juice originating from ruptured juice vesicles to travel to the peel unobstructed. We also observed that in almost all cases BEC symptoms appeared within 15 minutes of induction. In some cases BEC symptoms took less than five minutes to appear.

In many instances the conditions to induce BEC were sufficient to cause juice vesicle rupture and juice leakage into the central core or the peel. However, no visible BEC symptoms were observed. These fruit were characterized by a healthy peel and/or core which absorbed juice and prevented it from reaching the peel. Although BEC symptoms were not visible in these fruit, the mechanical damage and presence of juice in the albedo can result in generation of off-flavors and predispose fruit to decay. BEC is a disorder that can essentially be eliminated by proper handling of grapefruit. When harvesting thin-peeled grapefruit during the warmest part of the day, reducing fruit field heat and reducing impacts both in the field as well as the packinghouse can greatly reduce the incidence of BEC.

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Proc. Fla. State Hort. Soc. 111:257-260. 1998.

CITRUS PLOIDY LEVEL INFLUENCE ON THE FLAVONOID COMPOSITION IN JUICE AND LEAF TISSUE IN GRAPEFRUIT CULTIVARS

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Additional index words. Citrus paradisi Macf., tetraploid, triploid, diploid, naringin, narirutin, leaves, fruit.

Abstract. **Diploid *Citrus paradisi* Macf. (grapefruit) cultivars (cvs.) were found to consistently contain higher levels of Brix %, acidity (i.e. citric acid/ml) and Brix/acid ratios in the juice than their tetraploid counterparts. In contrast, higher levels of narirutin, naringin, and neohesperidin were found in the fruit juices of tetraploid cvs. In naringin-dominate grapefruit leaf types such as 'Hall' and 'Seedy Marsh', naringin levels were higher in tetraploid leaves than in diploid leaves. 'Hall' Leaves had less narirutin but more neohesperidin in tetraploid leaves than in diploid leaves. However in 'Seedy Marsh' leaves the opposite occurred. Neohesperidin-dominate leaf type, 'Imperial' and 'Royal' cvs. have higher neohesperidin levels in tetraploid leaves than in diploid leaves while exhibiting less narirutin levels in tetraploid leaves than in diploid leaves. Triploid progeny expressed variable levels of flavonoids, some are several fold over that found in their diploid and tetraploid parents.**

The occurrence of polyploidy in *Citrus* and related genera has been reported since the 1920's (Cameron and Soost, 1968; 1969; Cameron, Soost and Olson, 1964; Soost and Cameron, 1981). Tetraploidy is the most common natural polyploidy. Crossing a diploid with a tetraploid results in triploids, which are often known for their high vigor and relatively high yields (Cameron and Soost, 1969). Triploids are essentially seedless. Some of the Citrus triploids have many favorable fruit characters but all were moderately or very high in acid, evidently as a result of having either tetraploid grapefruit or tetraploid lemon as one of the parents. Tetraploid pollen or seed parents are possible. Autotetraploids, spontaneous tetraploids appear to occur almost entirely as nucellar seedlings. Generally, tetraploid leaves are broader in proportion to their length than diploids and are considerably thicker. Leaf color tends to be darker. Wings of the petioles are usually broader in some varieties and they often fuse with the leaf blade (Cameron and Soost, 1968). Thorniness is more pronounced in tetraploids, vigorous shoots are less common and growth is slower and generally the tree is smaller, less erect, and more compact. Tetraploids are slower in fruiting and produce less fruit. Juice vesicles are tougher and the yield of juice on the basis of the whole fruit weight is much lower in tetraploids compared to diploids. The influence of polyploidy on the chemical composition of the leaves and fruit is unknown.

Among the plant phenolics, flavonoids are of particular interest, since they appear to function in all roles in which plant secondary metabolites have been implicated (Dakora, 1995). Flavonoids and the other phenolics derived from the phenylpropanoid biosynthetic pathway, have been reported as having a wide variety of physiological effects in both plants and animals, serving as enzyme activators and inhibitors, metal ch-

elators, antioxidants, free radical scavengers, transcription regulators, phytohormones, and as mutagenic, antimutagenic, carcinogenic, anticarcinogenic, cytotoxic, antineoplastic, anti-inflammatory and anti-allergenic substances. In plants they have been shown to function in protection from UV radiation damage; in mineral nutrition; in temperature and water stress; in pollination and seed dispersal (by their color properties); and as constitutive chemoprotective agents against other plants, microbial pathogens, fungi, insects, and herbivores.

