

# Development of Seedless Orange and Grapefruit Cultivars through Seed Irradiation

C.J. Hearn

U.S. Department of Agriculture, Agricultural Research Service, 2120 Camden Road, Orlando, FL 32803

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**Abstract.** Seed of 'Pineapple' orange [*Citrus sinensis* (L.) Osbeck] and 'Duncan' and 'Foster' grapefruit (*C. paradisi* Macf.) were exposed to gamma rays at 10, 15, 20, 25, and 30 krad. Seedling emergence was delayed. LD<sub>50</sub> levels were 10-15 krad for 'Pineapple', 15 krad for 'Duncan', and ≤10 krad for 'Foster'. The greater sensitivity of 'Foster' may have been due in part to higher seed moisture content at treatment. Seedless mutants were obtained following gamma irradiation and fruiting of small numbers of seed and seedlings. The frequency of seedless mutants of 'Pineapple' and 'Duncan' was highest following treatments of 20-25 krad.

Genetic improvement of oranges and grapefruit by conventional plant breeding methods has been limited, because these citrus forms are heterozygous and reproduce largely by nucellar embryony. Therefore, improvement has been largely through selection of naturally occurring somatic mutants (1). Irradiation may be of value as a tool to enhance the frequency of mutation of vegetatively propagated plants. Hensz (5) used X-rays and thermal neutrons on citrus seed and buds in attempts to induce somatic mutations. The 'Star Ruby' grapefruit (*C. paradisi* Macf.) was released as a seedless cultivar with improved color following thermal neutron treatment of seed (6). Gregory and Gregory (3) treated citrus seed and buds with X-rays in an effort to develop mutants with improved cold hardiness. Spiegel-Roy and Padova (8) determined the radiosensitivity of orange seed and buds to gamma rays from <sup>60</sup>Cobalt as a first step in a comprehensive mutation breeding program.

'Pineapple' orange and 'Foster' and 'Duncan' grapefruit produce fruit with excellent quality in Florida; however, their seedlessness makes them of limited value in the fresh fruit market. Because of their good quality traits, these cultivars were selected as candidates for seed irradiation with gamma rays in an attempt to produce mutant clones with fruit that are commercially seedless.

## Materials and Methods

Seed of nucellar 'Pineapple' orange and nucellar 'Foster' and 'Duncan' grapefruit were collected 11 May 1970, in Lake County, Fla. They were surface-disinfected with 8-hydroxyquinoline sulfate and allowed to air-dry for several hours. Dry weights of seed samples showed that 'Foster' contained 47.5% moisture, 'Duncan' 35.7%, and 'Pineapple' 32.9%. Seed were stored in sealed plastic bags in a refrigerator at 4°C until samples were irradiated at the Univ. of Florida, on 27 May 1970. Seed samples were exposed to a <sup>60</sup>Co source emitting 20 krads per minute of gamma rays. Dosage levels were 10, 15, 20, 25, and 30 krad. Sample sizes per treatment were 140 seed for 'Duncan' and 'Foster', 114 seed for 'Pineapple', and 100 seed for the controls. The seed samples were divided into 2 replications and planted in a greenhouse on 28 May 1970, in seed flats containing a 2:1 mixture of vermiculite and peat. The planting depth was 1-1.5 cm. Ambient temperatures in the greenhouse were about 30°C during the day and 25° at night.

The emerging seedlings were counted at 3-day intervals beginning 14 days after planting and continued for a 70-day period. The seedlings were grown in the greenhouse until they were transplanted in the field in Feb. 1972 where they have grown to the present. The populations consisted of 160 'Pineapple', 65 'Duncan', and 21 'Foster' trees planted at 1.5 × 6.1-m spacing. Fruit were examined for seed content as the trees began bearing in the 1977-1978 season. Fruit were examined for seed content during the 1979-1980 and 1980-1981 seasons. No fruit were available during the 1981-1982 and 1982-1983 seasons because of the Jan. 1981 and Jan. 1982 freeze injury.

