

Pollination Requirements of Pigmented Grapefruit (*Citrus paradisi* Macf.) from Northwestern Argentina

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ABSTRACT

The consequence of a proposed pollinator decline for agriculture is a subject of much ongoing debate. However, pollination requirements of many cultivated plants remain unknown. *Citrus* is complex in terms of pollination needs because of great variation in breeding systems among and within species, and even among and within cultivars. The objective of this study was to evaluate pollinator dependence of three cultivars of grapefruit (*Citrus paradisi* Macf.) planted in northwestern Argentina. Bagged flowering branches were assigned to different pollination treatments: emasculation, spontaneous self-pollination, hand self-pollination, and hand cross-pollination, and the results compared with those from open-pollinated flowers. We counted the number of pollen grains and pollen tubes in the style, fruit set, and seed production. We also assessed differences in germination rates of self- vs. cross-pollen grains. We found that hand- and open-pollinated flowers set about six times more fruit than emasculated and bagged (insect excluded) flowers. In addition, cross-pollen performed better in terms of grain germination and tube growth than self-pollen. Although being fully self-compatible, apomixis and wind pollination are not important factors for grapefruit reproductive success. Thus, insect pollinators represent a critical and potential limiting resource for seedless grapefruits from northwestern Argentina.

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Abbreviations: FAA, formalin–acetic acid–ethyl alcohol.

STUDIES ON CROP pollination by native and nonnative insects are becoming increasingly critical because of a perceived global decline in pollinator stocks, a subject of much current debate with great economical and conservation consequences (Ghazoul, 2005a, 2005b; Steffan-Dewenter et al., 2005). Insect pollination is determinant or beneficial for agriculture since more than 65% of the 1500 cultivated species are pollinated by animals (Roubik, 1995). However, detailed studies of crop pollination systems are incomplete or out of date (Klein et al., 2006). To adequately evaluate the importance of animal pollination for food production and the impact of pollinator losses due to different anthropogenic disturbances, one of the first steps is to determine the pollination needs of different cultivated plants (Klein et al., 2006). Even when some highly productive crops may benefit from insect pollination, generalizations on pollinator requirements are usually based on results obtained from one or a few cultivars (Crane and Walker, 1984; Free, 1993; Roubik, 1995), a practice that can lead to misleading conclusions and mismanagement with great potential economical losses (Steffan-Dewenter et al., 2005; Westerkamp and Gottsberger, 2000).

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The extensively cultivated genus *Citrus*, which includes such economically important crops as orange (*Citrus sinensis*), tangerine (*C. reticulata*), grapefruit (*C. paradisi* Macf.), and lemon (*C. limon*), exhibits tremendous variation in pollination requirements, making generalizations of little value. In *Citrus*, pollinator dependence and breeding systems vary among and within species, and even within cultivars of the same species (Cameron and Frost, 1968; Crane and Walker, 1984; Free, 1993; McGregor, 1976; Roubik, 1995). For instance, whereas 'Clementine' tangerine and some sweet oranges are completely or partially self-incompatible (Cameron and Frost, 1968; McGregor, 1976; Sanford, 1992)—thus requiring insects for pollination—spontaneous self-pollination prevails among 'Eureka' and 'Lisbon' lemons (Webber, 1946). This genus also exhibits large variation in the incidence of parthenocarpy (McGregor, 1976), and thus, the sexual process is not always necessary for fruit development. On the other hand, many cultivars of *Citrus* are known to be self-incompatible and therefore an appropriate supply of cross pollen and pollinating insects are needed for high fruit yields (Sanford, 1992).

