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Increased Embryo Viability of Early Ripening Peaches in Response to Daminozide, Maleic Hydrazide, and Thiourea

S.Z. El-Agamy[1] and W.B. Sherman
Fruit Crops Department, University of Florida, Gainesville, FL 32611

Additional index words. *Prunus persica*, embryo culture, seed germination, butanedioic acid mono-(2,2-dimethylhydrazide)

Abstract. **Trees and fruit of 6 early ripening cultivars of peach and nectarine [*Prunus persica* (L.) Batsch] were sprayed about 4 weeks prior to commercial harvest with either butanedioic acid mono-(2,2-dimethylhydrazide) (daminozide), maleic hydrazide, or thiourea. The date of 50% drop of overripe fruit was retarded one to 4 days. Chemical treatments increased germination of *in vitro* embryo culture of 'Fla. 3–1' and 'Flordaking' up to 27–32%. Seed germination from cracked pits of 'Fla. 7–3N', 'Fla. 7–4N', 'Earligrande', and 'Fla. M6–6N' generally increased up to 50%. The delayed fruit drop did not appear to account for all of the increase in germination. The results support an enhancement of embryo development in addition to that attributed to delayed fruit maturity.**

Early ripening resulting from a short fruit development period (FDP) is a major goal in most peach and nectarine improvement programs, but breeding is hampered by the inability to germinate seed from maternal parents with a short FDP. The earlier ripening cultivars generally have proportionally less embryo development and lower percentage viability (1). Embryos from fruit ripening less than 85 days from bloom generally need specialized culture for proper germination and growth, but even with embryo (1) or ovule (2) culture, the percentage of germination is proportional to FDP (7).

Peach embryo development generally occurs after stage I of fruit development (6). Methods to speed up embryo development or delay fruit development may be a means of increasing percentage of germination. For example, thiourea and maleic hydrazide were found to depress fruit development in banana and sporadically stimulate seed formation (3). Chemicals that would increase percentage of seed germination in peach would permit using shorter FDP maternal parents in breeding to obtain a larger population of seedlings with early ripening.

Fruit from single limbs in 2–3 trees of 'Flordaking', 'Fla. 3–1', and 'Earligrande' peaches and 'Fla. M6–6N', 'Fla. 7–3N', and 'Fla. 7–4N' nectarines were used to evaluate the effect of daminozide, maleic hydrazide, and thiourea on fruit ripening and seed formation in 1981 and 1982. Single and double applications of daminozide (2000 ppm), maleic hydrazide (1000 ppm), and thiourea (2000 ppm) were made prior to the final fruit swell (about 25–30 days before first commercial picking) in 1981 and 1982. Fruit and trees were sprayed using Tween 20 (0.5%) as a wetting agent.

When 50% of the fruit dropped, the rest were harvested. Pits from 20 to 30 fruits for each treatment were extracted and cracked and the seed were refrigerated at 5°C for 4 weeks in sterilized and humidified perlite medium. Seed were removed from cold treatment and placed at room temperature under 15 hr/day fluorescent light for 4 weeks. Germination (the emergence >2 cm of both the radical and plumule) was then determined.

The earliest-ripening cultivars—'Flordaking' and 'Fla. 3–1'—having a FDP <70 days did not germinate in 1981, whereas seed of other cultivars having a FDP ≥70 days did germinate. In 1982, embryos of 'Flordaking' and 'Fla. 3–1' were excised and cultured on modified Knops medium (4). They were left at room temperature for 20 days after planting to allow embryo maturation. The embryos were then moved to a cold dark room where the temperature was maintained at 4–7°C for 4 weeks before germination. 'Fla. 3–1' (50 days FDP) had small embryos (4.3 to 5.7 mm length) surrounded by large amounts of endosperm at 50% fruit drop. These embryos doubled in length within 2 weeks in culture before they were placed in cold treatment. Cotyledons of 'Flordaking' (65 day FDP) were full-size, softened, and slightly opaque and no endosperm was visible. Later-ripening cultivars (≥70 days FDP) had full-size, soft, and white embryos.

The 1981 and 1982 germination data for seeds not embryo-cultured resulting from daminozide, maleic hydrazide, and thiourea sprays on the 4 cultivars were similar and thus combined in Table 1.

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[1]Current address: Fruit Crops Department, Assiut University, Assiut, Egypt.

