Pseudo-xenic Effect of Allied *Annona* spp. Pollen in Hand Pollination of cv. 'Arka Sahan' [*(A. cherimola* × *A. squamosa)* × *A. squamosa*]

S.H. Jalikop¹ and Ravindra Kumar

Division of Fruit Crops, Indian Institute of Horticultural Research, Hessarghatta, Bangalore, Karnataka 560089, India

Additional index words. Annonaceae, fruit set, metaxenia, pollination, pollen profile, pollen source

Abstract. Annonaceous fruits are characterized by a serious problem of fruit set, and hand pollination is commonly practiced for improving the set. Because we observed that allied *Annona* spp. are freely crossable and often produce attractive F_0 fruits, the possibility of using their pollen in hand pollination was investigated. A total of 1080 flowers of cv. Arka Sahan in 2003 and 3420 in 2004 were pollinated with *A. atemoya* Hort., *A. cherimola* Mill., *A. reticulata* L., *A. squamosa* L., and self-pollen. Pollen source had significant effects in both or either year on all 14 fruit traits studied except days to ripening. *A. squamosa* pollen gave the highest fruit set (greater than 91%) and the heaviest fruits (greater than 600 g). Good to moderate fruit set was recorded with *A. reticulata* and self-pollen (31% to 86%); and with *A. atemoya* and *A. cherimola* pollen, the set was poor (4% to 13%), whereas natural set was as low as 2%. 'Arka Sahan' fruits resulting from *A. squamosa* pollen showed quicker fruit maturity (128 days) and least weight loss on ripening (13%). Evaluation of fruit pulp quality revealed that pollen source and year had significant effects on the total soluble solids and acidity but not their ratio. Mean number of seeds per 100 g fruit was found to vary (6 to 11) with pollen source used. *A. squamosa* and *A. reticulata* pollen had higher seed count with good to moderate fruit size and symmetrical shape, but those from the rest of the pollen sources neither possessed good size nor shape. Results clearly showed that 'Arka Sahan' could be successfully hand pollinated with *A. squamosa* pollen. The differential ability of pollen sources to fertilize number of ovules and set seeds per fruit rather than the pollen genotype had a significant effect on many fruit traits like size, symmetry, skin thickness, and total soluble solids, which we describe as "pseudo-xenia." Useful pseudo-xenic effect is worth exploiting in fruit culture.

Six of 120 species of *Annona* have pomological significance. Cherimoya (*A. cherimola* Mill.), sugar apple (*A. squamosa* L.), and the hybrid between the two, atemoya, which has been assigned horticultural species status (*A. atemoya* Hort.), are the most popular ones (Batten, 1990; Peña et al., 2002). The other less important annona species producing edible fruits are bullock's heart (*A. reticulata* L.), Ilaia (*A. diversifolia* Staff), and sour sop (*A. muricata* L.).

The common constraint limiting commercial production of annonaceous fruits is low flower to fruit ratio resulting from inadequate pollination (Gazit et al., 1982; Peña et al., 2002). Neither self- nor cross-pollination takes place effectively. Self-pollination is hindered by the presence of marked protogyny (Kahn

et al., 1994), whereas cross-pollination by insects is limited because the flowers are not brightly colored and lack fragrance and nectar (Escobar et al., 1986; Peña et al., 2002). Because pollen grains occur in clumps and are sticky, wind pollination is not common (George et al., 1989). Moreover, annona pollen at dehiscence generally contains a good proportion of dyads and tetrads intermingled among individual viable and non-viable pollen grains (Saavedra, 1977).

To circumvent poor fruit set, either hand pollination (Escobar et al., 1986; Melo et al., 2004; Saavedra, 1977) or use of insect pollinators (Gazit et al., 1982; George et al., 1989; Peña et al., 2002) is recommended, but the latter gives erratic results and is not efficient when pollination from a specific pollen source is desired. Hand pollination improves fruit set and substantially boosts fruit yield per tree by increasing the fruit size and number, the major yield components (Pritchard and Edwards, 2006). However, it is labor-intensive and should be adopted in varieties that produce premium quality fruits by choosing an efficient pollen source. The influence of pollen on seed (xenia) and fruit (metaxenia) is well known in many crop species (Denney,

1992), including annona, in which Kahn et al. (1994) observed that fruit set, morphology, and quality are dictated by choice of pollen parent. The rewards will more than compensate if desirable xenic effects manifested at the F_0 level, like heterosis manifested at the F_1 level, is exploited.

'Arka Sahan', the annona variety on which different pollen species were tested, is an outstanding recombinant isolated from the family of 'Island Gem' (*A. cherimola* × *A. squamosa*) × 'Mammoth' (*A. squamosa*) and released for cultivation (Jalikop and Kumar, 2000). However, like other annonas, it yields fewer fruits with variable shape and size. Our hybridization program showed that most of the edible *Annona* spp. were crosscompatible (Kumar and Jalikop, 2000), and some interspecific crosses exhibited good set producing big, attractive F_0 fruits. This motivated us to test allied annona species as pollenizers. The objective of the present research was to study the effects of *Annona* spp. pollen on the set, size, shape, and quality of 'Arka Sahan' fruits and to find the most suitable pollen source for hand pollinating this variety.