In particular, the pollination requirements and breeding system of grapefruit are poorly understood, and the available information is confusing. Some studies state that cross-pollination is not necessary for fruit set (Krezdorn, 1986a; Roubik, 1995), whereas others demonstrate that the transfer of cross-pollen mediated by insects increases fruit production (Burger, 1985b). In some cultivars, most seeds are produced by asexual means, but this information does not necessarily indicate the absence of benefits derived from insect pollination (McGregor, 1976). In 'Marsh' grapefruit, for example, open-pollinated flowers set about twice as many seeds and four times as many fruits as spontaneously, self-pollinated flowers (Wright, 1937, in McGregor, 1976). 'Star Ruby' grapefruit (a seedless deep red, fleshed-fruit cultivar) produced a reduced number of functional pollen grains, and few fruits developed if flowers were not hand-pollinated (Burger, 1985b). Crane and Walker (1984) also stated that pollen transfer by insects increases fruit set in grapefruit.

In Argentina, citriculture is practiced mostly in the northwestern and northeastern regions of the country. In particular, grapefruit was brought from Jamaica to northern Argentina in 1911 (Dansa, 2001), and an increasing number of important cultivars have now been selected and propagated locally from naturally occurring mutations (Dansa, 2001). Feral Africanized honeybees (*Apis mellifera*) and other stingless and solitary bees (e.g., *Trigona argentina* and *Augochloropsis cupreola*) visit grapefruit flowers in northwestern Argentina (Chacoff and Aizen, 2006). However, there is a lack of studies on the reproductive biology and relevance of insect pollinators for fruit production, both for the local cultivars or for others cultivated in the region.

The objective of this study was to assess experimentally the pollination requirements of three pigmented (red seedless) cultivars of grapefruit planted in northwestern Argentina to evaluate the possible effects of insect and cross-pollination on fruit production. Two are regional cultivars of great economic importance. We also examined differences in pollen grain germination and tube attrition of self- vs. cross-pollen, to evaluate whether postpollination events may favor outcrossing.

MATERIALS AND METHODS

The Tree Species

Grapefruit originated in the Caribbean (Gmitter, 1995; León, 2000) by natural hybridization between pummelo (*C. grandis* Osbeck) and sweet orange (*C. sinensis* Osbeck) (Moore, 2001; Nicolosi et al., 2000). Variation among grapefruit cultivars has arisen both naturally and through induced somatic mutations, which were propagated asexually (Gmitter, 1995). Cultivated *Citrus* are mostly shrubs or small trees with dense foliage and flowers, which are either solitary or grouped in small clusters. The conspicuous white corolla is composed of five distinct petals and alternating sepals. The flowers produce a strong scent when mature. The androecium consists of 20 to 40 stamens with partially fused filaments and yellow anthers. A nectariferous disc inside a ring of stamens secretes abundant nectar (Schneider, 1968). A globose, yellowish stigma terminates the style, which connects to a superior ovary containing 8 to 15 fused carpels, each enclosing two rows of ovules. Flowers are simultaneously hermaphroditic, releasing pollen when the stigma is receptive. Flowers open early during the morning and remain receptive until dusk of the following day.

In the study region, grapefruit trees bloom for approximately 45 d, between the end of August (late winter) and the end of September (midspring). Fruits start ripening during the austral fall and can remain on the tree for several months after maturation. Therefore, grapefruit harvest starts in March and continues until mid-August.

We recorded 50 species of insects visiting grapefruit flowers. However, about 95% of the visits were made by the alien feral, Africanized honeybees, with species of stingless (e.g., *T. argentina* and *Tetragonisca angustula*) and solitary bees (*Xylocopa eximia* and *Megachile* sp.) being the next most common visitors. Flowers received an average of 1.4 visits h⁻¹ during daytime (Chacoff and Aizen, 2006).

Study Area

We studied grapefruit cultivars planted in the Upper Bermejo River Basin, near Orán city, Salta province, northwestern Argentina (23°28' S, 64°24' W), in a zone corresponding to the premontane lowland forests of the Yungas (400–600 m above sea level) (Cabrera, 1976). Annual rainfall in this region averages 733 mm and varies between 280 and 1224 mm (Brown et al., 2001). *Citrus* plantations occupy 13500 ha of the Upper Bermejo River Basin, producing mostly grapefruit (57%) and sweet oranges (32%) (Peralta, 1999). Grapefruit from this region represents more than 30% of the Argentine production, and most of it (> 80%) is exported (Dansa, 2001; Federcitrus, 2003).