As a specific result of the plant's interaction with changes in its the environment, specific flavonoids and other phenolic metabolites are produced and accumulated (Dixon and Paiva, 1995; Hahlbrock and Scheel, 1989; Laks and Pruner, 1989; Snyder and Nicholson, 1990). The biosynthesis of new flavonoids is constitutive in plants, being internally controlled during normal growth and development, such as new vegetative leaf growth and reproductive organ development (Heller and Forkmann, 1988). Flavonoid biosynthesis may also be induced by exogenous stimuli, such as changes in light and temperature (Hahlbrock and Scheel, 1989). Biosynthesis and/or modification of constituative flavonoids can be triggered, or elicited, by damage to the plant caused by physical agents (wind, freezing, water stress, ozone, heavy metal ions, certain herbicides), herbivore attack (insects, grazing animals) and microbial invasion (bacteria, fungi) (Dixon and Paiva, 1995; Dixon et al., 1992; Ebel, 1986; Ebel and Mithofer, 1998; Nahrstedt, 1990; Nicholson and Hammerschmidt, 1992).The purpose of this investigation was to examine the flavonoids as a function of the chromosome number in juice and leaf tissue for several grapefruit cultivars.

Materials and Methods

The following *Citrus paradisi* Macf. (grapefruit) cvs were employed in the study: 'Hall', 'Imperial', 'Royal', and 'Seedy Marsh'. Plant material was obtained from Citrus Variety Collection at the University of California, Riverside. Approximately 200 mg of leaf tissue was weighed and placed into a 1.5 ml plastic centrifuge tube. One ml of 50% DMSO-methanol was added, and the tissue was chopped up finely with a spatula. The extraction mixture was centrifuged and filtered (0.2 micron filter) prior to chromatography.

The chromatographic system consisted of two Shimadzu pumps (LC-6A) and a Shimadzu automatic sampler (SIL-6A) controlled by a Shimadzu controller (SCL-6A). The 25 cm reverse phase column was a Whatman Partisil 5 ODS-3. The detection system was a Hewlett Packard 1040 diode array detector. The detector was set to measure spectra from 220 to 400 nm and record the chromatograms as absorbance at 285 nm. The attenuation varied but was usually set at 0.1 absorbency units full scale. At a flow of 1.0 ml/min the gradient elution schedule consisted of an initial 2 min. of 80% 0.01 M H_3PO_4 and 20% methanol followed by a linear gradient to 100% methanol in 55 min. Quantification of the individual compounds was based on integrated areas and an external standard (Vandercook and Tisserat, 1989).

An aliquot of juice was titrated potentiometrically for total acidity (Vandercook, Price and Harrington, 1975). The results were calculated as mg anhydrous citric acid per ml. The Brix values were measured by refractometer and are uncorrected for acid.

Results and Discussion

Ploidy is defined as the number of sets of chromosomes present in the cells of the plant: haploid, diploid, triploid, tetraploid, etc. In citrus, breeding has generated several plants that are triploid and tetraploid in addition to the normal diploid form. Tetraploidy, 4N, is the most commonly occurring form of citrus ploydoidy. It is derived from nucellar embryogeny. Triploidy, 3N, occurs in the offspring of a diploid and tetraploid cross.