## Results and Discussion

Seedling emergence was delayed by irradiation treatment and the delay period generally was related to the irradiation dosage. This is in agreement with citrus seedling emergence data by Gregory and Gregory (3) following X-ray treatments of 1 to 10 krad. The 30-krad treatment was lethal to all seed in the experiment. Gregory and Gregory (3) found 10 krad to be lethal to fully hydrated grapefruit seed; Spiegel-Roy and Padova (8) found that 20 krad of gamma rays was lethal to orange seed containing 72% to 77% moisture. Gamuzov (2) found that 30 krad from X-rays reduced germination of lemon [*C. limon* (L.) Burm. f.] seed by 77.6%, and 50 krad was lethal. The moisture content was not reported.

The maximum seedling emergence (Fig. 1) of the 'Pineapple' control was attained after 35 days, while 67-70 days were required for seed treated at 10, 15, and 20 krad. Only 3.5% of seedlings emerged after 25 krad and these emerged within 57 days. The final average seedling emergence percentages were statistically different at the 5% level between each treatment (Duncan's multiple range test). The LD<sub>50</sub> for 'Pineapple' seed was between 10 and 15 krad, but probably closer to 15 krad. Spiegel-Roy and Padova (8) reported the LD<sub>50</sub> of 'Shamouti' orange seed at 10 krad.

The maximum seedling emergence (Fig. 2) of the 'Duncan' control was attained after 35 days. The maximum emergence from seed treated at 10 krad had occurred at 56 days, while those at 15 krad required 70 days, those at 20 krad required 63 days, and those at 25 krad required 49 days. Only 14% of the seedlings emerged after treatment at 20 krad and 2% emerged after 25 krad. The final average seedling emergence percentages were statistically different at the 5% level between each treatment. The LD<sub>50</sub> for 'Duncan' seed was 15 krad. Hensz (5) reported an LD<sub>50</sub> between 5 and 10 krad for grapefruit seed treated with X-rays. The optimum treatment for X-ray-treated

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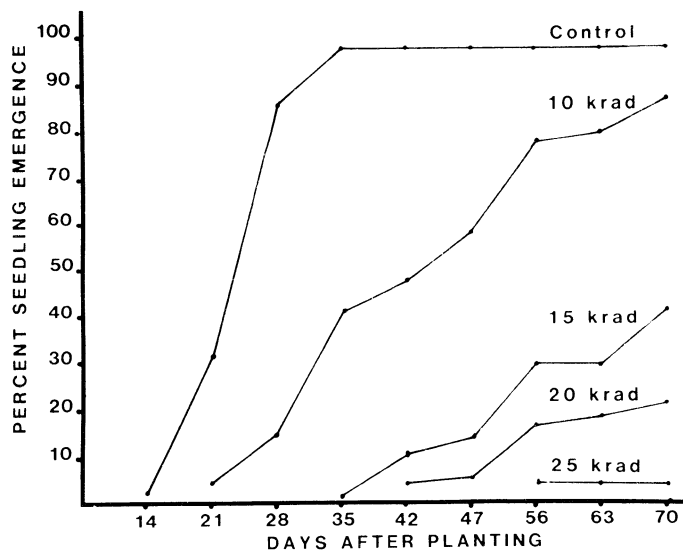


Fig. 1. The effect of seed irradiation on seedling emergence of 'Pineapple' orange.

'Duncan' seed by Gregory and Gregory (3) was 7 krad and no seedlings emerged after 10 krad. They did not report seed moisture content. Further, they stated that "citrus seed do not permit drying and, therefore have to be irradiated fully hydrated."

The seedling emergence curves for 'Pineapple' and 'Duncan' (Fig. 1 and 2) were very similar; however, the 'Duncan' seedlings showed a tendency to earlier emergence. The moisture content of seed of these cultivars was similar at treatment time.