Materials and Methods

Study site and pollination. The study was carried out at the Experimental Farm of Indian Institute of Horticultural Research, Bangalore, India, during May to Oct. 2003 and 2004. The site is located at an elevation of 890 m in mild-tropics (13°58'N, 77°37'E). Flowers in female stage (those having receptive gynoecia) in 8-year-old trees of 'Arka Sahan' raised on *A. reticulata* rootstocks were pollinated with *A. atemoya*, *A. cherimola*, *A. reticulata*, *A. squamosa*, and 'Arka Sahan' in the morning (0600 to 0900 HR). During our pilot study to know the best time for pollination, it was noted that on a given day, some 'Arka Sahan' trees produced many and others far less number of flowers with receptive gynoecia. Treatments (pollen sources) were therefore spread across flowers available collectively in 12 trees because it would not have been possible to secure required number of flowers (30 per day per treatment) for some treatment if pollen sources were to be assigned treewise. Everyday, receptive moist stigmas of 150 flowers selected at random were evenly smeared with the pollen using a fine-tipped brush. Pollen was gathered from the flowers in the male stage by tapping and rubbing the flowers between the fingers in a 50-mL plastic cup. To avoid contamination, separate earmarked cups and brushes for each pollen source were used and hands were cleaned with alcohol before starting the next treatment. Each hand-pollinated flower was labeled with the pollen source, date of pollination, and a serial number. Altogether, 1080 flowers in 2003 (19 to 24 May) and 3420 flowers in 2004 (13 May to 4 June, except four holidays) were pollinated. Besides, to measure the set, size, and quality of fruits resulting from the natural pollen source, 30 flowers subjected to natural pollination were also labeled everyday.

Received for publication 1 Nov. 2006. Accepted for publication 10 Feb. 2007.

Thanks are due to Director, Indian Institute of Horticultural Research, Bangalore, India, for providing facilities, Drs. N.K. Krishna Kumar, B.N.S. Murthy, and R.S. Kulkarni for helpful suggestions, and anonymous reviewers for critical and constructive comments.

¹To whom reprint requests should be addressed; e-mail jalikop@iihar.ernet.in.

Pollen assay. A few anthers from the flowers picked in the morning were squashed into a drop of 1% aceto-carmin stain. Specimens were examined using a light microscope and pollen scored as viable (stained), nonviable (unstained), dyads, and tetrads. Six fields of 100 grains of each pollen parent were counted in 2004 and the mean was calculated.

Data collection. All flowers from each treatment were left untouched, but fruit development was monitored at regular intervals. Fruit set was recorded at maturity in fruits in which interspace between the carpels had widened and turned cream in color. A sample of 30 fruits (15 each in 2003 and 2004) per treatment was randomly selected from the fruits harvested as and when they were mature to record the following data: 1) date of harvest; 2) fruit weight in grams; 3) date of ripening, when the entire fruit was soft; 4) ripe fruit weight in grams; 5) fruit symmetry, visually graded as conical, oblate, oblique, and irregular; 6) skin surface, differentiated visually according to whether the exposed surface of the carpel on the fruit was flat, bulged, or intermediate between flat and bulged; 7) skin thickness, measured in millimeters using a vernier calipers at three random points on the skin; 8) seeds/fruit; and 9) seed weight/fruit. Fruit quality was estimated by considering total soluble solids (TSS), recorded in °Brix using a handheld refractometer (Erma, Tokyo) and titratable acidity (TA), estimated independently on 15 fruit samples by titrating 10 mL juice against 0.1 N NaOH and expressed as total titratable acidity percent. Using certain observations, we derived: 1) fruit set percent (fruit set × 100) ÷ number of flowers pollinated; 2) days to fruit maturity, time lag between pollination and fruit harvest; 3) days to ripening, time lag between fruit harvest and ripening; 4) percent weight loss on ripening (difference between fresh and ripe fruit weight × 100) ÷ fresh

fruit weight; 5) number of seeds/100 g fruit weight (seeds/fruit × 100) ÷ fruit weight; 6) mean seed weight, total seed weight/fruit ÷ number of seeds/fruit; and 7) TSS acidity ratio, TSS ÷ TA.