Two distinct types of grapefruit occur based on their flavonoid composition: the naringin dominant grapefruit and the neohesperidin dominant grapefruits. In the neohesperidin-dominant cultivars 'Imperial' and 'Royal', the tetraploid cultivars generally have higher concentrations of flavonoids in the leaves than the diploid cultivars as shown in Table 1. This is especially true in the 'Imperial' cultivars. Notice that the concentration of neohesperidin is over three times higher in the tetraploid than in the diploid. This difference is less pronounced in the 'Royal' cultivar, although the trend also occurs. In general, the flavonoid concentrations of the juice of the neohesperidin dominant cultivars were fairly low compared to those occurring in the leaf. It is interesting to note that the naringin levels are now generally higher than the neohesperidin levels in the juice samples as compared to that occurring in the leaf. The juice characteristics are shown in Table 2. Brix levels of mature fruit for both the tetraploid were less than in the diploid cultivars of the neohesperidin dominant grapefruits but were generally high, measuring above 12 in all cases. The acid levels were somewhat higher in the neohesperidin dominant cultivars, over that of the naringin dominant lines. This is especially true in the tetraploid 'Imperial' line that has a high acid concentration of 26.1 mg per ml.

Turning to the naringin dominant cultivars, 'Seedy Marsh' and 'Hall', there is again just slightly higher concentrations of the flavonoids in the tetraploid leaves over that of the diploid leaves as shown in Table 1. Note that the concentration of naringin and neohesperidin are nearly equal in these leaves. The levels of the flavonoids in the juice of the naringin dominant cultivars are fairly low as compared to that occurring in the leaves. Interestingly, there are no measurable levels of neohesperidin in the juice for any these samples although it was quite prominent in the leaves. In both cases, the levels of naringin are higher in the tetraploid than in the diploid. Analysis of juice chemical characteristics of the naringin dominant cultivars show pretty good Brix levels, similar to that of the neohesperidin dominant cultivars, and somewhat lower acid levels over that of the neohesperidin dominant cultivars.

Figures 1 and 2 show the analysis of flavonoids in the leaves and fruit of several triploid progenies derived from crossing a diploid parent, 'Siamese acidless' pummelo, and a tetraploid parent, 'Seedy Marsh' grapefruit. Both of these

Table 1. Flavonoids in leaves of diploid and tetraploid grapefruit cultivars. NRT-narirutin, NRG-naringin, NHP-neohesperidin.

Cultivar	Ploidy	NRT (mg/g)	NRG (mg/g)	NHP- (mg/g)
Naringin Dominant Types:				
Seedy Marsh	2N	0.68	2.38	3.14
Seedy Marsh	4N	0.73	3.45	2.71
Hall	2N	0.67	2.09	2.93
Hall	4N	0.60	3.96	3.15
Neohesperidin Dominant Types:				
Imperial	2N	0.71	0.95	1.66
Imperial	4N	0.59	1.36	6.51
Royal	2N	0.91	1.12	2.43
Royal	4N	0.79	1.10	3.33

Table 2. Flavonoid content and physical characteristics of juice from diploid and tetraploid grapefruit cultivars.

Cultivar	Ploidy	Brix (%)	Acidity (citric acid/ml)	Brix Acid Ratio	NRT (mg/ml)	NRG (mg/ml)	NHP (mg/ml)
Naringin Dominant Types:							
Seedy Marsh	2N	12.5	18.4	6.8	0.07	0.11	—
Seedy Marsh	4N	11.8	21.6	5.4	0.08	0.15	—
Hall	2N	11.1	17.0	6.5	0.10	0.24	—
Hall	4N	11.6	20.5	5.6	0.16	0.43	—
Neohesperidin Dominant Types:							
Imperial	2N	12.9	16.0	8.0	0.10	0.13	0.03
Imperial	4N	12.6	26.1	4.8	0.19	0.35	0.07
Royal	2N	13.1	17.3	7.6	0.05	0.08	0.03
Royal	4N	12.8	19.4	6.6	0.07	0.15	0.05

