PHENOLOGY, BREEDING SYSTEM AND FRUIT DEVELOPMENT OF ARGAN [*ARGANIA SPINOSA*, SAPOTACEAE] CULTIVATED IN ISRAEL[1]

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PHENOLOGY, BREEDING SYSTEM AND FRUIT DEVELOPMENT OF ARGAN (*ARGANIA SPINOSA* SAPOTACEAE) CULTIVATED IN ISRAEL. *Economic Botany 52(2):161–167. 1998.* *Argan (*Argania spinosa*) is an evergreen tree native to southwestern Morocco appreciated for its edible, high nutritional oil, extracted from the kernels of the drupe-like fruit. Aspects of its reproductive biology were studied with the aim to domesticate the tree as an oil crop. Flowering of fertigated trees cultivated in the Negev Highlands of Israel was confined to the spring months. The flowers were protogynous. Results of different pollination treatments showed that a pollen vector was necessary for pollination and that fruit set was significantly higher in cross and open pollination (7–9%) than in self pollination (0.5%). The lower fruit set obtained in self pollination was related to postzygotic discrimination. Pollen transfer by wind was restricted to short distances. Flies, mainly of the family Calliphoridae, visited the flowers and were found to be covered with argan pollen. Fruits ripened nine months after anthesis, exhibiting bisigmoidal growth curve.*

*Phénologie, système de reproduction sexuelle et développement du fruit de l'arganier (*Argania spinosa, *Sapotaceae) cultivé en Israël L'arganier (*Argania spinosa*) est un arbre endémique du Sud-Ouest marocain apprécié pour l'huile comestible et à haute valeur nutritive extraite des graines de son fruit à caractère de faux drupe. Nous avons étudié certains aspects de la biologie reproductive de l'arganier dans le but de domestiquer cet arbre pour son huile. La floraison d'arbres irrigués poussant en Israël dans les hauteurs du Negev est restreinte au printemps. Les fleurs sont protogynes, le style emergeant de la fleur avant l'anthèse. Les résultats de différents traitements de pollinisation démontrent la nécessité d'un vecteur pour la pollinisation, et aussi que le nombre de fruits posés est significativement plus élevé dans les cas de croisements et de pollinisation ouverte (7–9%) que dans les cas d'auto-pollinisation (0.5%). La réduction observée dans le nombre de fruits posés par l'auto-pollinisation est attribuable à une discrimination post-zygotique. Le transport du pollen par le vent est restreint à de courtes distances, et l'intervention de mouches (Calliphoridae) dans la pollinisation a été démontrée. Les fruits exhibent une courbe de croissance bisigmoïde et atteignent l'état mûr neuf mois après l'anthèse.*

Key Words: phenology; flower; fruit; pollen; pollination.

Argan (*Argan spinosa* (L.) Skeels) is a medium-sized thorny evergreen tree of the Sapotaceae family native to a wide semiarid area in south-west of Morocco. It supplies a variety of necessities to the local inhabitants of its distribution area: the kernels of the drupe-like fruit yield a fine edible oil, the wood is used for firewood, and leaves and fruits are foraged by goats and camels (Morton and Voss 1987; Nouaim et al. 1991; Prendergast and Walker 1992). The oil is highly appreciated in Morocco, where it commands a price twice as high as olive oil. The unsaturated fatty acid fraction of argan oil is comparable with that of olive oil, but has a higher linoleic acid and a lower oleic acid content (Farines et al. 1984; Huyghebaert and Hendricks 1974).

There have been no attempts to cultivate argan for its oil (Morton and Voss 1987; Prendergast and Walker 1992). In a feasibility study

[1] Received 20 February 1997; accepted 21 November 1997.

conducted in the Negev desert of Israel, argan trees propagated from seeds exhibited a high growth rate and precocious yielding (Nerd et al. 1994). The plants, which were irrigated the year round, started to bear fruits in the third year, and maximal yields were harvested from the sixth year: 30 kg of fruit (sun dried) per tree for the best specimens. However, estimated oil production for high yielders was 0.9 kg per tree, which is less than one-fifth of the oil produced by olive trees of the same age growing in the area. The oil content was in the range reported for fruits harvested in the wild, namely 50 to 56% of kernel dry wt (Nouaim et al. 1991). The low oil yield was related to the low total kernel weight. The predominant fruit components were the pericarp and the drupe wall, each accounting for 44–48% of total fruit dry weight (fruit dry wt ranged between 3.5 and 7 g). The kernels, on the other hand, made up only 5–8% of total dry wt. These results suggest that improving total kernel weight per fruit is essential for the successful introduction of argan as a horticultural crop.