Statistical analysis. The experiment was arranged in a completely randomized design with 15 (for fruit measurements) or five (pollen profile score) replications/treatment (pollen sources) for each year. However, the 30 pollinated flowers per treatment per day represented one replication for analysis of data on fruit set. The influence of date of pollination was minimized by effecting pollination on continuous dates over a short period of time and to obtain a more accurate estimate of treatment effects, a large number of flowers were pollinated (750 per treatment). Because *A. cherimola* produced no fruits in 2003 and 14 fruits in 2004, it was included only in the 2004 analysis. Before analysis of variance, the data in percentage were arcsine-transformed to meet the assumption of a normal distribution (Zar, 1996) and Duncan's multiple range test was used for mean separation. Data were analyzed year-wise and also year × pollen source interaction was computed. Linear correlation was computed for relevant pairs of traits. For the qualitative parameters, a weighted mean was calculated by assigning weightage in the order of preference as conical = 4, oblate = 3, oblique = 2, and irregular = 1 for fruit shape; and flat carpel = 3, intermediate between flat and bulged carpel = 2, bulged carpel = 1 for skin surface. The data on these two traits were subjected to categorical analysis (Gacula and Singh, 1984) and χ^2 was worked out using data pooled over the years.

Results

Effects of *Annona* spp. pollen on fruit set, size, skin thickness, and seed traits. In 2003 and 2004, pollen source significantly influ-

enced the fruit set (Table 1). In both the years, pollen of *A. squamosa* produced maximum set of more than 91%. *Annona cherimola* pollen did not set fruit in 2003 and gave a very poor set in 2004. Naturally pollinated flowers recorded a meager set of 1.66% in 2003 and 1.17% in 2004. Pollen effect on fruit weight was highly significant ($P \leq 0.01$). Fruits pollinated by *A. squamosa* pollen were the heaviest and those pollinated by *A. atemoya* were the lightest. Small- to moderate-sized fruits obtained from 'Arka Sahan', *A. atemoya*, and natural pollen source had the thinnest skin and in contrast, the large fruits formed from *A. squamosa* pollen registered the thickest skin. Generally, the number of seeds/fruit and per-unit fruit weight followed a similar trend. Those containing more seeds produced by *A. squamosa* pollen in both the years and *A. reticulata* in 2004 had symmetrical, large fruits, whereas the fruits having fewer seeds had asymmetrical, small fruits. Furthermore, a significant ($P \leq 0.01$) linear correlation between seed number and fruit size ($r = 0.84$) and seed number and fruit symmetry ($r = 0.67$) was also observed in this study.

Effects of *Annona* spp. pollen on pulp quality. In 2003, a dry year, TSS in the fruit juice was generally higher (31.98 to 33.34 °Brix) and was unaffected by the pollen treatments. In 2004, pollen treatment source significantly influenced this trait with *A. reticulata* pollen producing fruits having the lowest TSS. Interestingly, in 2003, the same source recorded the highest TSS value. Titratable acidity was significant at $P \leq 0.05$ in both the years, but in 2004, it exhibited less variation.

Effects of *Annona* spp. pollen on days to fruit maturity and ripening and weight loss on ripening. Days to fruit maturity, days to ripening, and weight loss on ripening were consistent over years as suggested by the nonsignificant pollen source × year interaction (Table 1). However, a discernible differences

Table 1. Influence of pollen of allied *Annona* spp. on fruit set, quality, and seed traits of Arka Sahan during 2003 and 2004.

Year/ Pollen source (ps)	Fruit set (%)	Fruit weight (g)	Skin thickness (cm)	Number of seeds/ fruit	Mean seed weight (g)	Seeds/ 100g fruit	T S S (°Brix)	T A (%)	T S S : T A ratio	Days to maturity	Days to ripening	Weight loss on ripening (%)
2003 ²												
<i>A. atemoya</i>	13.33 ^{ab} _y	216.33 ^a	0.17 ^{ab}	18.13 ^c	0.27 ^a	10.60 ^b	32.83	0.25 ^a	137.66	127.46 ^a	6.00	19.44
<i>A. cherimola</i>	0.00	—	—	—	—	—	—	—	—	—	—	—
<i>A. reticulata</i>	30.55 ^{bc}	283.13 ^b	0.19 ^{bc}	16.54 ^{bc}	0.37 ^c	7.32 ^a	33.34	0.34 ^b	107.92	131.26 ^b	5.80	19.36
<i>A. squamosa</i>	91.33 ^d	439.06 ^d	0.21 ^c	33.87 ^d	0.30 ^{ab}	9.71 ^b	32.88	0.27 ^{ab}	121.06	128.06 ^a	5.87	17.90
Arka Sahan	44.44 ^c	284.54 ^{bc}	0.16 ^a	13.07 ^{ab}	0.29 ^{ab}	5.85 ^a	32.11	0.28 ^{ab}	121.25	132.26 ^b	6.00	18.33
NP ^x	1.66 ^a	203.93 ^a	0.17 ^{ab}	10.60 ^a	0.31 ^b	7.12 ^a	31.98	0.25 ^a	150.56	130.00	5.26	19.84
2004 ²												
<i>A. atemoya</i>	8.43 ^b	164.40 ^a	0.19	10.07 ^a	0.33	8.07 ^{bc}	27.61 ^{bcd}	0.33 ^{abc}	85.89	130.40 ^{ab}	5.80	16.72 ^b
<i>A. cherimola</i>	4.31 ^a	180.29 ^a	0.20	8.57 ^a	0.37	6.18 ^a	28.94 ^d	0.33 ^{bc}	91.67	137.36 ^c	6.14	16.64 ^b
<i>A. reticulata</i>	85.88 ^d	456.47 ^c	0.21	36.80 ^c	0.38	9.42 ^c	24.89 ^a	0.31 ^{ab}	81.85	133.67 ^{bc}	6.27	13.12 ^a
<i>A. squamosa</i>	91.96 ^d	612.67 ^d	0.20	47.20 ^d	0.36	9.18 ^c	27.08 ^{bc}	0.34 ^{bc}	81.10	127.93 ^a	6.20	12.84 ^a
Arka Sahan	65.68 ^c	295.47 ^b	0.17	15.40 ^b	0.36	6.92 ^{ab}	25.99 ^{ab}	0.28 ^a	92.85	137.27 ^c	6.13	15.85 ^{ab}
NP ^x	1.17 ^a	185.00 ^a	0.19	10.66 ^a	0.37	6.98 ^{ab}	27.71 ^{cd}	0.35 ^c	81.69	139.00	5.73	17.49 ^b
2003	**	**	**	**	**	**	NS	*	NS	**	NS	NS
2004	**	**	NS	**	NS	**	**	*	NS	**	NS	**
p s × year	**	**	NS	**	NS	**	**	**	NS	NS	NS	NS