The seedling emergence curves for 'Foster' grapefruit (Fig. 3) show rapid emergence of the control and low emergence from the treated seed. Only 16% emergence occurred after 10 krad, only 1% after 15 krad, and none at higher dosages. The final average emergence percentages were statistically different at the 5% level between each treatment (control, 10 krad, and 15 krad). The LD<sub>50</sub> of 'Foster' seed was less than 10 krad and the sensitivity may have been due to the high moisture content of the seed. Samples of 'Foster' and 'Duncan' grapefruit were collected on 28 Dec. 1982 and air-dried for 2 durations under identical conditions. Moisture determinations were made after each drying period and the moisture contents were identical for the cultivars.

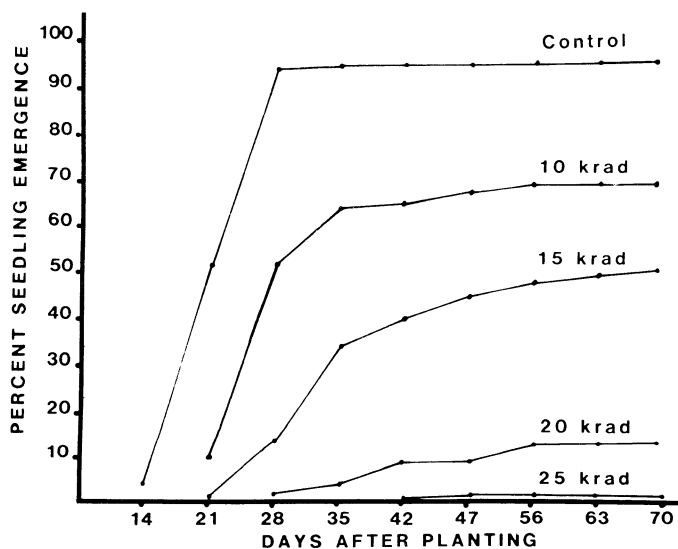


Fig. 2. The effect of seed irradiation on seedling emergence of 'Duncan' grapefruit.

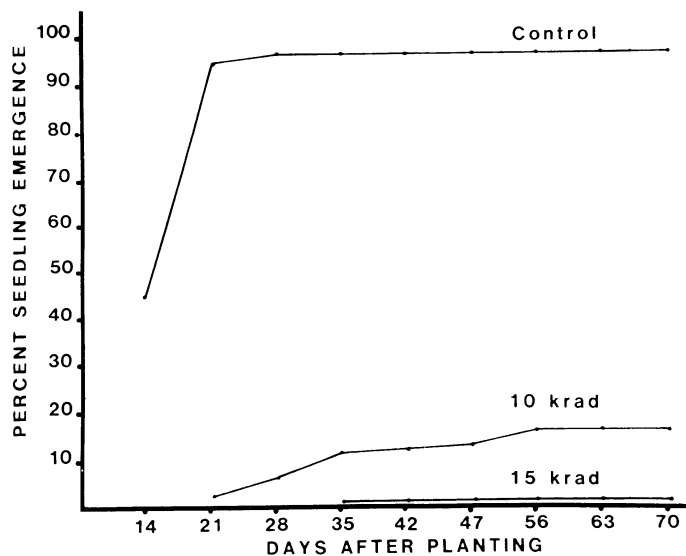


Fig. 3. The effect of seed irradiation on seedling emergence of 'Foster' grapefruit.

They contained 39% and 35% moisture, respectively, after the drying periods. No difference between cultivars was expected.

Root and shoot terminals of germinating seedlings from irradiated seed were stubby and calloused. This suggested injury or death to some of the terminal cells. The effect appeared to be more severe as the treatment increased. This could explain the delay in seedling emergence. Small, distorted leaves developed after the affected plants emerged and internodes were so short that the plants had a rosette appearance. The small plants had appearances similar to that observed by Haskins and Moore (4) following seed X-ray treatments. After a period of time, a single leader shoot emerged which exhibited apical dominance. As the shoot grew, the newly emerged leaves and internodes appeared normal as if the plants had outgrown the irradiation effects. Control plants grew vigorously following emergence and were substantially taller.