An understanding of the reproductive biology of the argan is thus essential for the formulation of programs for breeding and selection and for rational orchard management. Published work on the reproductive biology of argan focuses on flower and fruit morphology (Cornu 1897; Perrot 1907), or else on flowering and ripening periods and fruit drop in the argan's native area (Belmouden, Bani Aameur, and Dupuis 1995; Ferradous, Bani Aameur, and Dupuis 1995; Nouaim et al. 1991). In the present study we concentrate on the phenology, pollination requirements, and fruit development of argan cultivated in the Negev Highlands (Ramat Negev) of Israel.

Materials and Methods

Experimental Orchard

Our study was carried out in 1992–1994 on seed-propagated trees planted in 1985 at Ramat Negev, which is situated in the Highland region of the Israeli Negev desert (Nerd et al. 1994). The seeds were obtained from trees growing in the botanical garden of Mikwe Israel (in Israel). Climatological data for the study period are presented in Fig. 1. The region is distinguished by low winter temperatures, which can drop to extremes of −7°C, and by a low annual rainfall

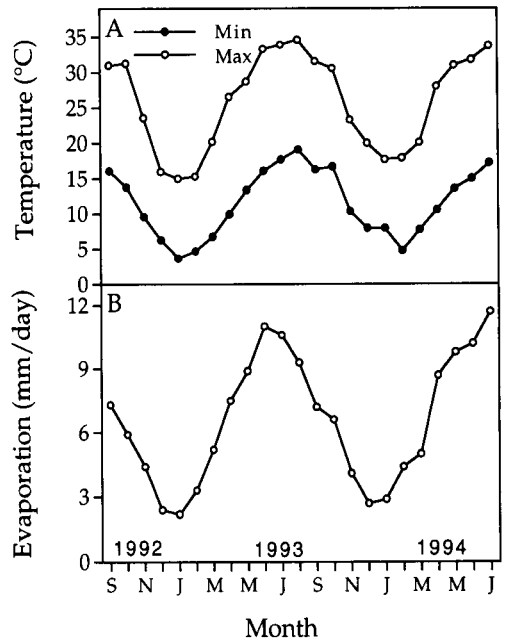

Fig. 1. Temperature (A) and pan evaporation rates (B) during the study period at Ramat Negev. Values are means of daily measurements.

confined to winter and averaging 90 mm. The trees were irrigated the year round by drippers to keep the soil wet, with good quality water (EC = 1 dS/m) containing NPK fertilizer (Nerd et al. 1994).

Phenology

Tips of shoots of the last annual growth (15 shoots per tree) were tagged in the winters of 1992/3 and 1993/4 in six randomly selected trees. Time of appearance of flowers, their position along the shoot, and shoot elongation period were recorded. Flowering period was also recorded for the rest of the orchard (16 trees).

Floral Phases and Breeding System

Flower Opening and Stigma Receptivity

Argan flowers are small (0.4–0.5 cm width) and sessile. Morphologically (Cornu 1897; Perrot 1907), the flower consists of five hairy green sepals, a pale green corolla with five lobes, five stamens alternating with five staminodes, and one pistil; the superior hairy ovary contains two to four loculi with one ovule in each loculus. The process of floral opening and receptivity of the stigma (pollen germinability on the stigma) at various floral phases were examined in the

spring of 1993 in cut shoots removed from three trees and placed in 5% sucrose solution in a room with max/min temperature of 24°C/17°C. Stigma receptivity was determined in flowers on the cut shoots enclosed in paper bags according to the procedure described by Ascer and Peloquin (1966). Flowers at different developmental phases (10 flowers for each phase for each tree) were removed from the plants and placed in Petri dishes containing 1.5% agar and were immediately pollinated with fresh pollen collected from other trees in the orchard. After 24 h the pistil was cut and fixed in FAA (40% formaldehyde, glacial acetic acid and 80% ethanol, 1:1:8 v/v/v). Pistils were rinsed with water, softened in 8 N NaOH, squashed, and stained with 1% aniline blue in 0.1 N K_3PO_4, to enable detection of tube growth by fluorescent microscopic observation (Martin 1959).

