Journal of the American Pomological Society 59(2):97-103 2005

Maternal and Paternal Effects on the Incidence of Double Kernelled Fruits in 'Guara' Almond[1]

M. González[2], S.F. Oller del Águila[3] and J. Cuevas[3,4]

Abstract

'Guara' is a self-compatible almond of recent introduction that in a few years has become the most planted cultivar in Spain. However, 'Guara' produces a high percentage of fruits with two seeds. The occurrence of double kernels in almond fruits seriously depreciates the price of nuts as the resulting seed are destined to less profitable uses. In the present work, the effects of pollination treatment, shoot vigor, and the blooming time on the production of double kernelled fruits in 'Guara' were determined. Our results indicated that the paternal genotype did not have a significant effect on pollen tube growth, fertilization rate, fruit set, and double kernel appearance on 'Guara'. No significant effect of shoot vigor was observed, despite differences in flower density and size. Early flowers were more prone to the production of double kernels than late flowers. Early flowers were also significantly heavier. Results suggest a positive effect of the size of the flower on the development of both primary and secondary ovules leading to a higher production of double kernels.

Almond (*Prunus dulcis* (Mill.) DA Webb.) is a typical Mediterranean crop widely planted in Spain, where its importance is not only economic, but also social, since almond is mostly located in depressed areas with scarce agricultural resources and strong migratory pressures (1). Spain is the leading almond producer in Europe and the second in the world, after USA, with 16 % of the world's total (7).

The wide diversity of Spanish almond germplasm represents a great genetic richness. However, such variability negatively affects commercialization, because products are not uniform (9). Almond breeding programs in Spain have focused on self-compatibility and late bloom. As a result of these programs, some selections such as 'Guara', 'Aylés', 'Moncayo', 'Masbovera', 'Glorieta', 'Francolí', 'Antoñeta' and 'Marta' have been obtained and offered to farmers. More than 80 % of the new plantations and topworkings have used these new cultivars, with 'Guara' being the most planted (9). 'Guara' is a self-compatible late blooming cultivar which is able to produce high yields in the absence of insect pollinators (17). Its kernels are oval, medium-sized, sweet and with very good commercial quality. Despite its advantages, 'Guara' is not free from defects, including the presence of 10-20 % of double kernelled fruits (9). Double seeds have irregular shape that are not suitable for uses where the whole kernel is visible. Therefore, cultivars prone to produce a high percentage of double kernels face reluctance of farmers to plant them.

Most members of Rosaceae subfamily Prunoideae, including almond, produce drupes that develop from the single pistil of a perigynous flower. Drupes are single-seeded. However, the almond pistils contain two ovules; the so-called primary ovule is more advanced in its development, whereas the secondary ovule is often delayed (3, 13). The secondary ovule is normally not fertilized and aborts. The degeneration of the secondary ovule normally begins after pollination, but before the fertilization of the primary ovule takes place (13). If the degeneration of the secondary ovule does not occur, both ovules can be fertilized and two kernels are formed inside the shell (10). In apricot, the degeneration of the secondary ovule also happens before fertilization of the primary ovule and is independent of pollination (15). Rodrigo and Herrero (15) interpret secondary ovule abortion as a programmed developmental process. This interpretation

[1] We wish to thanks A. Oliva and AgroMañán for their assistance and to E. Baeza who made the translation.

[2] Estación Experimental de Cajamar "Las Palmerillas". Autovía del Mediterráneo, Km 416.7, 04710, El Ejido, Almería, España.
[3] Dpto. Producción Vegetal. Universidad de Almería. La Cañada de San Urbano s/n, 04120, Almería, España.
[4] Corresponding author e-mail: jcuevas@ual.es

considers the formation of two seeds within a single endocarp as an accident from a maternal plant point of view, since two seeds dispersed together will presumably negatively affect seedling growth and survival.