The seedlings were transplanted to the field in 1972 for fruiting. An occasional 'Pineapple' and 'Duncan' seedling had a few fruit in the 1977-1978 and 1978-1979 seasons, but not enough for reliable evaluations. During these first 2 seasons, 20 'Pineapple' plants had fruit with reduced seed content. Only 2 of these were among the 13 with the lowest average seed content during the 2 subsequent seasons. Six 'Duncan' plants had reduced seed content in 1977-1978, but only one of these was among the 3 with the lowest average in subsequent seasons. Apparently the first and 2nd crops of only a few fruit may not give reliable estimates of seediness of subsequent crops. This agrees with a report by Hensz (7) on 'Hudson' and 'Foster Pink' grapefruit. Data on seedlessness during the first 2 crops were disregarded for this reason.

Seediness of fruit of 'Pineapple' seedling selections during the 1979-1980 and 1980-1981 seasons is presented in Table 1. Average seed content was 3.3 or less and the maximum was 9 during 2 consecutive seasons. Citrus fruit containing 0-9 seed usually are considered "commercially seedless" (7). These selections will be evaluated in field plantings. Average seed content per fruit and the range for each selection showed little variation during the 2 seasons. Data on seed content of the 'Pineapple' control are included for comparison. Its seasonal variation was greater than that of the irradiated selections.

Three mutant selections of 'Duncan' grapefruit with fruit having a maximum of 9 seed during 2 consecutive seasons are listed

Table 1. Seediness of fruit from 13 mutant seedling selections of 'Pineapple' orange resulting from seed irradiation.<sup>4</sup>

Selection no.	Dosage krad	Season			
		1979-1980		1980-1981	
		Avg no. seed per fruit	Range	Avg no. seed per fruit	Range
9-93	25	2.8	0-5	2.0	0-6
9-94	20	0.6	0-3	0.6	0-2
9-95	20	2.7	0-6	2.1	0-5
9-109	20	0.9	0-4	1.7	0-5
9-116	20	2.2	1-4	1.8	0-3
9-119	20	0.7	0-2	3.2	0-9
9-125	20	2.8	0-2	3.3	0-8
10-8	20	0.9	0-3	2.1	0-4
10-29	15	2.4	0-7	2.8	0-6
10-40	15	0.3	0-1	1.3	0-3
10-56	15	1.7	0-4	2.7	0-6
10-98	10	0.7	0-2	1.3	0-3
10-122	10	0.6	0-2	0.9	0-2
Pineapple control		17.5	11-25	22.1	13-31

<sup>4</sup>Data based on random samples of 20 fruit per single-tree selection each season.

in Table 2. Seasonal variation in average seed content and range was small. These 3 mutants were selected from a population of 65 plants.

Only a few of the 'Foster' seed germinated following irradiation and 21 plants were established in the field. One of these had reduced seed content, but the maximum content of one of the fruit was greater than 9.

Important considerations in this irradiation research were: 1) the optimum dosage, and 2) the smallest number of resulting plants necessary for a reasonable probability of finding seedless mutants. The 2nd consideration is particularly important because citrus trees require considerable space and time before results are obtained. Only 570 seed of 'Pineapple', 700 of 'Foster', and 700 of 'Duncan' (1970 total) were treated in this experiment. Hensz (5) treated 32,000 seed of 6 cultivars with either X-rays or thermal neutrons, but did not report the numbers of each cultivar. He reported that 5900 seedlings were grown from these seed, but did not report the number grown to fruiting age. Since 3 of the 6 cultivars produce "commercially seedless" fruit, production of seedless mutants was not the objective of that

Table 2. Seediness of fruit from 3 mutant seedling selections of 'Duncan' grapefruit resulting from seed irradiation.<sup>4</sup>