The pollen samples used for the stigma receptivity study were routinely checked for in vitro pollen germination by the suspension culture technique (Shivanna and Rangaswamy 1992) modified for argan by Irijimovich (1995). The medium used was a water solution containing 30% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)_2 \cdot 4H_2O$, 100 ppm KNO_3, and 200 ppm $MgSO_4$. The pollen (~10 mg) was mixed with 0.5 ml medium in a 10-ml flask. The sealed flask was agitated in the dark for 24 h at 25°C, then sampled for microscopic examination. Grains were scored as germinated when tube length exceeded the diameter of the grain itself.

Pollination Treatments

The effect of different pollination treatments on fruit set was studied in the spring of 1994 in four trees at the orchard. Shoots with newly produced floral buds were trimmed of all but 20 floral buds each and treated in one of the following ways: 1) enclosed in paper bags (autonomous self pollination); 2) enclosed in paper bags, flowers being pollinated at anthesis with pollen of the same tree (hand self pollination); 3) enclosed in paper bags, flowers being pollinated at anthesis with pollen of another tree (hand cross pollination); 4) left uncovered (open pollination). Each treatment was applied to 10 shoots per tree. Bags were removed at the end of anthesis, and green fruitlets were counted eight weeks later (at Ramat Negev such fruitlets usually developed into fruits). In parallel, in vivo pollen germination and tube growth in self and foreign pollen were studied in three trees. Treatments 2 and 3 described above were applied to each tree. Pistils removed 0, 12, 24, 48 and 72 h post pollination (eight per tree in each replication) were treated and examined as in the stigma receptivity study. In each pistil, the length of at least six tubes was measured.

Pollen Vectors

The behavior of insects in the orchard was studied in the spring of 1994. Insects trapped with an insect net were identified by Dr. Z. Klein of the Agricultural Research Organization (Bet-Dagan, Israel). In order to check pollen transfer by wind, 2-m-high poles were placed at all four cardinal points (N, S, E, W) around three trees at distances of 1.5 and 6.5 m from the tree. Ten microscope slides covered with a thin layer of vaseline were placed along each pole to trap pollen. The number of pollen grains adhering to each slide was determined under a binocular after a 24-h exposure.

Fruit Measurements

Fruits were tagged at the beginning of June 1994 (May flowering) on four trees for the periodic sampling. The fruits, ca. 100 per tree, were chosen for uniformity in their initial length. At each sampling date, fruit length and diameter and the fresh weight of fruit components (pericarp, drupe and kernels) were determined for six fruits per tree. Dry weight of the kernels (oven dried at 70°C) was determined during the period of rapid kernel growth, the dry matter being used for measuring oil content by extraction with petroleum ether (A.O.A.C. 1990).

Ovule Number and Seed Set

Ovules were counted under a microscope in transections of ovaries dissected in the spring of 1994 from flowers of 12 trees (30 flowers per tree). Kernels were counted in mature fruits of the same floral flush at the same sample size.

Results and Discussion

Flowering and shoot elongation were recorded for two consecutive years in well-watered argan trees planted in the Negev Highlands (Fig. 2). For the investigated trees (n = 6), the main flowering period was confined to 3–4 weeks in the spring (end of April and May), while the shoot growth period was less regular and depended on year or tree. In general, significant

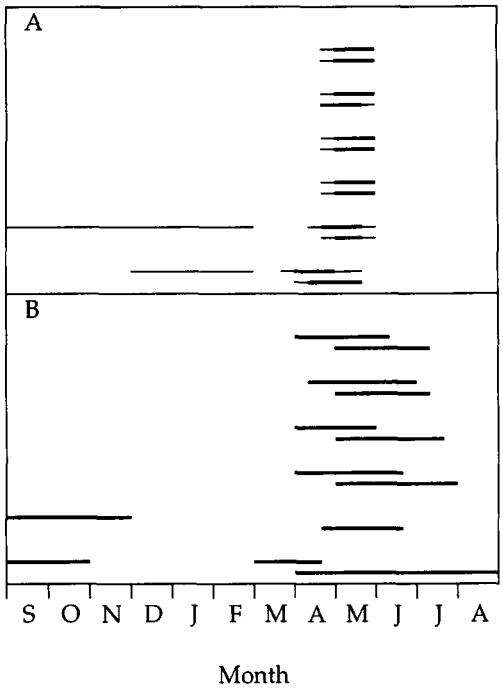

Fig. 2. Periods of flowering (A) and shoot elongation (B) in six argan trees cultivated at Ramat Negev. For every pair of lines corresponding to a single tree, the upper line indicates data for 1992–1993, and the lower line data for 1993–1994. The thicker sections of the lines in the upper figure indicated periods when the trees were heavily covered with flowers.

growth occurred in the spring and early summer. Flower clusters were usually initiated on shoots of the previous growth season. Our observations coincide with those reviewed by Nouaim et al.