²*A. cherimola* produced no fruits in 2003 and in 2004 only 14 fruits were obtained.

³All values are mean of 15 fruits, except fruits developed from *A. cherimola* pollen in which 14 fruits were observed. Within columns, means followed by different superscript letters are significantly different at the 0.05 *P* level according to Duncan's multiple range test.

^xNatural pollination.

^{ns,*,**}Nonsignificant or significant at *P* = 0.05 or 0.01, respectively.

($P \leq 0.05$) resulting from pollen source for days to fruit maturity were noted in 2003 and 2004. *Annona squamosa* pollen produced the largest fruits which, interestingly, took less number of days to attain maturity (≈ 128 d). *Annona cherimola* pollen resulted in not only smaller fruits, but also required a maximum mean number of days to maturity (≈ 137 d). Mean days to fruit ripening varied within a narrow range (5.3 to 6.3 d) and was non-significant. The fruits originating from *A. cherimola*, *A. atemoya*, and a natural pollen source lost more weight on ripening, whereas those from *A. squamosa* and *A. reticulata* (the large fruited) lost the least. Fruits with more fresh weight losing less weight on ripening was also established by the negative significant correlation between fresh weight and ripe fruit weight ($r = -0.42$; $P \leq 0.05$).

Effects of *Annona* spp. pollen on fruit morphology. Fruit morphology was studied by considering the qualitative data recorded on fruit symmetry and skin surface (Table 2). *Annona squamosa* treatment imparted conical symmetrical fruits registering a very high weighted mean of 3.93/4.00 over the 2 years. In other words, 29 of 30 sampled fruits had the desirable symmetry (Fig. 1). *A. cherimola* and self-pollen resulted mostly in misshapen fruits. Fruits with all three carpel types were recorded at a varying frequency in all the treatments, but there was no significant pollen effect on surface morphology of carpels.

Pollen profile of the pollen source used was examined to match it with fruit set. It may be seen from Table 3 that interspecies variation was significant for viable, nonviable, tetrad, and dyad pollen.

Discussion

In annona, hand pollination is critical for good fruit set. Current annona breeding programs are directed toward developing varieties by converging allied species and hence, exploring pollen sources for hand pollination cutting across species boundaries is important. The present article describes the effects of pollen of allied *Annona* spp. on fruit and seed characteristics in an interspecific hybrid 'Arka Sahan'. The results showed that the pollen source had significant effects in both or either year on all the 14 fruit traits studied except days to ripening.

The overall fruit set trend in 2003 and 2004 resulting from different pollen treatments was similar, although the former was a drought year with deficit rain (531 mm), whereas the latter year received good rain (965 mm). The poor fruit set by *A. reticulata* and no fruit set by *A. cherimola* pollen in 2003 could be attributed to lower precipitation (George and Nissen, 2002; Pinto et al., 2005). Furthermore, the fruit set was found to significantly vary with the pollen source. The previous workers depending on pollen treatment in different *Annona* spp. have reported variable effects on fruit set: 1) in *A. cherimola*, set was 84% to 96% with self-pollen but with mixed pollen, it was 38% to 69% (Richardson and Anderson, 1996); 46.6% when self-

Table 2. Weighted mean for shape and skin surface of Arka Sahan fruits developed from pollen of *Annona* spp. and natural pollen averaged over 2003 and 2004.