The determinants of double kernels including its inheritance are not clear (16). It is well known that certain cultivars produce a high percentage of double almonds (5, 12). According to Egea and Burgos (6), the cultivars with small differences in ovule development show higher propensity to produce double kernels. Apart from the long-known maternal effect, pollen genotype may affect the frequency of fertilization of both ovules (3). Asensio and Socías i Company (2) cited the need for more analyses on the influence that the pollen that accomplishes the fertilization, and the pollen-pistil interaction might have on double kernel incidence. On the other hand, a strong environmental influence on the production of double kernel fruits is inferred from the marked annual differences within cultivars or seedlings (5, 12, 16). Asensio and Socías i Company (2) point out as determinant the environmental conditions during the last stage of ovule development and during pollination. Egea and Burgos (5) concluded that warm pre-bloom temperatures decrease the incidence of double kernelled fruits, and that the small increase in temperature achieved by bagging experimental shoots decreases the percentage of doubles in 'Malagueña'.

The aim of the present work is to determine the effect that the pollination treatment, fruiting wood with different vigor (spurs and shoots with different length and flower density), and blooming date may have on the percentage of double seeds in 'Guara'.

Materials & Methods

The effect of the different factors implicated in the appearance of double kernelled fruits was studied in three different experiments carried out during 2002, in a private, solid, dry-farming block of 'Guara' located in Tahal (Almería, SE of Spain). For each experiment, four-year-old trees grafted on GF677 almond X peach hybrid were used. The experimental design was randomized complete blocks for all trails, considering every tree as a block, and selecting a total of five trees for each experiment.

To determine the paternal effect, three pollination treatments, self-, cross- and open-pollination, were applied on every flower from 10 one-year-old shoots per tree and pollination treatment. Self-pollination was achieved through isolation of the shoots with micro-perforated bags that excluded almond pollinators. To ensure the deposition of its own pollen over the stigma, fresh 'Guara' pollen was collected and applied on intact flowers with a paintbrush every day during bloom. Cross-pollination was performed with 'Ferragnes' pollen on 'Guara' flowers previously emasculated at balloon stage to exclude self-pollen (8). 'Ferragnes' is a cultivar that produces a very low percentage of doubles (6). Open-pollination was achieved through the exposure of the branches to the action of pollinating insects. Two strong healthy beehives were in the vicinity of the experimental trees. Simultaneously blooming 'Ferragnes' trees were cultivated next to the 'Guara' block providing opportunity for cross-pollination. Bloom of 'Guara' extended from February 6 to February 28, 2002, while 'Ferragnes' trees bloomed approximately from February 4 to February 26, 2002. Fruits from tagged shoots were harvested on August 8, 2002. Immediately, the shell was manually broken and the fruits classified according to the number of seeds contained. The proportion of double kernelled fruits in the different treatments was compared by means of a Chi-square test.

For the determination of the effect of the vigor of the fruiting wood on the percentage of double kernelled fruits, 10 fruiting structures per tree, either spurs or short or long one-year-old shoots, were tagged. Their characteristics are listed in Table 1. Flowers were open-pollinated. Fruits were harvested August 8, 2002 and processed as above.

In the last experiment, the percentage of double kernelled fruits in early and late flowers was compared. For each blooming type, 10 long one-year-old shoots per tree were marked. To differentiate fruits developed from early flowers, all the closed buds were eliminated on February 14, 2002, leaving only opened flowers. In this way all the fruits were developed from flowers that opened February 14, 2002 or before. Late flowers were selected in another set of shoots, by eliminating all

Table 1. Length and flower density of the different fruiting organs. Mean ± SE.

Fruiting organ	Length (cm)	Flower density (flowers/cm)
Long 1-year-old shoots	43.2 ± 2.3	0.36 ± 0.04
Short 1-year-old shoots	13.3 ± 0.5	0.34 ± 0.03
Spurs	3.1 ± 0.2	1.97 ± 0.06

opened flowers on February 21 and leaving only closed buds. With this procedure, all the fruits had their origin in flowers that opened after February 21. Flowers were open-pollinated. After harvest, fruits with one or two kernels were separated, and processed as before.