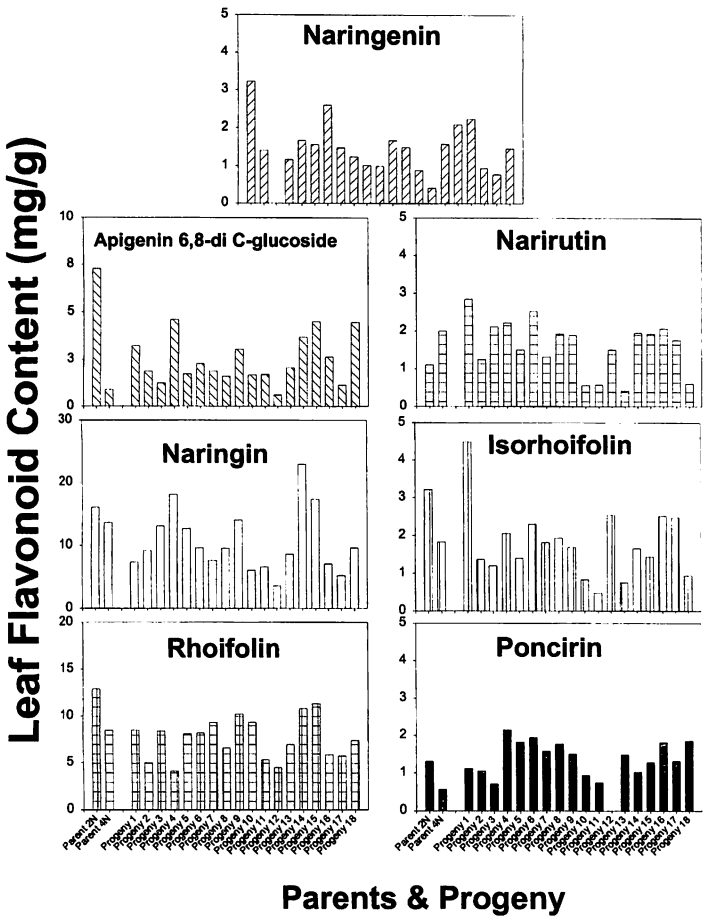

Figure 1. Flavonoid concentrations in the leaves of triploid progeny and the diploid and tetraploid parents. Concentrations are mg/g dry weight.

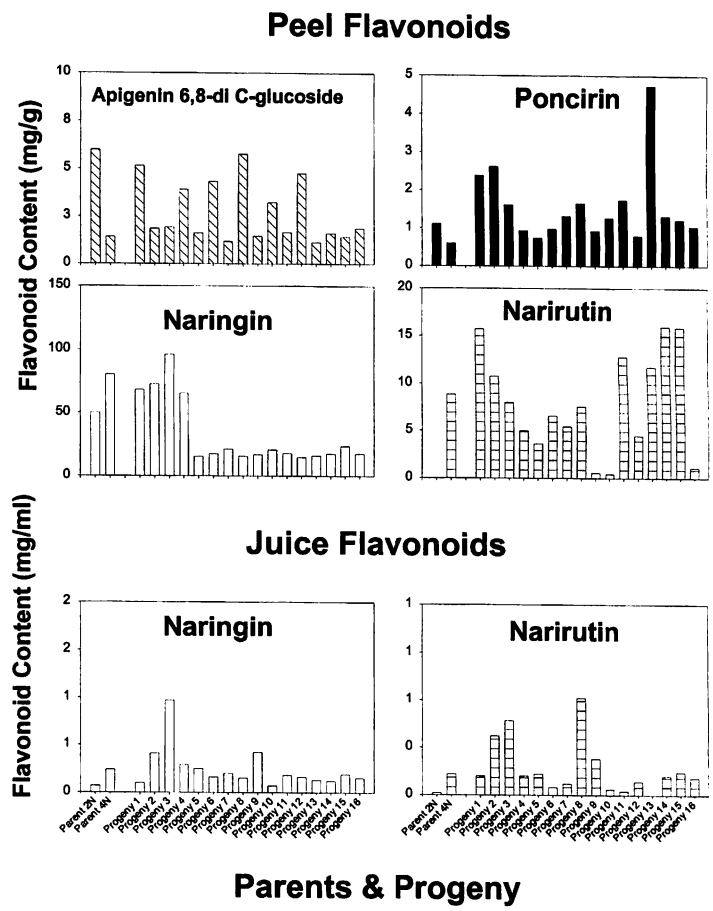

Figure 2. Flavonoid concentrations in the peel and juice of triploid progeny and the diploid and tetraploid parents. Concentrations in the peel are mg/g dry weight. Concentrations in the juice are mg/ml.

parents are naringin dominant types. The flavonoid analysis of the parents, appear in the first two bars in each panel of the three figs., show the typical pattern, that is the levels of the flavonoids in tetraploid parent is higher than that of the diploid parent. This is especially noticeable in the narirutin and naringin concentrations.

