Pruned trees consistently produced larger and heavier nuts (Table 1 and Fig. 2). Average nut volumes from pruned and unpruned trees were 0.40 and 0.32 cm^3, and the average nut weights were 0.33 and 0.20 g respectively. Percent kernel was nearly the same and percent full nuts was much greater for nuts coming from pruned trees as compared to nuts from unpruned trees. Unauthorized harvesting precluded accurate determination of seed yield. However, it is doubtful that seed number was affected since primodia were initiated prior to pruning.

Basal pruning may prove to be a valuable technique for stimulating quality of piñon nut production due to an increase in nut size and decreased percentage of poor nuts. Fewer empty nuts and increased weight will reduce labor investment per unit weight of harvested seed.

Basal pruning will spare cone bearing branches in the upper crown and will remove lower branches likely to be net consumers rather than producers of energy. Pruning can be easily combined with other operations such as Christmas tree harvest and thinning or fuel wood cutting.

Table 1. Effect of pruning treatment on weight, percentage kernel and percentage filling of piñon nuts.

Treatment	Avg nut wt (g/nut)	Kernel[z] (%)	Full nuts[z] (%)
Unpruned	0.20	55	84
Pruned	0.33**	54	99**

[z]Based on 50 nut sample.

**Significantly different from unpruned at 1% level.

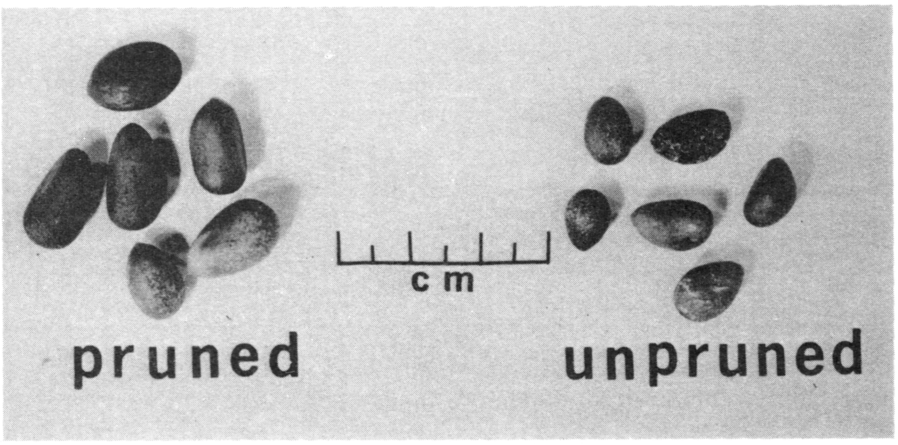

Fig. 2. Nut sizes from purned and unpruned piñon trees.

Literature Cited

1. Barger, R. L. and P. E. Folliott. 1972. Physical characteristics and utilization of major woodland tree species in Arizona. USDA For. Ser. Res. Paper RM-83, p. 38-44. Rocky Mtn. For. & Rge. Expt. Sta., Ft. Collins, Colo.
2. Botkin, C. W. and L. B. Shires. 1948. The composition and value of piñon nuts. *N. Mex. Agr. Expt. Sta. Bul.* 344.
3. Fisher, J. T. and J. M. Montano. 1977. Management of Piñon for Ornamentals, Christmas Trees and Nut Production. p. 35-40. *In* Ecology, Uses, and Management of Pinyon-Juniper Woodlands. USDA For. Ser. Gen. Tech. Rpt. RM-39. Rocky Mtn. For. & Rge. Expt. Sta., Ft. Collins, Colo.
4. Lanner, R. M. 1977. The eradication of pinyon-juniper woodland. *Western Wildlands* 3:12-17.
5. Little, E. L., Jr. 1940. Suggestions for selection cutting of piñon trees. USDA For. Serv., Southwest For. & Range Expt. Sta., Res. Note 90., Tucson, Ariz.
6. _____. 1938. Food analysis of piñon nuts (A compilation of existing data). USDA For. Serv., Southwest For. & Range Expt. Sta., Res. Note 48., Tucson, Ariz.
7. Schmidtling, R. C. 1975. Fruitfulness in conifers. Nitrogen, carbohydrate, and genetic control. USDA Forest Serv. Proc. Third N. Amer. For. Biol. Workshop. p. 148-164, Colo. State Univ. Fort Collins.
8. Wilson, L. F. and V. J. Rudolph. 1970. Early basal pruning to control pests. *J. For.* 68:632-634.
9. Varnell, R. J. 1969. Female-strobilus production in a slash pine seed orchard following branch pruning. Proc. Tenth South. Conf. For. Tree Improv. p. 222-228.