The pollen-pistil interaction and the level of fertilization under self- and cross-pollination was assessed by fluorescent microscopy according to Pimienta and Polito (14). To determine pollen tube growth dynamic and the level of fertilization, 40 flowers per treatment were harvested at 3 and 5 days after pollination. Open-pollinated flowers were not analysed because of lack of controls on the moment and circumstances in which pollen transfer occurred. To learn more about the reasons for the possible differences among flowers on different types of fruiting wood or with different blooming dates, we estimated the size of the flower as the dry weight of the newly opened flower with its petals. Constant dry weights of 25 sampled flowers per tree and treatment were obtained after 72 hours in a drying oven set at 60 °C.

Results

Effect of the pollination treatment. Unrestricted open-pollination increased significantly seed set with respect to hand cross- and self-pollination ($P < 0.001$). Under open-pollination treatment 58 % of flowers set fruit versus 34 and 28 % measured in self- and cross-pollinated flowers, respectively. Despite such effects on seed set, no significant differences were observed between the different pollination treatments in the occurrence of doubles ($\chi^2 = 0.94$, $P = 0.23$). That is to say that the type of pollination did not affect the seed pattern in 'Guara' (Table 2). Nevertheless, in the case of self-pollination a tendency to produce a higher percentage of double kernelled fruits was observed. For open- and cross-pollination a majority of single kernelled fruits were produced showing no deviations in the seeding pattern.

The pollen-pistil interaction also did not show any major difference, between self- and cross-pollination in the number of pollen tubes found in the middle part, or in the base of the style (Table 3). The number of pollen tubes was high (between 14 and 27) in the middle

Table 2. Effects of pollination treatment on seeding pattern of 'Guara' almond. 2x3 Contingency table.

Seed number	Cases	Open-pollination	Cross-pollination	Self-pollination
	Observed	84.00	46.00	48.00
1	Expected	82.44	44.97	50.59
	χ^2	0.03	0.02	0.13
	Observed	4.00	2.00	6.00
2	Expected	5.56	3.03	3.41
	χ^2	0.44	0.35	1.97
	(%)	4.55	4.17	11.11
	Total	88	48	54

χ^2 total = 2.94, No deviation from expected distribution ($P = 0.23$, df = 2)

Table 3. Effects of cross- versus self-pollination on pollen tube number and fertilization. Mean ± SE.

	Pollen tube number in the style after 3 days		Pollen tube number in the style after 5 days		Fertilization (%)	Fertilization (%)
Treatment	Middle	Base	Middle	Base	1 ovule	2 ovules
Self-pollination	26.5±8.4	3.7±1.8	26.5±10.5	4.0±1.7	83	17
Cross-pollination	20.4±6.1	3.7±1.3	13.9±8.1	2.9±2.2	83	17

part of the style in both treatments and small at the base of the style, where only between 3-4 pollen tubes were detected on average in both treatments. The pollination treatment did not affect the percentage of flowers with one or two ovules penetrated by a pollen tube. The percentage of flowers in which fertilization of both ovules was observed was around 7 %, which in turn represented 17 % of the flowers in which fertilization took place (Table 3). This level is higher than the average value (7 %) of double kernelled fruits found in this experiment (Table 3). A certain level of seed abortion, mostly affecting to the second smaller seed, was detected when cracking endocarps in this and in the next experiments; however, seed failure to complete development was not rigorously quantified.