Pollen source	Weighted mean	
	Fruit shape	Skin surface
<i>A. atemoya</i>	2.3**	2.1
<i>A. cherimola</i>	1.8**	2.0
<i>A. reticulata</i>	3.0**	2.1
<i>A. squamosa</i>	3.9**	1.8
Arka Sahan	2.7*	2.2
Natural pollination	2.3 ^z	2.1
χ^2 (overall)	87.8**	4.1 NS

^zSignificance compared with hand- with naturally pollinated fruits as determined by separate pairwise χ^2 values.

NS,*,**Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively. Weighted mean calculated by assigning weightage in the order of preference as conical = 4, oblate = 3, oblique = 2, and irregular = 1 for fruit shape; flat carpels = 3, intermediate between flat and bulged carpels = 2, and bulged carpels = 1 for skin surface.

pollen was used but with atemoya pollen, it was 4.7% (Duarte and Escobar, 1998); 2) in *A. atemoya* with *A. squamosa* pollen, the set was 80.5% (Melo et al., 2004); 3) in *A. squamosa*, set was 90.9% to 100% with self-pollen (Cogez and Lyannaz, 1996); and 4) in *A. muricata*, set ranged from 31.2% to 37.5% (Khalid, 1992). Kahn et al. (1994) showed that both maternal and paternal parents have significant effects on percentage of fruit set.

Kahn et al. (1994) and Pritchard and Edwards (2006) reported significant pollen effect on fruit weight, which is in accordance with the findings made in the present study. Weight of fruits sired by *A. atemoya* or *A. cherimola* was on par with unpollinated, naturally set fruit suggesting that hand pollinating with pollen of these species is not worth attempting, whereas *A. squamosa* pollen produced the heaviest fruits besides giving the best fruit set; hence, it could be regarded as an attractive pollen source.

In agreement with the results in the present study, many previous workers in annona (e.g., Kahn et al., 1994) have shown a positive association between seeds/fruit and fruit size and seeds/fruit and fruit symmetry. In 2004, *A. squamosa* pollen produced 47 seeds/fruit and the corresponding figure was 11 with natural pollination, which showed that nearly 80% ovules degenerate or develop parthenocarpically into small seedless segments for want of pollen. Seedless segments in the flesh improve consumptive quality of the fruit, but to achieve perfect fruit symmetry, a high percentage of ovules must be fertilized and set seeds because it promotes good carpellary wall development (Richardson and Anderson, 1996). Hence, any attempt to reduce the seeds/fruit below a threshold would be at the expense of size and symmetry.

Relatively more seeds ($\approx 9/100$ g fruit) detected in the fruits developing from *A. squamosa*, the pollen source that gave the best fruit set, should nevertheless be acceptable because a threshold limit of less than 10 seeds/100 g flesh is set as a selection criterion in the Australian Annona Breeding Program (George et al., 2002). Contrary to our findings, Richardson and Anderson (1996) observed that seed/100 g flesh was not influenced by pollen source in cherimoya. This

Fig. 1. Top: Hand-pollinated 'Arka Sahan' fruits produced from the best pollen treatment, *A. squamosa* (note the large size symmetrical fruits). Bottom: Unpollinated naturally set fruits (note the small size and misshapen fruits).

might be the result of the intraspecific pollen source used by them. Furthermore, the significant year interaction observed in this study for seed number could be attributed to season-sensitive factors governing the seeds/fruit like ovule and pollen fertility besides food reserves in the plant (Gorchov, 1988). Additionally, this trait is also affected by relative humidity through better pollen germination and extended stigma receptivity.

Annona reticulata pollen provided evidence for a xenic effect by producing the heaviest seeds. Likewise, 'Arka Sahan' seeds generated from *A. atemoya* pollen were the lightest in both years. Foreign pollen stimulating production of heavier or lighter seeds has been reported in many crops, including cherimoya (Kahn et al., 1994), mandarins (Bukiya, 1990), faba bean (Duc et al., 2001), and sunflower (Goud et al., 2000). Relatively lighter seeds recorded on the whole in 2003 may be attributed to smaller fruits produced as a result of drought.

Possibly, higher seed number in the fruits emerging from *A. squamosa* pollen could have triggered rapid fruit development leading to faster fruit maturity. It is well documented that developing seeds synthesize hormones (mostly auxin) required for fruit development (e.g., Dag and Mizrahi, 2005), creating a strong sink. Annona fruits have sigmoid growth with a climacteric ripening habit and, once ripe, have a short postharvest life (Batten, 1990; Cautin and Agusti, 2005). The ripening being a cellular process that

Table 3. Pollen profile of edible *Annona* spp. and Arka Sahan.