HortScience 15(6):728–729. 1980.

Identification of Nucellar and Zygotic Seedlings of *Citrus* with Leaf Isozymes[1]

Robert K. Soost and Timothy E. Williams
Department of Botany and Plant Sciences, University of California, Riverside, CA 92521
Andrew M. Torres
Department of Botany, University of Kansas, Lawrence, KS 66045

Additional index words. apomictic, hybrid

Abstract. **The distinction between apomictic nucellar and zygotic 5-month-old seedlings of *Citrus* was made using two genetically defined isozyme markers: phosphoglucose isomerase and phosphoglucose mutase. Of 123 seedlings of attempted crosses of 'King' (seed parent) x 'Parsons Special', 18 had the 'King' genotype and were therefore nucellars. Segregation ratios for both markers were as expected among the zygotics.**

Several economically important species of *Citrus* either produce asexual nucellar embryos exclusively or a polyembryonic mixture of nucellar and recombinant sexual embryos (3). One of the major problems in citrus breeding is to distinguish between these while the seedlings are young (ca. 2 months). Nucellar gene combinations are already available in the seed parent and resources spent growing plants with them could be saved with early identification. Other workers (1, 2, 4, 5) have examined various biochemical characteristics in attempts to determine the genetic origin of seedling populations prior to fruiting (ca. 5 years).

Torres et al. (6) proposed that some isozymes would provide excellent single gene markers because they are colinear with the gene, codominant, little affected by the environment and are identical in leaves of young and mature plants of the same cultivated variety. The rationale for using isozymes for addressing the problem and the electrophoretic techniques were noted. The genetic control of 3 enzyme systems coded by 4 genes with 12 codominant alleles was analyzed in citrus and near relatives. Among the variable isozyme systems were phosphoglucose isomerase (PGI) and phosphoglucose mutase (PGM). The former is specified by the gene *Pgi-1* and the latter by *Pgm*. For each enzyme, *F* is used to specify a fast migrating subunit, *S* a slow migrating subunit and *I* an intermediate one. The *W* subunit for *Pgi-1* was named for the cultivar 'Willial' in which it was first

[1]Received for publication June 27, 1980. This research was supported in part by the USDA under agreement number 5901-0410-9-0310-0.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

located (6). It migrates faster than the *F* band.

Here we wish to show how these markers were used to analyze a 5-month-old seedling population of the polyembryonic seed parent 'King' tangor (probably *C. sinensis* (L.) Osbeck × *C. reticulata* Blanco) pollinated by 'Parsons Special' mandarin (*C. reticulata*). The genotype of 'King' is *F/F* for *Pgi-1* and *F/S* for *Pgm* (Table 1, Fig. 1), and of 'Parsons Special' is *W/S* for *Pgi-1* and *F/I* for *Pgm*. Note that *Pgi-1* by itself could provide positive nucellar vs. zygotic identification because the presence of the isozymes specified by *W* or *S* could alone document the zygotic contribution of 'Parsons Special'. However, the 2 markers together afford an interesting double check because a seedling with the nucellar ('King') *F/F* genotype for *Pgi-1* must always also have the *F/S* genotype for *Pgm*. Two further conditions can be predicted and tested: one is that among the zygotic seedlings the segregation ratio of *W/F:F/S* for *Pgi-1* should be 1:1; the second is that the *Pgm* ratio for the *F/F:F/I:F/S:S/I* genotypes, after the nucellars are subtracted from the *F/S* class, should be 1:1:1:1. In brief, zygotic seedlings of 'King' must be either *W/F* or *F/S* for *Pgi-1* and 1 of 4 possible *Pgm* genotypes. While *Pgm* alone could not be used to distinguish all nucellars from zygotics, any seedlings of the three *Pgm* genotypes, F/F, F/I or S/I, would be zygotics since they too demonstrate the gametic contribution of 'Parsons Special'.

The results of the analysis of a sample of 123 seedlings are given in Table 2. The 18 seedlings that were *F/F* for *Pgi-1* were also, as predicted, *F/S* for *Pgm*. Thus 14.6% of the sample was undoubtedly nucellar. Of the remaining 105 seedlings we would expect half to be *W/F* for *Pgi-1* and the other half to be *F/S*. The observed 61:44 ratio did not differ significantly from the expected (χ^2 = 2.15, p = 0.10). The observed segregation ratio of the *Pgm* zygotic seedling genotypes, less the 18 *F/S* nucellars (Table 2), agrees very well with the expected (χ^2 = 0.69, p = 0.70).

Table 1. *Pgi-1* and *Pgm* genotypes of 'King', 'Parsons Special', nucellar and zygotic seedlings.

