The sexual, monoembryonic seed parents used here would be homozygous recessive *pp*. If the tetraploid pollen parents are heterozygous, the hybrid progenies should segregate 1 *PPpp*:4 *Pppp*:1 *pppp* if chromosome-type segregation and no crossing over are assumed. If *P* were fully dominant, a phenotypic ratio of 5 poly- to 1 sexual monembryonic plants would be expected. But if a higher proportion of the plants are sexual, incomplete dominance with modifying genes or environmental variation may be altering the phenotype of some simplex (*Pppp*) plants. More sexual plants would also be expected if 2 complementary dominant genes were needed to produce asexual polyembryony. Modifying genes would still need to be postulated if all

Table 1. Numbers of tetraploid hybrid *Citrus* trees having indicated percentages of multiple embryos in their seeds.

Cross number	Tetraploid polyembryonic pollen parent[z]	Distribution of trees				
		Percentages of seeds with multiple embryos				
		0	1-3	4-7	8-100	Totals
	Hybrids identified as tetraploid by chromosome number					
1	Sweet orange			2	5	7
2	Paper Rind orange	4	3	2	13	22
3	Ruby orange	1			4	5
4	King tangor	4	8	2	8	22
5	Royal grapefruit		1	1	5	7
6	Dancy tangerine			1	1	2
	Totals	9	12	8	36	65
	Additional probable tetraploids					
3	Ruby orange		1	1	1	3
4	King tangor	3	5	3	19	30
	Totals	3	6	4	20	33

[z]The seed parent was diploid, monoembryonic 'Sukega' grapefruit in all crosses except number 6, where it was 'Temple' tangor.

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plants with less than 8% polyembryony are considered to be strictly sexual.

Until critical populations can be scored for the presence or absence of nucellar seedlings, the exact genetic basis of nucellar embryony cannot be determined. However, it is clear that monoembryonic tetraploids can be obtained from crosses involving polyembryoyonic tetraploids, and that sexual plants may be recovered in proportions greater than would be expected on the basis of a single dominant gene.

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Influence of Canopy Depth on Susceptibility of 'Marsh' Grapefruit to Chilling Injury[1]

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Abstract. **'Marsh' grapefruit (*Citrus paradisi* Macf.) harvested from the exterior canopy of the tree were more susceptible to chilling injury (CI) at 4.4°C than grapefruit harvested from the interior canopy of the same tree. No differences were found in the levels of total soluble carbohydrates, reducing sugars, and sucrose in the peels of the fruit from the 2 canopy depths. An unusual pattern of CI, remarkably similar to the pattern of citrus rust mite [*Phyllocoptruta oleivora* (Ashm.)] damage, was observed in several of the exterior canopy fruit. Although the fruit had no visible rust mite damage at harvest, fruit remaining on the tree developed the characteristic bronzing associated with rust mite injury within 2 to 3 weeks after the test fruit were harvested. It is suggested that environmental and biotic factors predispose grapefruit to CI.**

Susceptibility of grapefruit to CI at 4.4°C is seasonal with fruit harvested early (October-December) and late (April-June) generally being more susceptible to CI than fruit harvested at midseason (4, 5, 10). The mechanism of the midseason resistance is not known, but may be related to soluble metabolites or metabolic activity of the peel (5, 10).

Fruit in various canopy positions are exposed to widely differing microclimates during growth and maturation (3, 11). Differences were observed in the internal quality of fruit from the sunlit exterior canopy positions and the shaded interior canopy positions, especially early in the season (11). For this reason, it was of interest to determine if canopy depth influenced the susceptibility of grapefruit to CI.

'Marsh' grapefruit were harvested separately from the interior and exterior canopies of trees on 2 different dates during the 1979-80 season. Fruit were harvested in mid-November 1979 from 4 trees and combined into one interior and one exterior canopy sample. In mid-January, fruit were harvested from 3 trees and maintained as separate samples instead of being combined as was done for the November harvest. The fruit were washed with detergent, rinsed, and air dried. Forty fruit of each lot were stored at 4.4°C and CI (peel pitting) was rated at weekly intervals as previously described (4, 10).

For carbohydrate analyses 5 fruit were randomly selected from each lot, the flavedo (colored) portion of the peel was removed, and sugars were extracted in 80% ethanol as previously described (10). Total soluble carbohydrates were determined by the anthrone method (6) and reducing sugars and sucrose were determined by gas-liquid chromatographic procedures (7) modified as follows: 1 ml of the resin treated ethanol extract was dried and 1 ml of Tri-Sil (Pierce Chemical Co., P. O. Box 117, Rockford, IL 61105) added. The sample was sonicated for 20 min followed by centrifugation. Two and one-half μl of the sample were injected into the gas chromatograph.

Fruit harvested from the exterior canopy were more susceptible to CI than fruit harvested from the interior canopy (Fig. 1). The fruit harvested in November from both the exterior and interior canopies were more susceptible to CI than fruit harvested in January. All of the exterior fruit and 60% of the interior fruit harvested in November had CI lesions after only 4 weeks of storage at 4.4°C (Fig. 1).

Although seasonal resistance of grapefruit to CI was previously related to high reducing sugar levels in the peel (5, 10), no differences were found in the levels of either total soluble carbohydrates, reducing sugars, or sucrose in the peel of exterior and interior canopy fruit (data not shown). These results do not necessarily rule out the possibility that reducing sugars play a role in the resistance of grapefruit to CI. While the interior fruit developed greater resistance to CI, the exterior canopy fruit may have been predisposed to CI by an environmental or biotic factor absent in the interior canopy.

An unusual pattern of pitting was observed in several of the exterior canopy fruit harvested in January which contributed to both the speed of development and severity of CI. Instead of being randomly located and widely scattered over the surface of the fruit, a typical characteristic of CI in grapefruit (Fig. 2A), the pits were restricted to and generally covered an entire hemisphere of the fruit (Fig. 2B, C, D). CI of these fruit was more rapid (pitting occurring in less than 2 weeks) and

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