Six red and very red seedless cultivars (Henninger Ruby, Río Red, Rouge La Toma, Flame, Foster Seedless, and Star Ruby) are the most commonly cultivated (Peralta, 1999).

We studied the pollination requirements of three seedless, pigmented cultivars in four commercial plantations. These cultivars included (i) Rouge La Toma, a very red cultivar that was selected and propagated from a naturally mutated Henninger's Ruby tree at La Toma plantation (Salta Province, northwestern Argentina); (ii) Río Red, a very red cultivar generated in 1963 by irradiation of buds of Ruby Red seedlings in Texas (Gmitter, 1995); and (iii) Foster Seedless, a red cultivar that arose from a natural mutation in a Foster tree at the INTA experimental station at Yuto (Ing. Palacios, personal communication, 2003; Jujuy Province, northwestern Argentina) and is now cultivated widely in the Upper Bermejo River Basin (Peralta, 1999). We selected these cultivars because of their relatively high economic importance and abundance in the study area.

Field Sampling

Pollination Experiments

We studied 6 to 12 trees in each plantation to assess their dependence on cross-pollination and their degree of self-compatibility. For Rouge La Toma, we selected 10 trees in the La Toma plantation during 2000 and 9 trees in the Prodonor plantation during 2001; for Río Red, we studied 12 trees in the Citrusalta plantation during 2001 and 2002. For this last cultivar, six trees were surveyed during 2001; the same trees were used in the next year, except for one tree that did not flower profusely the second year and thus was replaced. We also added six new Río Red trees to our study during 2002. Foster Seedless was represented by six trees in the Peña Colorada plantation during 2002. Trees in each plantation were selected randomly. More details on the plantations and cultivars are provided in Table 1.

On each plant, we enclosed 10 flowering branches with pollination bags made of nylon mesh a few days before flower anthesis. The purpose of the mesh bags was to isolate flowers from the insects but to be permeable to any windborne pollen. Each branch enclosed 8.7 ± 6.6 flowers (mean \pm 1 SE). Following standard breeding system protocols (described in detail in Kearns and Inouye, 1993), each branch was assigned randomly to one of five different manipulative pollination treatments, with two branches per treatment: (Treatment 1) to test for apomixis, we emasculated bagged flower buds by removing anthers with a pair of fine forceps and applied no pollen to the stigmas; (Treatment 2) to test for spontaneous self-pollination, we bagged flowers that were not manipulated in any other way; (Treatment 3) to test for potential geitonogamous pollination and self-compatibility, we hand-pollinated flowers in bagged branches by brushing dehisced anthers from five other flowers

from the same plant; (Treatment 4) to test for allogamous pollination and cross-compatibility, we hand-pollinated flowers in bagged branches with a mixture of pollen from five donor trees from the same plantation and cultivar; and (Treatment 5) to assess natural levels of pollination and reproductive success, we exposed flowers in nonbagged branches to pollinators. Hand-pollinations were performed every day or every other day as flowers dehisced, and pollen was applied to apparently receptive stigma by brushing anthers or using toothpicks, when necessary. Despite the clonal nature of these plantations, isoenzymatic assays showed the existence of sizable amounts of genetic variation within plantations (N. Chacoff et al., unpublished data), probably due to somatic mutations that frequently occur in *Citrus* (Gmitter, 1995; Moore, 2001). Thus, Treatments 3 and 4 were not equivalent and probably involved pollen of different genetic quality.