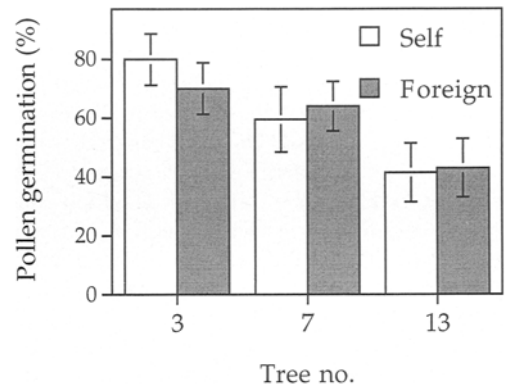

Fig. 4. In vivo germination of self and mixed foreign pollen of argan. Values are means ± SE for eight flowers in each of three trees.

(1991), showing that in Morocco argan flowers in April and May. However, according to Ferradous, Bani Aameur and Dupuis (1995), flowering in certain areas in Morocco can begin earlier and last longer, namely from October to March; early (October–November) and late flowering trees (February–March) were observed, and some trees reportedly flowered throughout the wet season. Although detailed environmental data were not presented by the authors they emphasized that availability of water in the soil was important for flowering.

Flower phases indicate protogynous dichogamy. The first sign of flower development is the appearance of the stigma at the top of the closed bud. A few days later (up to four) the style

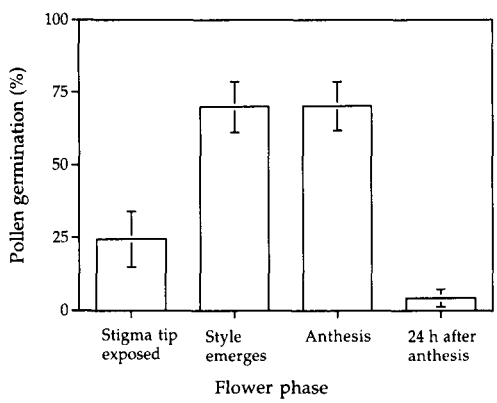

Fig. 3. In vivo pollen germination (stigma receptivity) at various floral phases of argan. Values are means ± SE of three trees. Mixed foreign pollen was used.

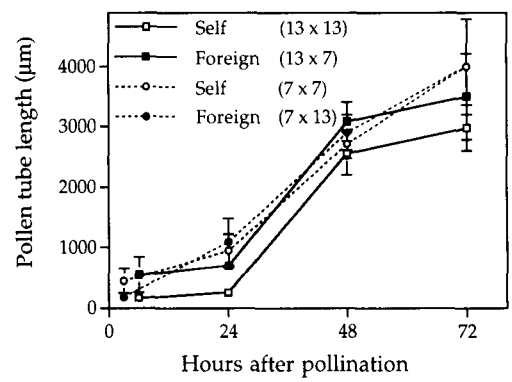

Fig. 5. In vivo tube elongation of self and foreign pollen of argan. Values are means ± SE for eight flowers in each of three trees. Numbers in brackets are the no. of female and male trees, respectively, participating in the cross.

TABLE 1. EFFECT OF POLLINATION MODE ON FRUIT SET (% OF TAGGED FLOWERS) IN ARGAN TREES GROWING AT RAMAT NEGEV. FRUIT SET WAS DETERMINED SIX WEEKS AFTER ANTHESIS. VALUES ARE MEANS ± SE FOR FOUR TREES.

Mode of pollination	Fruit set (%)
Control (nontreated bagged shoots)	0
Hand self pollination	0.51 ± 0.2 a
Hand cross pollination	6.47 ± 1.9 b
Open pollination	8.87 ± 1.2 b

Data followed by different letters within the column differed significantly at $P \leq 0.05$.

emerges, after which the sepals open and the anthers release their pollen. Flowers stay open for two days, and then the corolla abscises. Pollen germination on the stigma (stigma receptivity) was low (24%) before emergence of the style and high (60–70%) after its emergence and throughout anthesis (Fig. 3). Percentages of in vitro germination for the pollen samples used in the stigma receptivity study averaged 63.3 ± 1.2%, well within the range recorded for in vivo pollen germination at advanced stages of pistil development (Fig. 3). This indicates that our in vitro method (Irijimovich 1995) is useful for predicting pollen quality in argan. The viability of argan pollen collected from wild stands in Morocco, as tested by acetocarmine staining (Belmouden, Bani-Aameur, and Dupuis 1995), was usually found to be high (68.3–99.5%); this value, however, was not correlated with ability to germinate.