Parent or progeny	Genotype[z]	
	Pgi	*Pgm*
King	*F/F*	*F/S*
Parsons Special	*W/S*	*F/I*
Nucellar sdlgs.	*F/F*	*F/S*
Zygotic sdlgs.	*W/F, F/S*	*F/F, F/I* *F/S, S/I*

[z]Migration speed of subunit W>F>I>S

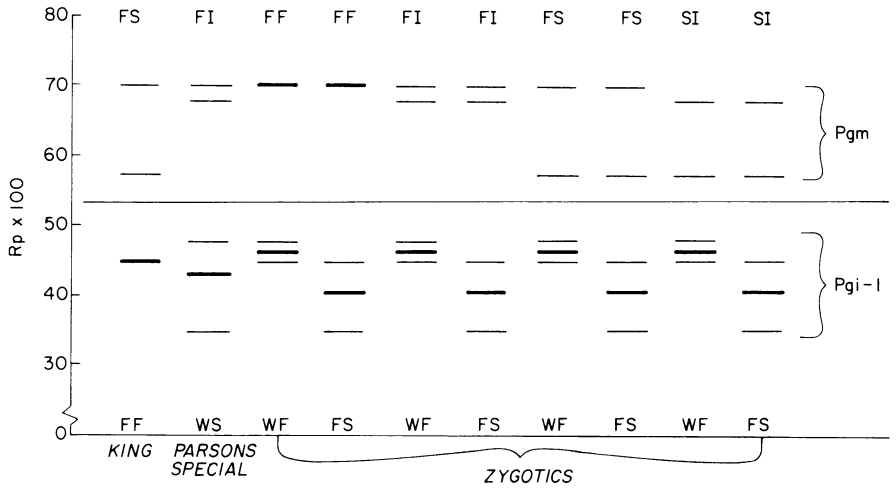

Fig. 1. Schematic illustration of 'King', 'Parsons Special' and the 8 zygotic phenotypes for *Pgi-1* and *Pgm*. Origin at 0, anode toward the top, *Pgi-1* genotypes below, those of *Pgm*, above.

Table 2. Genotypes of 'King' × 'Parsons Special' seedlings.

No. of seedlings	No. of seedlings						
	Pgi-1			*Pgm*			
	F/F	*W/F*	*F/S*	*F/F*	*F/I*	*S/I*	*F/S*
Observed	18[z]	61	44	26	26	24	29+18[z]
Expected		52.5	52.5	26.25	26.25	26.25	26.25

[z]Nucellar seedlings of the 'King' genotype.

Older, fruiting populations of 'King' as the seed parent were identified by Frost and Soost (3) principally on morphological bases, as having 21% nucellars out of 391 plants. Our analysis shows 14.6%. Given the variable production of nucellars (3) and the likelihood that weak seedlings were not field planted in the Frost and Soost populations, the percentages are considered to be in close agreement.

This demonstration of the use of isozymes to help solve a long-standing citrus breeding problem emphasizes the importance of developing yet more markers so that young seedlings of each possible cross could be analyzed. If, for example, only *Pgm* markers had been available in the above case, only 62% rather than 100% of the zygotics could have been identified and which of the 47 plants with the *Pgm F/S* genotype were nucellars would not be determinable.

Literature Cited

1. Button, J., A. Vardi, and P. Spiegel-Roy. 1976. Root peroxidase isozymes as an aid in *Citrus* breeding and taxonomy. *Theor. appl. Genet.* 47:119-123.
2. Esen, A., R.W. Scora, and R.K. Soost. 1975. A simple and rapid screening procedure for identification of zygotic *Citrus* seedlings among crosses of certain taxa. *J. Amer. Soc. Hort. Sci.* 100:558-561.
3. Frost, H.B. and R.K. Soost. 1968. Seed reproduction: development of gametes and embryos. p. 290 to 324. *In* W. Reuther, L.D. Batchelor, and H.J. Webber (eds.) The citrus industry, Vol. II. Div. of Agricultural Sciences, Univ. of California, Berkeley.
4. Iglesias, L., H. Lima, and J.P. Simon. 1974. Isozyme identification of zygotic and nucellar seedlings in *Citrus*. *J. Hered.* 65: 81-84.
5. Tatum, J.H., R.E. Berry, and C.J. Hearn. 1974. Characterization of *Citrus* cultivars and separation of nucellar and zygotic seedlings by thin layer chromatography. *Proc. Fla. State Hort. Soc.* 87:75-81.
6. Torres, A.M., R.K. Soost, and U. Diedenhofen. 1978. Leaf isozymes as genetic markers in citrus. *Amer. J. Bot.* 65:869-881.