Pollen source	Pollen type (%)			
	Stained	Unstained	Tetrad	Dyads
<i>A. atemoya</i>	7.4 ^a	4.7 ^a	71.7 ^a	16.2 ^a
<i>A. cherimola</i>	14.1 ^a	56.3 ^b	5.2 ^b	24.5 ^b
<i>A. reticulata</i>	37.3 ^b	28.3 ^c	16.9 ^b	17.6 ^a
<i>A. squamosa</i>	42.9 ^b	5.6 ^a	12.0 ^b	39.5 ^c
Arka Sahan	15.6 ^a	19.8 ^d	33.5 ^c	31.0 ^b

Within columns, means followed by different superscript letters are significantly different at the 0.05 *P* level according to Duncan's multiple range test.

takes place throughout the tissue regardless of mass of fruit should have caused very large-sized fruits derived from *A. squamosa* and small ones from *A. atemoya* requiring the same period for ripening. Furthermore, the greater weight loss of small fruits during ripening could be attributed to relatively larger fruit surface area compared with bigger ones.

Interestingly, TSS was affected by pollen source in the wet year (2004) but not in the dry year (2003) possibly because of the impact of moisture stress on fruit size and hence TSS levels. Kahn et al. (1994) showed that pollen parent did not significantly influence TA levels in cherimoya fruits. However, the significant pollen effect noted for this trait in the present study could be because of the diverse pollen tested by us. TA was significantly affected by the years showing its sensitivity to environmental change. Similarly, González (1991) noted that the values for acidity recorded in Chile were always lower than those recorded in Spain for the same chirimoya germplasm. TSS:TA ratio largely reflects the taste (Saied et al., 2005) and also accentuates other flavors (Sistrunk and More, 1983). Pollen species and year did not significantly change this ratio. Generally, TSS was higher and TA levels were lower in 2003 and vice versa in 2004. Further greater TSS:TA ratio in 2003 was attributable to higher TSS values, which means the fruit pulp was more sweet and less sour during 2003. In the hand-pollinated atemoya fruits in Brazil, Melo et al. (2004) recorded a smaller TSS:TA ratio (37.2 to 49.8), whereas those recorded by us were quite high (81.1 to 150.6). This could be attributed primarily to the pulp quality characteristic of the atemoya variety studied by them. The original genotype of 'Arka Sahan' was selected based on TSS and TA and their blend in the naturally formed fruits. Hand pollination with *A. squamosa* pollen, unlike other pollen sources, did not significantly affect these fruit quality components.

In annona, shield-like or somewhat flat carpelled fruit skin is preferred to bulged carpelled skin (Pinto et al., 2005). A best treatment is the one that would give a weighted mean of 3.00/3.00 if all the sampled fruits were flat carpelled. However, none of the pollen sources influenced to reach a score close to this, and there was no significant pollen effect on carpel morphology. In accordance with our result, Kahn et al. (1994) observed in a study involving two maternal and three paternal cherimoya cultivars that there was no signifi-

cant paternal effect on surface morphology of carpel. However, they reported a significant pollen effect for another observation relating to carpel morphology, i.e., percentage of carpels with protuberances.

Annonaceae is the most primitive angiosperm family (Heywood, 1993; Peña, 2003), and a good proportion of pollen of *Annona* spp. is shed from the mature anthers with tetrad and dyad stages. *Annona squamosa* and *A. reticulata*, the pollen sources that gave good fruit set with more seeds, registered the highest viable pollen grains. Other workers also recorded good pollen viability of 94% (Sahoo et al., 2000) and germination of 80% (Sulikeri et al., 1975) in *A. squamosa*. *Annona cherimola* with abundance of non-viable pollen failed to give good fruit set. Rosell et al. (1999) reported low pollen viability as a cause for poor fruit set in this species. Poor fruit set by pollen of *A. atemoya* and 'Arka Sahan' may be because of higher proportion of tetrads, which have been shown to be incapable of germinating on the stigmatic surface in cherimoya by Saavedra (1977). A higher concentration of tetrads in *A. atemoya* and 'Arka Sahan' pollen may possibly be because of their interspecific constitution. It is concluded that good pollen gives good set and before field testing, screening based on pollen viability could be a useful exercise in identifying potential pollen sources.

Xenic effects. Results obtained in the present study demonstrate significant differential effects of pollen source on seed weight (xenia) and fruit traits like size, symmetry, skin thickness, and TSS (metaxenia). The use of the terms "xenia" and "metaxenia" is confusing. Denney (1992) has thoroughly reviewed the terminology and according to him, the term "xenia" covers all direct pollen effects in seeds and fruits, whether discerned in embryo, endosperm, or maternal tissues. He is also of the view that as "xenia" includes "metaxenia" and use of "metaxenia" is superfluous. The multicarpelled gynoecium of annona flowers contain many ovules (Biale and Barcus, 1970) and require a number of successful fertilization events for fruit set, growth, and development. Each seed is formed by fertilization of an ovule by one pollen grain (Jalipok and Kumar, 1990). The significant effect of *A. reticulata* and *A. atemoya* pollen on seed weight could be clearly regarded as xenia. However, the effect of different *Annona* spp. pollen on fruit tissue was largely because of differential efficacy of the pollen sources to fertilize a