Germination percentage on the stigma was similar for self and foreign pollen, but differing among trees (Fig. 4). Tube elongation rate was also similar, and in both instances the tube reached the base of the style within 72 h post pollination (Fig. 5). However, foreign pollen led to significantly higher fruit set, namely 6.5 ± 1.9% vs 0.5 ± 0.2% for self pollen (Table 1). It is therefore concluded that some degree of postzygotic discrimination probably occurs in argan. Table 1 also shows that the percentage of fruit set was similar for open and cross pollination.

Fruit set failed in bagged shoots, indicating that a pollen vector is needed for effective pollination. After a day with moderate north–west wind (6.5–10 km h^{-1}), pollen was detected on slides placed 1.5 m from the tree (8–28 grains cm^{-2} on slides facing south and east), but not on slides placed at a distance of 6 m. This finding indicated that pollen is dispersed by wind only to short distances. The flowers, which lack nectar but have a typical strong fragrance, attracted many insects, particularly flies of the family Calliphoridae (Table 2). The visiting insects were found to be covered with argan pollen but more studies are needed to elucidate their role in pollination. The dichogamous nature of the flower, its partial self sterility, and the need for a pollen vector appear to be traits that ensure fruit set by cross pollination.

Measurement of fruit growth parameters was initiated when fruits reached a length of 0.7–0.9 cm (beginning of June), fruits of this size tending to develop and reach maturity. Significant drop of flowers and small fruits occurred prior to this stage. Fig. 6A shows that dimensional growth (length and diameter) was rapid between July and August and then slowed, the fruits reaching their final size in winter, approximately nine months after anthesis. Fig. 6B shows that fresh wt growth of whole fruit was characterized by a double sigmoidal curve and consisted of three distinct stages: I) an early period of rapid growth, II) a period of slow growth, and III) a late period of rapid growth. A pronounced gain in fresh weight occurred for the pericarp in stages I and III, for the drupe in stages I and II, and for the kernel in stages II and III. The drupe wall hardened in stage II, and coloration occurred in stage III. Accumulation of dry matter and oil in the kernel coincided with its period of fresh weight increase (Fig. 7). This pattern of

TABLE 2. INSECTS VISITING FLOWERS OF ARGAN TREES AT RAMAT NEGEV.

Family	Species	Frequency (arbitrary)
Alleculidae	*Omphalus* sp.	+
Sryphidae	*Episyrphus balteatus* (De Geer)	++
Sryphidae	*Eristalinus aereus* (Scopoli)	++
Calliphoridae	*Chrysomya albicepes* (Wiedeaman) and other *Chrysomya* spp.	++++

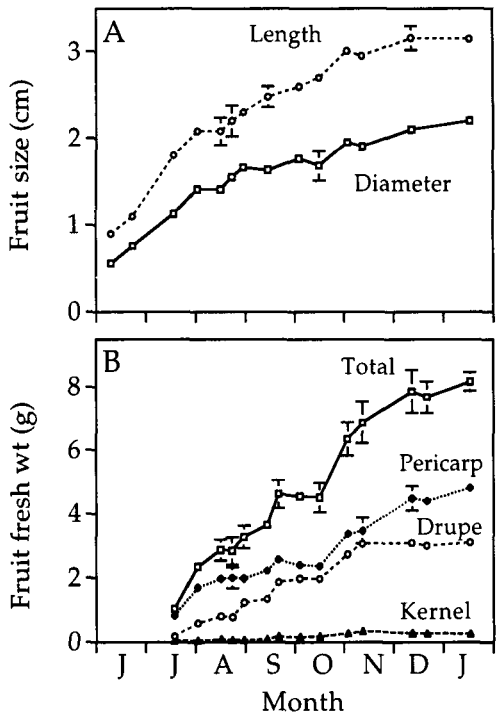

Fig. 6. Increase in fruit size (A) and fresh weight of fruit components (B) at Ramat Negev. Values are means ± SE for four trees.