One week after pollination, we collected styles from flowers in all five treatments just before they started falling naturally to estimate pollen receipt and pollen tube growth. Styles were fixed and stored in individual microcentrifuge tubes containing FAA (formalin-acetic acid-ethyl alcohol, 5:5:90). In the laboratory, styles were cleared in a 10 mL L^{-1} NaOH solution for 48 h and stained with 0.1% aniline blue in $0.1 \text{ mol L}^{-1} \text{ K}_3\text{PO}_4$ (Martin, 1959). Squashed preparations were examined with an epifluorescence microscope at 100 \times . For each style, we counted the number of pollen grains germinating on the stigma and the number of pollen tubes at the base of the style.

We estimated reproductive success from each treatment as final fruit set and seed set. Fruits were collected when ripe, in March, and developed seeds were counted. From these data, we determined fruit set (number of fruits/number of flowers) and seed set (number of seeds fruit⁻¹). Sample sizes for each variable, treatment, and cultivar are indicated in the Appendix.

Pollen Grain Germination Rates

To compare pollen germination rates of self- vs. cross-grains, we enclosed flower buds from branches of the Rouge La Toma cultivar (in La Toma plantation) during the flowering season of 2002. As buds opened, we manually pollinated virgin flowers with either cross- or self-pollen. Cross-pollen came from three different trees within the same plantation and cultivar. Flowers were rebagged after pollination. We collected and fixed styles in FAA at different times after pollination (2, 4, 8, and 24 h). Styles were stained as above and pollen grains that had germinated on the stigma counted with an epifluorescence microscope. In this particular experiment, we could not estimate rates of pollen-tube growth because no pollen tubes reached the base of the style within 24 h. In grapefruit, the period from pollination to fertilization has been reported to be from 2 to

Table 1. Characteristics and location of the commercial grapefruit (*Citrus paradisi* Macf.) plantations studied.

Plantation	Cultivar	Area cultivated with <i>Citrus</i>	Area cultivated with grapefruit	Age of plantations	Locality	Location
		ha	ha	yr		
Citrusalta	Río Red	290	68	10	Colonia Santa Rosa	23°28'15" S 64°24'20" W
12/80	Rouge La Toma	215	133	12–14	Colonia Santa Rosa	23°21'44" S 64°18'18" W
Manero	Rouge La Toma	300	100	20	Colonia Santa Rosa	23°25'33" S 64°26'30" W
Peña Colorada	Foster Seedless	156	70	25	Aguas Blancas	22°47'58" S 64°22'13" W

12 d (Eti and Stosser, 1992; Ngo et al., 2001). In total, 223 styles were collected, which represented a mean of 28 styles **treatment⁻¹** (pollen origin and time combination).

Data Analysis

We analyzed data using two-way mixed model ANOVA with two fixed factors, cultivar and pollination treatment. The tree factor was nested within cultivar and thus considered random. Year was also considered as a random factor (Bennington and Thayne, 1994). We performed orthogonal contrasts to assess significant differences among treatments. The contrasts were (i) emasculation vs. spontaneous self-pollination (Treatments 1 vs. 2), (ii) emasculation and spontaneous self-pollination vs. hand and open pollination (Treatments 1 + 2 vs. 3 + 4 + 5), (iii) hand (self and cross) vs. open pollination (Treatments 3 + 4 vs. 5), and (iv) hand cross- vs. hand self-pollination (Treatments 3 vs. 4) (Table 2).

This ANOVA model was used to analyze the following response variables: (i) number of pollen grains germinating on the stigma, (ii) number of pollen tubes reaching the base of the style, (iii) fruit set (fruit/flower ratio), and (iv) seed set (seeds [fruit ratio⁻¹]). The variable fruit set was transformed using the arcsine transformation ($\arcsin \sqrt{X}$) to correct for normality and homocedasticity (Sokal and Rohlf, 1995). In contrast, seed set, a Poisson-distributed variable, was analyzed with generalized, rather than general linear, models, using the logarithm as the link function and a Poisson error distribution (Littell et al., 1996). Because models were not completely balanced, Satterthwaite's approximation method was used to estimate degrees of freedom and thus to identify the denominator of the *F* tests (Littell et al., 1996). Although we combined results from two different plantations for the Rouge La Toma cultivar, we would have reached the same conclusions if plantation were included as a random effect on the models (results not shown).