number of ovules and set seeds. Mainly, the pollen profile of the pollen sources (Table 3) affected the number of seeds per fruit, which in turn altered many fruit traits. In several other crops also, seed number in fruit has been shown to influence fruit size, shape, peel thickness, fruit ripening, and so on (Amoros et al., 2003; Bucchieri and di Vaio, 2004; Dag and Mizrahi, 2005; Gorchov, 1988; Ketsa, 1988; Ozga et al., 2002;). Furthermore, xenic effects on seed and fruit characters have been reported in *Annona* previously by Rokba et al. (1982) and Kahn et al., (1994). The indirect significant effects of pollen treatments observed in this study on fruit tissues is simply because of how well a source is capable of pollinating and for which we assign the term "pseudo-xenia." The marked changes in fruit traits resulting from the influence of pollen genotype is "xenia," whereas that resulting from a pollen source through significant change in seeds per fruit could be described as "pseudo-xenia." Useful pseudo-xenic effects merit realization in commercial fruit culture, when feasible.

In conclusion, it can be mentioned that 'Arka Sahan' growers can expect better economic returns by hand pollinating with *A. squamosa* pollen because it gives the best fruit set, size, and symmetry without significantly affecting the fruit quality. Among the other pollen sources tested, *A. reticulata* and 'Arka Sahan' can be rated as good, *A. atemoya* poor, and *A. cherimola* very poor.

Literature Cited

- Amoros, A., P. Zapata, M.T. Pretel, M.A. Botella, and M. Serrano. 2003. Physico-chemical and physiological changes during fruit development and ripening of five loquat (*Eriobotrya japonica* Lindl.). Food Sci. Tech. Intl. 9:43–51.
- Batten, D.J. 1990. Effect of temperature on ripening and post-harvest life of fruit of atemoya (*Annona cherimola* Mill. × *A. squamosa* L.) cv. 'African Pride'. Scientia Hort. 4:129–136.
- Biale, J.B. and D.E. Barcus. 1970. Respiratory patterns in tropical fruits of Amazon Basin. Trop. Sci. 12:93–104.
- Bucchieri, M. and C. di Vaio. 2004. Relationship among seed number, quality and calcium content in apple fruits. J. Plant Nutr. 27:1735–1746.
- Bukiya, Z.M. 1990. Effect of the pollen of different pollinators on seed weight in early mandarins. Subtropicheskie Kul'tury 1:67–71.
- Cautin, R. and M. Agustí. 2005. Phenological growth stages of the cherimoya tree (*Annona cherimola* Mill). Scientia Hort. 105:491–497.
- Cogez, X. and J.P. Lyannaz. 1996. Manual pollination of sugar apple (*Annona squamosa*). Tropical Fruits Nwsl. 19:5–6.
- Dag, A. and Y. Mizrahi. 2005. Effect of pollination method on fruit set and fruit characteristics in vine cactus *Selenicereus megalanthus* ('yellow pitaya'). J. Hort. Sci. Biotechnol. 80: 618–622.
- Denney, J.O. 1992. Xenia includes metaxenia. HortScience 27:722–728.
- Duarte, O. and O. Escobar. 1998. Improving fruit set of cherimoya (*Annona cherimola* Mill.) cv. Cumbe, by autogamous and allogamous hand