Effect of the fruiting wood. The different kinds of fruiting shoots (either spurs or short and long one-year-old shoots) did not show significant effects on the seed pattern of 'Guara' (χ^2 = 0.86, P = 0.65). Thus, the number of seeds in a single fruit was not affected by the type of wood on which the fruit developed (Table 4). This result was obtained despite marked differences in flower density (Table 1) and in spite of the highly significant, although modest, differences in flower dry weight (P < 0.001). Short one-year-old shoots produced the heaviest flowers (49.9 ± 0.9 mg; Mean±SE). The lightest flowers were produced in the spurs (45.6 ± 0.8 mg). Long one-year-old shoots developed flowers with an average weight of 47.7 ± 0.4 mg. The average percentage of double kernelled fruits found in this experiment was 11 %. Final seed set was not significantly different among the different kind of fruiting organs (P = 0.19). Long and short one-year-old shoots set a 47 and a 45 % of pollinated flowers, whereas only a 33 % of flowers became fruit in the spurs.

Effect of the blooming date. Timing of anthesis had a significant effect on the seed

Table 4. Effects of fruiting organs vigor on seeding pattern of 'Guara' almond. 2x3 Contingency table.

Seed Number	Cases	Spurs	Short shoots	Long shoots	Total
1	Observed	59.00	86.00	295.00	440
	Expected	61.22	86.05	292.74	
	χ^2	0.44	0.35	0.00	
2	Observed	10.00	11.00	35.00	56
	Expected	7.79	10.95	37.59	
	χ^2	0.63	0.00	0.14	
	(%)	14.49	11.34	10.61	11.29
	Total	69	97	330	496

χ^2 total = 0.86, No deviation from expected distribution (P = 0.65, df = 2)

Table 5. Flowering date effects on seeding pattern. 2x2 Contingency table.

Seed number	Cases	Early flowers	Late flowers	Total
1	Observed	83.00	150.00	233
	Expected	89.41	143.59	
	χ^2	0.46	0.29	
2	Observed	16.00	9.00	25
	Expected	9.59	15.41	
	χ^2	4.28	2.66	
	(%)	16.16	5.66	9.68
	Total	99	159	258

χ^2 total = 7.69; Significant deviation from expected distribution, (P adjusted (Yates) = 0.01; df = 1)

pattern of the almond 'Guara' cultivar (χ^2 = 7.69, $P < 0.01$). Early flowers produced more double kernelled fruits (16 %) than expected (Table 5). Late flowers formed an appreciably lower number of double kernelled fruits (6 %) than expected. Early flowers were significantly heavier ($P < 0.001$), with an average dry weight for each flower of 46.5 ± 0.7 mg, against a value of 33.2 ± 1.6 mg obtained for the late flowers. On the contrary, no differences in seed set were found between early and late flowers ($P < 0.99$). A 32 % of early flowers set fruit versus a 31 % found in late flowers.

Discussion

Almond is predominantly allogamous, although 'Guara' is reportedly self-compatible and autogamous (17). Yet, in many cultivated species where self-compatibility has been introduced, pollen tube growth under cross-pollination is frequently faster and more abundant than under self-pollination leading to higher levels of cross-fertilization. It is, therefore, reasonable to inquire about the effects of cross-pollination on the incidence of double kernelled fruits in 'Guara'. However, contrary to our expectations, the results clearly indicate that neither cross- nor open-pollination increased the levels of doubles in 'Guara' (Table 2). These results coincide with those obtained by Kumar and Das (11) who observed that the source of the pollen did not have any effect on the percentage of double seeds in 15 different almond cultivars. The absence of difference in the dynamic of the pollen tube growth, concerning speed and abundance, between self- and cross-pollination, previously documented by Socías i Company and Felipe (17), is confirmed in the present work (Table 3). The lack of differences in the growth of the pollen tube and the coincident levels of fertilization and fruit set also confirm the well-known self-compatibility of 'Guara' and suggest that the double kernel character is primarily maternal, with little, if any, effect of the pollen source. It could be argued, however, that it is not the pollen genotype but the intensity of the pollination and the more abundant pollen tube growth what determines the levels of double kernels. According to Grasselly and Gall cited by Egea and Burgos (5), the environmental conditions that favor pollination increase the appearance of double kernels in almond. In this sense, Palasciano et al. (12) observed in 'Ferrante', 'Filippo Ceo', 'Scorza Verde' and 'Tuono' a higher percentage of double kernelled fruits under artificial pollination (more than 100 pollen grains per stigma) compared to natural pollination (about 20 pollen grains per stigma). Similarly, Cousin and El Maataoui (3) justified the tendency of 'Lauranne' to produce double kernelled fruits by the high number of pollen tubes in the base of its style (an average of eight pollen tubes) in opposition to the reduced number (three pollen tubes) observed by Pimienta et al. (14)