Table 2. Results of the two-way ANOVA of the influences of pollination treatment and cultivar on number of germinated pollen grains, number of pollen tubes reaching the bases of styles, fruit set (no. fruits flower⁻¹) and seed set (no. seeds fruit⁻¹). Differences among pollination treatments were assessed performing orthogonal contrasts (Sokal and Rohlf, 1995). The contrasts are (i) emasculation vs. spontaneous self-pollination (Treatments 1 vs. 2), (ii) emasculation and spontaneous self-pollination vs. hand- and open-pollination (1 + 2 vs. 3 + 4 + 5), (iii) hand (self- and cross-) vs. open-pollination (Treatments 3 + 4 vs. 5), and (iv) hand cross- vs. hand self-pollination (Treatments 3 vs. 4).

	Pollen grains		Pollen tubes		Fruit set [†]		Seed set	
	df [†]	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>
Treatment	4, 82.2	36.2****	4, 64.2	30.2****	4, 194	2.5*	4, 24.6	0.3
Cultivar	2, 23.4	0.9	2, 26	4.6*	2, 35.3	0.4	2, 14.3	1.6
Treatment × cultivar	8, 81.4	1.7	8, 62.9	1.9	8, 166	1.1	5, 27.3	0.7
1 vs. 2	1, 79.3	1.6	1, 61.8	0.35	1, 209	0.1		
Treatment 1 + 2 vs. 3 + 4 + 5	1, 82.1	134.3***	1, 64.9	116.4***	1, 200	8.6**		
contrasts 3 + 4 vs. 5	1, 91.5	5.4*	1, 75	0.5	1, 191	1.3	1, 25.2	0.4
3 vs. 4	1, 81.3	1.4	1, 63.8	8.6**	1, 180	0.2	1, 28.9	0.3

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

****Significant at the 0.0001 probability level.

[†]Numerator, denominator degrees of freedom.

[‡]ANOVA performed on transformed data according to arcsine (\sqrt{X}).

We analyzed germination rates of self- vs. cross-pollen also using a general linear mixed model. Here, we considered time since pollination and pollen type (self vs. cross) as crossed fixed factors, and tree and its interactions with time and pollen type as random effects.

All the analyses were performed in SAS. We used the MIXED and GLIMMIX procedures for the general and generalized models, respectively (SAS Institute, 2004).

RESULTS

Flowers of the study cultivars of grapefruit received large pollen loads under natural conditions. Open-pollinated flowers had (mean ± [1 SE]) 56.2 (± 6.9) germinated grains on the stigma and 9.8 (± 1.1) pollen tubes reaching the ovary. Only 21 out of 642 open-pollinated flowers developed fruits; these fruits contained an average of 3.1 (± 0.9) seeds.

Pollination Experiments

Pollen loads on the stigmas differed greatly among pollination treatments, but they did not differ among cultivars (Table 2). Open- and hand-pollinated flowers had on average four times more pollen grains germinating on their stigmas than spontaneously self-pollinated flowers and eight times more than emasculated flowers (Fig. 1a). The number of germinated pollen grains on stigmas of emasculated flowers did not differ from that of self-pollinated flowers (emasculation vs. spontaneous self-pollination contrast, Table 2). Although emasculation was performed immediately before flower anthesis, a few pollen grains had accidentally fallen onto the stigmas when we removed anthers (see Appendix). Stigmas from open-pollinated flowers had 21% fewer germinating pollen grains than

those from hand-pollinated flowers (cross- and self-pollination vs. open-pollination contrast, Table 2), whereas the number of pollen grains found in hand self-pollinated flowers did not differ significantly from the number found in hand cross-pollinated flowers (Fig. 1a). The differences among pollination treatment in number of germinated grains were relatively consistent among cultivars (nonsignificant treatment × cultivar interaction, Table 2; see also Appendix).