Selection no.	Dosage krad	Season			
		1979-1980		1980-1981	
		Avg no. seed per fruit	Range	Avg no. seed per fruit	Range
9-31	20	4.5	1-8	5.7	2-8
9-34	20	1.0	0-3	1.8	0-4
9-83	15	4.6	2-9	3.0	1-9
Duncan control		51.3	47-66	52.4	48-65

<sup>4</sup>Data based on random samples of 15 fruit per single-tree selection in 1979-1980 and 20 fruit in 1980-1981.

research. Later, Hensz (7) reported that 580 'Hudson' and 540 'Foster Pink' grapefruit seedlings had fruited. This is the only report found on the fruiting of citrus plants that originated from irradiated seed. The other reports dealt only with irradiation effects on germination and seedling emergence.

In this experiment, a population of only 160 'Pineapple' seedlings was established in the field for fruiting. Further studies of the 13 selections in Table 1 show that 1 resulted from the 25-krad treatment (1 per 7 plants). Seven selections resulted from the 20-krad treatment (1 per 6.6 plants), 3 resulted from 15-krad treatment (1 per 16 plants), and 2 resulted from 10-krad treatment (1 per 31 plants). There is no apparent relationship between irradiation dosage and seed content among the selected 'Pineapple' mutants since some with low seed content resulted from low as well as high dosage (Table 1).

Among the 65 plants of 'Duncan' grapefruit, 1 of the 3 seedless selections resulted from the 25-krad treatment (1 per 3 plants). One resulted from 20-krad treatment (1 per 22 plants), and 1 resulted from 15-krad treatment (1 per 40 plants).

The LD<sub>50</sub> has been suggested as an efficient level for irradiation treatment (7). The above data suggest that the most efficient dosage was greater than the LD<sub>50</sub> level for 'Pineapple' and 'Duncan'.

Variations in tree vigor, growth habit, and fruit traits were apparent, but reliable inferences could not be made on the single-tree mutants growing in close plantings.

The results of this research indicate that commercially seedless mutants of seedy orange and grapefruit cultivars can be obtained following gamma irradiation of seed. Seedling emergence was delayed by irradiation and the LD<sub>50</sub> for 'Pineapple' orange and 'Duncan' grapefruit seed was about 15 krad. Seed of 'Foster' grapefruit were more sensitive to irradiation, as only a few seedlings were obtained and none produced seedless fruit. This greater sensitivity may have been due to the higher moisture content at treatment time. This may help to explain the differences in LD<sub>50</sub> of citrus seed reported by others. Some of the variation may be because of the type of irradiation or cultivar differences. About 8% of the surviving seedlings from 'Pineapple' orange seed produced seedless fruit, whereas 4.7% of those from 'Duncan' grapefruit produced seedless fruit. The frequency of seedless mutants was higher following treatments of 20 to 25 krad. This suggests that the most efficient dosage for seedlessness was higher than the LD<sub>50</sub> level.

The seedless mutants have been propagated to determine their horticultural characteristics while further overcoming juvenility (thorniness and vigorous vegetative growth).

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## Influence of Storage Temperature and Ethylene on Firmness, Acids, and Sugars of Chilling-sensitive and Chilling-tolerant Tomato