in 'Nonpareil', a cultivar that hardly produces double kernelled fruits. Our results do not agree with such an argument. In our work, the growth of the pollen tube was similar under both pollination treatments and equivalent to that observed in 'Nonpareil' by Pimienta et al. (14). However, in our experiments the fertilization of both ovules was observed in 17 % of the fertilized flowers (Table 3).

Despite 17 % of flowers with both ovules being fertilized, only an average of 7 % of double-seeded fruits were found at harvest. The decrease in the proportion of double kernels along fruit development was, at least partly, due to seed abortion, that was found to affect more often to the smaller developing seed. Unfortunately, no comparison of seed abortion incidence was made between single- and double-seeded fruits. However, in olive (*Olea europaea* L.), also commonly a single-seeded drupe, we have found that the presence of a second developing seed within the same endocarp increases seed abortion (González and Cuevas, in prep.).

The double kernel although primarily a cultivar characteristic has an important environmental component in almond. In this sense, the literature points out that the frequency of double seeds is largely variable for a given genotype between years and locations (5, 12, 16). In our experiments, the percent of doubles has reached levels somehow below average values for 'Guara' (9). The differential contribution that early or late flowers make to the incidence of double kernelled fruits (Table 5) suggest that a variation in fruit set between these two kind of flowers, for instance due to frost damage or an insufficient pollination during a certain stage of the bloom, will have a variable impact on annual variations depending on flower type affected.

Socías i Company and Felipe (18) indicate that a good nutritional status may also favor secondary ovule development and increase the levels of double kernelled fruits. In this sense, our hypothesis has been that factors improving flower quality can increase the frequency of double-seeded fruits. In our experiments, flower quality was estimated by its dry weight. Although we found significant differences among different fruiting organs, they were small (5 % in weight) and did not have a significant influence on the frequency of double kernelled fruits among different shoots (Table 4).

The results were different when flower dry weight differences were more pronounced. Our results show that early flowers produce significantly more double kernelled fruits than late, significantly lighter, flowers (Table 5). Differences in flower weight were important in this experiment, with increases of 40 % in flower dry weight observed in early flowers with an 3-fold increase in the appearance of double kernelled fruits (19.2 % versus 5.7 %). Socías i Company and Felipe (18) also observed in 'Guara' that the first flowers produced more fruits with two seeds: 18.5 % of double seeds in early flowers versus 6.8 % and 3.6 % obtained in those flowers with middle and late bloom, respectively. Levels of double kernelled fruits were also similar to the results reported in this paper. To explain this trend, Socías i Company and Felipe (18) suggest that better nurtured early flowers extend the viability of the secondary ovule allowing so the fertilization of both ovules. Coinciding with such an argument, we have compared flower size and double kernel appearance in different experiments and we have found that large early flowers with a dry weight over 45 mg generate an average of 13 % of doubles, whereas late flowers, with a noticeably lower size (33 mg), only produced 6 % of doubles. Such coincidence suggests the existence of a flower size threshold value below which the development of the secondary ovule and its fertilization is less likely. Poorer pistil development is more frequent in late and shaded flowers and in flowers formed on weak shoots (Felipe, 2000). As noted by Socías i Company and Felipe (18), it might look paradoxical, but our results and theirs indicate that more fecund flowers increase the proportion of less profitable double kernelled fruits, suggesting that flower quality and fruit quality may be, indeed, conflicting objectives in almond.