Variation in the number of pollen tubes paralleled those of germinating pollen grains, except for hand self-pollinated flowers, which produced 43 and 31% fewer pollen tubes reaching the base of the ovary than cross- and open-pollinated flowers, respectively (Fig. 1b and cross- vs. self-pollination

contrast in Table 2). This result indicates lower tube attrition of cross- than self-pollen. Cultivars differed significantly in the mean number of pollen tubes reaching the base of the style, mainly because twice as many pollen tubes grew in the styles of open- and cross-pollinated flowers of the Rouge La Toma than in those of the other two cultivars (Appendix). However, the general effect of pollination treatment did not differ among cultivars (nonsignificant treatment \times cultivar interaction, Table 2).

Emasculated and spontaneously self-pollinated flowers rarely set fruit ($\sim 0.14\%$), and their fruit set was three to six times lower than those of open- and hand-pollinated flowers (Fig. 1c). Fruit and seed production of hand-pollinated flowers (self- and cross-) did not differ significantly from those of open-pollinated flowers (Fig. 1c–d). Hand-pollination with self- or cross-pollen resulted in equivalent fruit set, despite a trend for cross-pollinated flowers to produce more fruit than self-pollinated flowers (Fig. 1c). Fruit set and seed production did not differ significantly among cultivars, and all cultivars responded similarly to the different pollination treatments (nonsignificant treatment \times cultivar interaction; Table 2 and Appendix). However, open-pollinated flowers from the Río Red cultivar did not produce more fruit than spontaneously self-pollinated flowers (Appendix).

Pollen Grain Germination Rates

Cross-pollen germinated faster than self-pollen. Differences in the number of germinated pollen grains between self- and cross-pollinated flowers developed over time ($F_{3,35.5} = 43.44$, $P < 0.001$, Fig. 2). However, differences between treatments became apparent 24 h after the beginning of the experiment, when we found a significantly larger number of germinated cross- than self-pollen grains ($F_{1,45.3} = 5.99$, $P < 0.05$, Fig. 2).

DISCUSSION

Grapefruit trees produce hermaphroditic flowers and anther dehiscence occurs when the stigma is still receptive. In this study, we found that emasculated and bagged flowers before anthesis were one-sixth less likely to set fruit than open- or hand-pollinated flowers. In addition, the three cultivars had viable pollen that germinates on the stigma and, at least in the two local cultivars, pollen must be transferred by insects for fruit production. Despite limited capacity for spontaneous, within-flower self-pollination and autogamous fruit production, these cultivars were fully self-compatible. The results presented here show that although apomixis and parthenocarpy may be common in *Citrus* (Frost, 1946; Roubik, 1995; Webber, 1946), insect pollination can be critical to maintain high yields.

Pollination, pollen tube growth, fertilization, and subsequent seed development are the steps involved in the sexual reproduction of the vast majority of flowering plants

(Richards, 1986). However, there are species in which fruit development occurs without sexual reproduction, that is, parthenocarpy (Richards, 1986). These fruits, consequently, are seedless or have seeds whose embryos develop apomictically (Gmitter, 1995). For instance, the Red Blush grapefruit cultivar is highly parthenocarpic (Kretdorn, 1986a). However, there are varying degrees of parthenocarpy in *Citrus*, and some parthenocarpic cultivars require at least the stimulus from pollen tube growth or even sexual fertilization and subsequent seed abortion to develop fruit (Kretdorn, 1986a). Although we did not conduct an embryological analysis to determine the origin of the seeds (zygotic or apomictic), we can conclude that animal pollination and pollen tube development are important factors for fruit production in all three study cultivars.