J. Manzano-Mendez,<sup>1</sup> J.R. Hicks,<sup>2</sup> and J.F. Masters<sup>3</sup>

*Vegetable Crops Department, Cornell University, Ithaca, NY 14853*

*Additional index words.* *Lycopersicon esculentum*, heat tolerance, ripening

**Abstract.** Two cultivars and 2 experimental chilling-tolerant lines of tomato (*Lycopersicon esculentum* Mill.) were harvested mature-green and stored for 15 days at 5°, 20°, and 35°C with or without the introduction of ethylene; portions of the high and low temperature samples were moved to 20° for an additional 10 days. Samples were analyzed for firmness, sugars, and acids. Fruit of the chilling-tolerant tomato lines were firmer than the commercial cultivars in all temperature treatments. Ethylene enhanced softening in the chilling-sensitive cultivars only at 20°, while the chilling-tolerant lines showed an effect only at 35°. The chilling-tolerant lines appeared to be more heat-tolerant than the sensitive cultivars. Sugar and organic acid analyses were not as clear-cut, often revealing a tendency for the cherry-sized fruit to behave similarly to each other and different from the normal-sized fruit. The chilling-tolerant lines held at 5° or moved from 5° to 20° had lower monosaccharide levels than the corresponding sensitive cultivars. This also was true when fruit were moved from 35° to 20°. 'New Yorker' tomato had low levels of malate after exposure to 35°, which resulted in a high citrate/malate ratio not evident in the other 3 cultivars. Phosphoric acid levels were higher in the chilling-tolerant tomato fruit and increased with increasing storage temperature. Line 281 deviated from the other 3 cultivars in that, in general, acids increased and sugars decreased with increasing storage temperature.

Normal fruit ripening in tomato is affected by low temperatures (5, 15) and exposure to high temperatures inhibits the development of red color and softening (11, 12, 19). While the rates of softening and color development have been predicted mathematically at temperatures from 12° to 27°C, the model did not describe accurately the situation outside of this range (23). This emphasizes the limited conditions under which normal ripening will occur.

Tomato fruit ripen relatively quickly with many simultaneous changes, making it difficult to understand the effect of each change. A number of reports have concentrated on isolating various facets of ripening through studies of tomato mutants that do not ripen normally (24). Another approach has been to store tomato fruit in controlled atmospheres, where substrates of glycolysis and the citric acid cycle change as in ripening fruit while color development and some enzyme synthesis does not proceed until the fruit are transferred to a normal atmosphere (9, 10). A 3rd method has been to store tomato fruit at temperature extremes and note the effect (11, 12, 15, 17, 19).

Low temperature or chilling tolerance has been demonstrated in some tomato lines (14, 17), and there is evidence that chilling tolerance indicates an ability to endure a wide range of temper-

atures rather than just the low end of the scale (1, 20). To test this idea, we compared the firmness and levels of sugars and organic acids in 2 chilling-tolerant tomato lines and 2 commercial cultivars at 5°, 20°, and 35°C. Since the production of ethylene has been shown to be reduced at high temperatures (16), it was introduced as a variable.

### Materials and Methods

Two chilling-tolerant breeding lines, 281 (cherry) and 79-546 (normal size), were compared with the chilling-sensitive cultivars 'Early Cherry' (cherry) and 'New Yorker' (normal size). Fruit was harvested mature-green and the 3 field replications for each line or cultivar were kept separate and used as treatment replicates. Each replicate contained 2-3 kg of fruit. The tomato fruit were held at 5° or 35°C in a flow-through air system with or without ethylene (about 50 ppm) for 15 days. Control fruit received the same treatments at 20°. Control fruit were analyzed after 15 days and samples were taken from all other treatments for analysis. The remainder of the fruit held at 5° and 35° was partitioned so that fruit that had received air during the first 15 days of storage were divided into 2 equal samples, one to be exposed to ethylene, the other not. Those that were exposed originally to ethylene were divided similarly. These fruit were moved to 20° to ripen for 10 days and then were analyzed.

Fruit firmness was determined by compression with a 500-g weight for 5 sec, using a penetrometer (GCA/Precision Scientific) modified for deformation testing (2, 18). Fruit were sliced, frozen, freeze-dried, and ground in a Wiley Mill after deformation testing.

Sugars were analyzed by extracting 0.5 g of each freeze-dried sample with hot 80% ethanol, evaporating the ethanol under

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<sup>1</sup>Visiting Fellow. Present address: Universidad Centro Occidental, P.O. Box 400, Barquisimeto-Lara, Venezuela, S.A.

<sup>2</sup>Associate Professor.

<sup>3</sup>Research Technician.