- pollination. Proc. Interamerican Soc. for Tropical Hort. 41:162–165.
- Duc, G., A. Moessner, F. Moussy, and C. Mousset Declas. 2001. A xenia effect on number and volume of cotyledon cells and on seed weight in faba bean (*Vicia faba* L.). Euphytica 117:169–174.
- Escobar, T.W., R.D. Zareter, and S.A. Bastida. 1986. Floral biology of artificial pollination of sour sop *Annona muricata* in Canca valley of Colombia. Acta Agron. 36:7–20.
- Gacula, M.C. and J. Singh. 1984. Statistical methods in food and consumer research. Academic Press, New York.
- Gazit, S., I. Galon, and H. Podoler. 1982. The role of nitidulid beetles in natural pollination of Annona in Israel. J. Amer. Soc. Hort. Sci. 107:849–852.
- George, A.P., R.H. Broadley, R.J. Nissen, S.D. Hamill, and R. Drew. 2002. Breeding new varieties of atemoya (*Annona* spp. hybrids). Acta Hort. 575:323–328.
- George, A.P. and R.J. Nissen. 2002. Effects of environment, season and crop load on gas exchange and plant water status of *Annona* spp. hybrid cultivar African Pride. J. Hort. Sci. Biotech. 77:131–136.
- George, A.P., R.J. Nissen, D.A. Ironside, and P. Anderson. 1989. Effects of nitidulid beetles on pollination and fruit set of *Annona* spp. hybrids. Scientia Hort. 39:289–299.
- González, R. 1991. Estudio de material vegetal en el banco de germplasma de Cherimoya de la Estacion Experimental 'La Mayora.' E.U.I.T.A. Sevilla, Espana.
- Gorchov, D.L. 1988. Effects of pollen and resources on seed number and other fitness components in *Amelanchier arborea* (Rosaceae: Maloideae). Amer. J. Bot. 75:1275–1285.
- Goud, S.I., K. Giriraj, and S. Vijayakumar. 2000. Studies on immediate effect of foreign pollen (xenia) on resulting F_0 seed characteristics in sunflower (*Helianthus annuus* L.). Helia 23:59–64.
- Heywood, V.H. 1993. Flowering plants of the world. Oxford Univ. Press, New York.
- Jalilokop, S.H. and P.S. Kumar. 1990. Use of a gene marker to study the mode of pollination in pomegranate (*Punica granatum* L.). J. Hort. Sci. 65:221–223.
- Jalilokop, S.H. and P.S. Kumar. 2000. New fruit varieties for arid regions, pomegranate 'Ruby' and custard apple 'Arka Sahan'. Indian Hort. 45:19–20.
- Kahn, T.L., C.J. Adams, and M.L. Arpaia. 1994. Paternal and maternal effects on fruit and seed characteristics in cherimoya (*Annona cherimola* Mill.). Scientia Hort. 59:11–25.
- Ketsa, S. 1988. Effects of seed number on fruit characteristics of tangerine. Kasetart J. Natural Sci. 22:225–227.
- Khalid, M.Z.M. 1992. Self-compatibility in *Annona muricata* (Linn.) clones. Special Publication Taichung District Agricultural Improvement Station 29:445–448.
- Kumar, P.S. and S.H. Jalilokop. 2000. Cross-compatibility among *Annona* species. Indian J. Hort. 57:309–313.
- Melo, M.R., C.V. Pommer, and R. Kavati. 2004. Natural and artificial pollination of atemoya in Brazil. Acta Hort. 632:125–130.
- Ozga, J.A., R.V. Huizen, and D.M. Reinecke. 2002. Hormone and seed-specific regulation of pea fruit growth. Plant Physiol. 128:1379–1389.
- Peña, J.E. 2003. Pollinating insects of tropical fruits. Manejo Integrado de Plagas y Groecologia. 69:6–20.
- Peña, J.E., H. Nadel, M. Barbosa-Pereira, and D. Smith. 2002. Pollinators and pests of *Annona* species, p. 197–221. In: J.E. Peña, J.L. Sharp, and M. Wysoki (eds.). Tropical fruit pests and pollinators. CAB Intl. Pub., U.K.
- Pinto, A.C. de Q., M.C.R. Cordeiro, S.R.M. de Andrade, F.R. Ferreira, H.A. de C Filgueiras, R.E. Alves, and D.I. Kinpara. 2005. Annona species, International Centre for Underutilized Crops, University of Southampton, Southampton, U.K.
- Pritchard, K.D. and W. Edwards. 2006. Supplementary pollination in the production of custard apple (*Annona* sp.)—The effect of pollen source. J. Hort. Sci. Biotechnol. 81:78–83.
- Richardson, A.C. and P.A. Anderson. 1996. Hand pollination effects on the set and development of cherimoya (*Annona cherimola*) fruit in a humid climate. Scientia Hort. 65:273–281.
- Rokba, A.M., N.H. El-Bakly, and N.E. Solet. 1982. Pollination studies in Annonas and their effects on fruit set, fruit maturity and fruit characters. Faculty of Agriculture, Shoubra El kheima—Cairo, Egypt. Research Bulletin No. 2117:118.
- Rosell, P., M. Herrero, and V. Galan Sauco. 1999. Pollen germination of cherimoya (*Annona cherimola* Mill.). In vivo characterization and optimization of *in vitro* germination. Scientia Hort. 81:251–265.
- Saavedra, E. 1977. Influence of pollen grain stage at the time of hand pollination as a factor on fruit set of cherimoya. HortScience 12: 117–118.
- Sahoo, S.C., J.M. Panda, and D. Mohanty. 2000. A note on pollen morphology, viability, pollination and fruit set in custard apple (*Annona squamosa* L.). Orissa J. Hort. 28:109–110.
- Saied, A.S., A.J. Keutgen, and G. Noga. 2005. The influence of NaCl salinity on growth, yield and fruit quality of strawberry cvs. 'Elsanta' and 'Korona'. Scientia Hort. 103: 289–303.
- Sistrunk, W.A. and J.N. More. 1983. Quality, p. 274–293. In: J.N. Moore and J. Janick (eds.). Methods in fruit breeding. Purdue Univ. Press, West Lafayette, IN.
- Sulikeri, G.S., U.G. Nalawadi, and C.D. Singh. 1975. Pollen viability studies in *Annona squamosa* (L). Current Res. 4:31–32.
- Zar, J.H. 1996. Biostatistical analysis, 3rd ed. Prentice Hall Inc., NJ.