Moreover, the arrival of pollen grains to the stigma is also important for seed formation. "Seedless" *Citrus* cultivars, as the majority of commercial grapefruits, have between zero and six seeds (Palacios, 1978). In some cultivars, seedlessness actually results from pollen sterility (nonfunctional pollen) and/or low levels of ovule fertility. A slow pollen tube growth in those cultivars producing viable pollen is apparently induced by inhibitors in the style, causing pollen tubes to abort before reaching the ovary, thus precluding sexual fertilization (Kretdorn, 1986a). Since high levels of ovule (female) sterility is common in all commercially seedless grapefruits (e.g., Marsh and Red Blush), there is no way to increase seediness appreciably (Kretdorn, 1986b). This may explain why fruits from the different pollination treatments contained a similar low number of seeds (Table 2, Fig. 1d). In addition, improved pollination did not enhance the number of seeds per fruit, even though it increased fruit set.

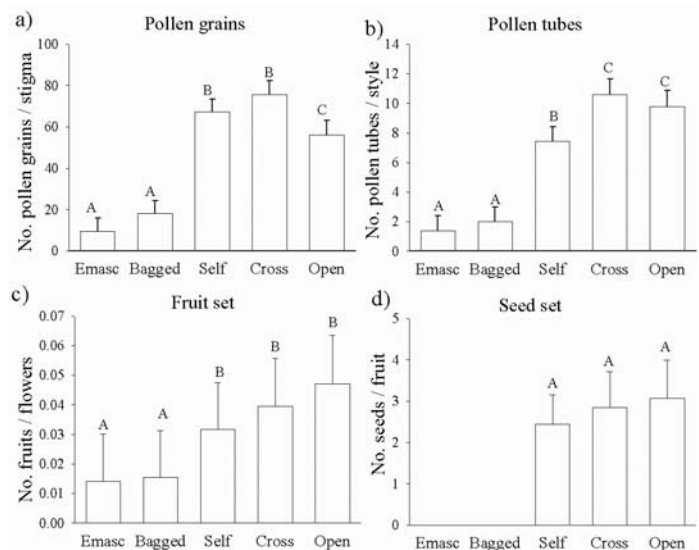


Figure 1. Least-square means of number of pollen grains: (a) pollen tubes, (b) final fruit set, (c) seed set, and (d) (± 1 SE). Different uppercase letters indicate significant differences among treatments ($P < 0.05$) according to the scheme of orthogonal contrasts (see "Data Analysis" and Table 2).

The few pollen grains found on the stigmas of flowers isolated with mesh bags demonstrate a limited capacity for spontaneous self-pollination and suggest that grapefruit pollen cannot be windborne. *Citrus* pollen is heavy and sticky and thus cannot be transported by wind (Kretdorn, 1986a; Sanford, 1992; Schneider, 1968). Therefore, the presence of pollinating insects becomes critical for high fruit yield in the study cultivars. The exception may be Río Red, the nonlocal cultivar, for which levels of fruit set resulting from spontaneous self-pollination did not differ from those of open-pollinated flowers. In this cultivar, animal pollination seems not to be a prerequisite for fruit production.

Hand-pollinated flowers with pollen from the same or other trees of the same cultivar exhibited a slight increase in pollen tube growth and fruit production with respect to open-pollinated flowers, indicating that these grapefruit cultivars may be limited by inadequate pollination. For some grapefruit cultivars, a high proportion of sterile pollen has been reported (Burger, 1982; Frost and Soost, 1968); however, for the cultivars studied here, we observed pollen germinating on the stigma and the development of pollen tubes that reached the base of the style. Our results on pollen germination rates and pollen tube growth show the existence of differences in pollination quality according to pollen origin. We found more cross- than self-pollen germinating 24 h after pollination. Furthermore, more cross-pollen tubes reached the ovary at flower senescence. Higher performance of cross- vs. self-pollen is common in many species (Aizen et al., 1990; Cruzan, 1989; Snow and Spira, 1991). Thus, under natural conditions of mixed pollination, cross-grains could outcompete self-grains in siring seeds and/or eliciting initial ovary swelling and fruit development. Although *Citrus* blooms profusely, mature fruits are produced from a very small percentage of flowers (0.1–3.5% in Bustan and Goldschmidt, 1998). Yet, the presence of abundant insect pollina-