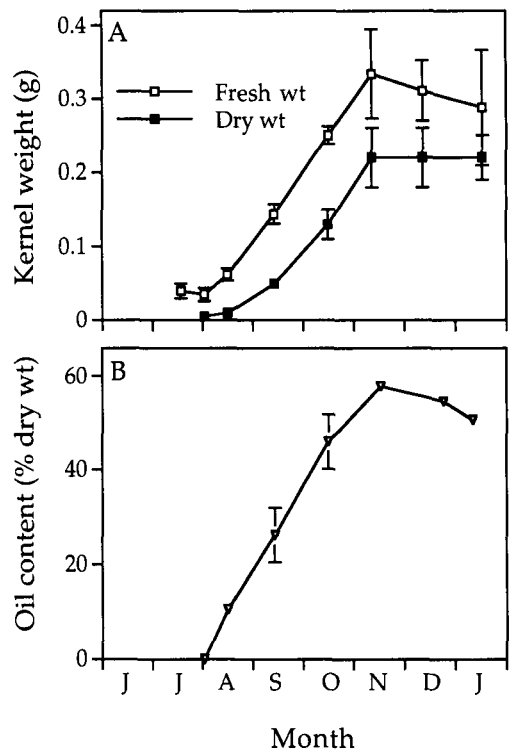

Fig. 7. Fresh and dry weight of argan kernel (A) and its oil content (B) during growth. Values are means ± SE for four trees.

fruit growth is similar to that shown for typical fleshy drupaceous fruits such as peach and apricot (Leopold and Kreidmann 1975).

According to our observations (unpublished) in a non-irrigated plot at a site located in the southern coastal plain of Israel (400 mm annual rainfall), where conditions are milder than at Ramat Negev, growth of fruitlets 0.2–0.3 cm long that set in the spring was interrupted during the summer on most trees. The fruitlets resumed growth in the autumn (September–November). Arrested fruit growth correlated with drought was also reported for stands in Morocco, where the fruit growth period can extend from 8 to 16 months (Belmouden, Bani Aameur, and Dupuis 1995). This flexible pattern of fruit development shifts fruit growth to seasons with favorable growth conditions and allows fruit production under the dry conditions of its natural habitat. However, this feature may be a disadvantage for a modern orchard, because it is associated with irregular fruiting and a long fruit growth period. Our study at Ramat Negev suggests that the continuous, short fruit developmental period obtained in our study is probably associated with watering.

The ovary of argan flowers contains two to four loculi with a single ovule in each. The drupe-like fruit may contain up to four kernels, but most frequently only one (Cornu 1897; Perot 1907). It was shown that fruits with a larger kernel number have also a larger total kernel weight (Nerd et al. 1994). Our results here indicated that ovaries contained two or three ovules, two ovules being the dominant number (80.4% of the total flowers examined), while the fruits contained one to three kernels, with one kernel being the dominant number (81.7% of fruits examined) (Table 3). The general trend was the abortion of one ovule. This was also seen in one unusual genotype (no. 21) that produced high percentage of flowers with three and four ovules (85%); 81% of the fruits had only two and three kernels. Studies are needed to find the effect of environment and inheritance on ovule production and kernel set.

TABLE 3. OVULE NUMBER IN FLOWERS AND KERNEL NUMBER IN FRUITS. THE VALUES ARE MEANS ± SE OF 12 TREES.

	Flowers with		Fruits with		
	2 ovules	3 ovules	1 kernel	2 kernels	3 kernels
% of total	80.4 ± 5.7	19.6 ± 5.7	81.7 ± 3.5	17.6 ± 3.2	0.7 ± 0.4

CONCLUSIONS

Several aspects of the reproductive biology of argan were examined with a view to promoting the development of the tree as a horticultural crop. The results of the breeding system studies indicate that argan flowers are partially dichogamous and that cross pollination promotes fruit set. A high degree of diversity may therefore be expected among the progeny of the same tree. Hence, it is not recommended that solid-clone orchards be established after selection of desirable clones for cultivation. Cultivation of argan trees leads to regular fruiting, with concentrated flowering in spring and continuous fruit development.

ACKNOWLEDGMENTS

We are grateful to Prof. Vasil Sarafis for his advice and J. Mouyal and E. Naus for their technical assistance. We also wish to thank the US-AID foundation (Middle East Regional corporation program) for financial support.

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