Interestingly, the flavonoid concentrations in the fruit of the triploid progeny are quite variable. In some cases, the flavonoid levels are easily five times higher in the triploid progeny than that of the tetraploid parent. While in other cases, the flavonoid levels of the triploid progeny are similar to that of the diploid parent. This is especially noticeable in the levels of naringin and narirutin in the fruit shown in Fig. 2, but less variability of the flavonoid concentrations in the leaves shown in Fig. 1. This observation re-inforces the theory that the genes controlling flavonoid accumulation is a mixture of dominant and recessive genes. In the tetraploid parent, there are four copies of the genes, and it logically follows that there are higher flavonoid levels over that of the diploid parent. This was also observed in the diploid and tetraploid forms of the same cultivar such as in 'Seedy Marsh' shown in Tables 1 and 2. The triploids, however, only get one set of genes from the diploid parent and two sets of genes from the tetraploid parent. This 'uneven' mix of genes seems to result in a varied expression of dominant and recessive genes in the progeny.

We suggest on the basis of this study that due to the bitter flavonoids levels are especially influenced by the parents and should be considered when conducting crosses. This study shows that ploidy level influences flavonoid content.

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Proc. Fla. State Hort. Soc. 111:260-263. 1998.

A DISCUSSION OF IN VITRO CONTAMINATION CONTROL OF EXPLANTS FROM GREENHOUSE AND FIELD GROWN TREES

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Additional index words. Plant tissue culture, sterilization, biocides, isothiazolones, antimicrobial, hypochlorous acid, stabilized chlorine isocyanurates.

Abstract. **Controlling fungal and bacterial contamination of woody plant material is important and extremely difficult from field sources. Greenhouse grown material has less decontamination problems with the field material. Isothiazolone biocides and sodium dichloroisocyanurate (NaDCC) were used singly and in combination to reduce microbial contamination to less than 5% in bud explants derived from field-grown citrus trees. Coating the explant with a slurry containing 20 mL·L^{-1} Plant Preservative Mixture (PPM), a mixture of two isothiazolones, and culturing on medium containing 5 mL·L^{-1} PPM resulted in 63% clean explants compared to >90% contamination with standard disinfestation procedures. Explants treated for 48 hr with 100 or 300 ppm NaDCC resulted in 83% and 96% clean explants, respectively. Phytoxicity problems were present at the 20 mL·L^{-1}.**

In vitro contamination by fungi, bacteria, or yeast is one of the most serious problems of commercial and research plant tissue culture laboratories (Leifert et al., 1994). The establishment of an in vitro culture requires the removal of culturable fungal and bacterial contaminants. Contamination can be especially troublesome form field obtained materials. The inability to adequately control contamination levels is the primary reason for failure of commercial laboratories (Leifert and Woodward, 1997). Chemical methods used include antibiotics and fungicides, alcohols, mercuric chloride, and oxidizing biocides such as halogen compounds (e.g., chlorine, bromine, and iodine) and hydrogen peroxide. Each method used balances factors such as the plant species, type of explant, phytoxicity, type of contaminant(s), and cost to obtain desired results.

Isothiazolones are a class of industrial biocides that have been used prophylactically in the form of Plant Preservative Mixture (PPM) in tissue culture medium to control microbial contamination (Niedz, 1998). PPM contains a mixture of two isothiazolones, methylchloroisothiazolinone and methylisothiazolinone (Guri and Patel, 1998). Adequate control of microbial contamination was achieved as long as inoculum levels were low. Also, little phytotoxicity was observed even at the highest concentrations tested of 2 mL·L^{-1}, twice the highest recommended rate by the manufacturer (Niedz, 1998). In this study, significantly higher isothiazolone levels are used to control the inoculum types and levels associated with greenhouse and field-grown plant material.

Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable. We thank Ms. Delores Lomberk, Mr. Scott Hyndman, and Mr. Gerald Mozoruk for their assistance in the collection, preparation, and in vitro culture of plant material, and Dr. Assaf Guri of Plant Cell Technology for generously providing PPM samples for testing.