Table 1. Effect of different chemical treatments on time of 50% fruit drop and germination percentage of embryos of 'Fla. 3–1' and 'Flordaking' and seed of 'Fla. 7–3N', 'Fla. 7–4N', 'Earligrande', and 'Fla. M6–6N' (1981 and 1982).

		Control		Daminozide (2000 ppm)		Maleic hydrazide (1000 ppm)		Thiourea (2000 ppm)	
Cultivar	FDP (days)	50% fruit drop (days)	Germination (%)	50% fruit drop (days)	Germination (%)	50% fruit drop (days)	Germination (%)	50% fruit drop (days)	Germination (%)
Fla. 3–1[z]	50	68	8	70	35*	72*	20	71*	25*
Flordaking[z]	65	77	28	79	60*	81*	34	79	60*
Fla. 7–3N	70	86	26	90*	19	90*	35	93*	59*
Fla. 7–4N	70	85	18	88*	55*	90*	36*	88*	63*
Earligrande	75	83	0	89*	53*	88*	81*	88*	25*
Fla. M6–6N	80	96	66	---[y]	---	---[y]	---	98	100*
Mean	68	82	24	83	44*	84	41*	86*	55*

[z]Only 1982 embryo culture data
[y]No treatment applied
*Significant at 5% from control

The objectives of using chemical treatments in this study were to enhance seed germination either by 1) delaying fruit drop to allow more time for embryo maturation or 2) speeding up embryo development in relation to fruit maturity.

In controls, 50% fruit drop occurred from 8 to 18 days (average of 1981 and 1982) after normal commercial harvest stage (Table 1). This variability was probably due to cultivar differences in physiological maturity rates of firmness. The softer cultivar at ground color break would have been more mature physiologically. Daminozide, maleic hydrazide, and thiourea delayed fruit drop from one to 4 days (Table 1), but generally had the least effect on the earliest-maturing cultivars.

Daminozide, maleic hydrazide, and thiourea generally delayed fruit drop, but this delay did not appear to account for all of the increase in seed germination. From our experiences in seed germination, an increase of 1–4 days in FDP of any early-ripening cultivars would not be expected to increase germination in the range of 30%. Daminozide, maleic hydrazide, and thiourea increased average germination in all cultivars 28%, 25%, and 31%, respectively, over the unsprayed control. The increase was present in embryos cultured both *in vitro* and in seed planted in perlite.

The chemical treatments caused no statistical differences in length of immature embryos of 'Fla. 3–1' and 'Flordaking' (data not shown). However, different germination rates were obtained from each treatment even though embryos were equal in size. This is not in agreement with Toledo et al. (5) where germination rate was affected by embryo size. The other 4 cultivars had embryos that filled so that no endosperm was evident; thus, embryo lengths were not measured.

Daminozide (1000 ppm), maleic hydrazide (2000 ppm), and thiourea (1000 ppm) with single and double spray applications (data not shown) were equally effective in delaying fruit drop and in increasing embryo germination. A second application of these 3 chemicals was made in 1982 one week after the first application but had little additional effect.

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In Vitro Shoot-tip Grafting to Eliminate Citrus Viruses and Virus-like Pathogens Produces Uniform Budlines

E.M. Nauer,[1] C.N. Roistacher,[2] T.L. Carson,[1] and T. Murashige[1]
University of California, Riverside, CA 92521

Additional index words. tissue culture, *Citrus*, citrus germplasm, virus diseases

Abstract. **Individual shoot-tip-grafted budlines of 'Willowleaf' mandarin (*Citrus reticulata* Blanco) and 'Temple' tangor [*C. reticulata* x *C. sinensis* (L.) Osbeck] were grown to fruiting for evaluation. Fruits of both cultivars were highly uniform among shoot-tip cultures, indicating that this technique for producing disease-free citrus germplasm is reliable and does not increase the production of variant budlines.**

Prior to development of shoot-tip grafting (5, 6, 7) and thermotherapy (1, 8) techniques, growing nucellar seedlings was the only method available for producing disease-free citrus cultivars from clones infected with virus or other graft-transmissible pathogens.

The primary disadvantage of producing citrus budlines through nucellar embryony is the phenomenon of juvenility (2). Young nucellar seedlings exhibit excessive thorniness, vigorous and upright habit of growth, slowness to fruit, alternate bearing in early years, and physical differences in fruit characteristics which are often detrimental in marketing the fruit. These characteristics may persist for many years and over many budded gen-

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[1]Department of Botany and Plant Sciences.
[2]Department of Plant Pathology.