Literature Cited

1. Arquero, O., A. Navarro, C. Navarro, J.L. Fernández, J.C. Gallego and A. Oliva. 2002. El cultivo del almendro en Andalucía. Fruticultura Profesional 126:5-14.
2. Asensio, M.C. and R. Socías i Company. 1996. Double kernels in almond: an open question. Nucis-

Newsletter 5:8-9.
3. Cousin, M. and M. El Maataoui. 1998. Female reproductive organs in self-compatible almond (*Prunus dulcis* (Miller) D.A. Webb) Lauranne and fertilization patterns. Sci. Hort. 72:287-297.
4. Dicenta, F., E. Ortega, J.A. Canovas and J. Egea. 2000. Hacia la plantación monovarietal de almendro con variedades autocompatibles. Fruticultura Profesional 111:11-18.
5. Egea, J. and L. Burgos. 1995. Double kernel fruits in almond (*Prunus dulcis* (Miller) D.A. Webb) as related to pre-blossom temperatures. Ann. Appl. Biol. 126:163-168.
6. Egea, J. and L. Burgos. 2000. Ovule differences between singled-kernelled and double-kernelled fruits in almond (*Prunus dulcis*). Ann. Appl. Biol. 136:291-295.
7. FAOSTAT. 2002. http://www. FAO.org
8. Felipe, A.J. 1977. Almendro. Estados fenológicos. Información Técnica Económica Agraria 27:8-9
9. Felipe, A.J. 2000. El almendro. I Material vegetal. Ed. Integrum. 461 pp.
10. Grasselly, C. and P. Crossa-Raynaud. 1984. El Almendro. Ed. Mundiprensa. Madrid. 495 pp.
11. Kumar, K. And B. Das. 1996. Studies on xenia in almond (*Prunus dulcis* (Miller) D.A. Webb). J. Hort. Sci. 71:545-549.
12. Palasciano, M., A. Godini and L. De Palma. 1994. Optimized self-pollination and production of double kernel in almond. Acta Hort. 373:215-217.
13. Pimienta, E. and V.S. Polito. 1982. Ovule abortion in 'Nonpareil' almond (*Prunus dulcis* (Miller) D.A. Webb). Amer. J. Bot. 69:913-920.
14. Pimienta, E., V.S. Polito and D.E. Kester. 1983. Pollen tube growth in cross- and self-pollinated 'Nonpareil' almond. J. Amer. Soc. Hort. Sci. 108:643-647.
15. Rodrigo, J. and M. Herrero. 1998. Influence of intraovular reserves on ovule fate in apricot (*Prunus armeniaca* L.). Sex. Plant Reprod. 11:86-93.
16. Spiegel-Roy, P. and J. Kochba. 1974. The inheritance of bitter and double kernel characters in the almond (*Prunus amygdalus* Batsch). Z. Pflanzen. 71:319-329.
17. Socías i Company, R. and A.J. Felipe. 1992. Self-compatibility and autogamy in 'Guara' almond. J. Hort. Sci. 67:313-317.
18. Socías i Company, R. and A.J. Felipe. 1994. Flower quality and fruit quality in almond: conflicting objectives?. In: Progress in Temperate Fruit Breeding, pp. 245-248. Eds. J. Schmith and M. Kellerhalls. The Netherlands: Kluwer Academic Publishers.

CALL FOR PAPERS
U.P. HEDRICK AWARD

A cash award of $300 with mounted certificate will be awarded to the winning student paper. Papers should be submitted to Dr. Robert Crassweller, Horticulture Department, Penn State University, University Park, PA 16803 by 30 May, 2005. See the journal for editorial style; paper length about 1000 words or 3-4 pages total. Papers can relate to any research aspect with fruit cultivars or rootstocks as influenced by environmental or cultural techniques. Breeding or the history or performance of new or old cultivars can be reviewed. Research and review papers will be judged separately.