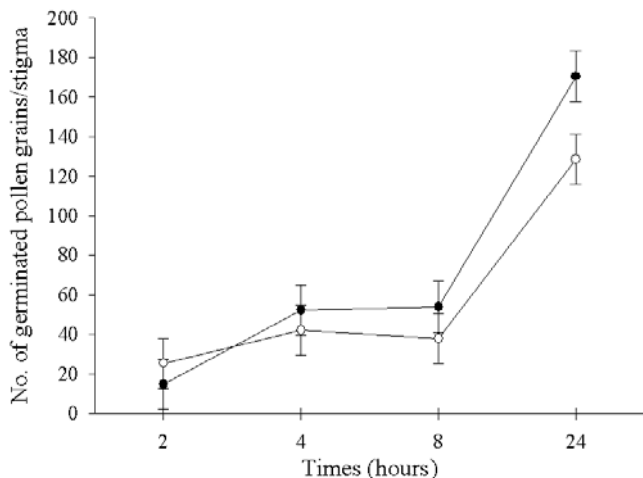


Figure 2. Mean (± 1 SE) number of germinated pollen grains on the stigma over time (hours) after self- and cross-pollinations. Significant statistical differences ($p < 0.05$) for specific times are indicated by the symbol *. Closed circles (●) correspond to cross-pollen; open circles (○) correspond to self-pollen.

tors is essential to guarantee maximum fruit set in our and other cultivars studied (Burger, 1985a; McGregor, 1976). For instance, trees from Star Ruby grapefruit that were exposed to bees inside cages produced more fruit than caged trees not exposed to bees (Burger, 1985a). More generally, fruit production, even in fully self-compatible plant species, may be strongly pollinator-limited (Klein et al., 2003a; Richards, 2001; Roubik, 2002a). Regrettably, most research in *Citrus* has been concentrated in breeding programs aimed at the search of adequate pollen donors in self-incompatible cultivars (Eti and Stosser, 1992; Ngo et al., 2001), or of plants resistant to different nutritional stresses, particularly to N and Mg deficiencies, or insect herbivory (e.g., Argov et al., 1999; Kretdorn, 1986b; Peralta, 1999). Thus, the degree of yield improvement that could be reached by encouraging efficient pollination and improving the pollination environment remains uncertain. Beekeepers usually perceive *Citrus* plantations as good nectar sources for honey production, whereas citriculturists are not much concerned about pollination requirements at all (Free, 1993; Sanford, 1992). In northwestern Argentina, most grapefruit pollination is conducted by feral Africanized honeybees (Chacoff and Aizen, 2006). Although these and other bees provide a valuable service, their abundance depends to some degree on the conservation of nearby forest remnants, where they nest and find nectar, and other resources throughout the year (Allen-Wardell et al., 1998; Kearns and Inouye, 1997; Klein et al., 2002).

The service that pollinators provide to agriculture is increasingly being taken into account in the economic cost structure associated to different crops (Constanza et al., 1997; Kenmore and Krell, 1998; Richards, 2001; Southwick and Southwick, 1992; Westerkamp and Gottsberger, 2000). Recent studies of pollination of coffee (*Coffea arabica*, *C. canephora*, and *C. robusta*) in Indonesia, Costa Rica, Brazil, and Panama reveal the importance of insect pollination and the conservation of the nearby forest for the fruit production of this self-compatible crops (De Marco and Monteiro Coelho, 2004; Klein et al., 2003b; Ricketts et al., 2004; Roubik, 2002b). Studies of native pollinators and the pollination requirements of crops, including some economically important ones, like coffee and *Citrus*, are still being developed. Conclusions drawn from these studies on the pollination requirements of crops could be linked to the conservation of habitat mosaics in which many pollinators thrive. The results reported in this study suggest that an adequate pollination service of these grapefruit cultivars provided by bees may be important to maintain and improve fruit production.

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APPENDIX

Least-square means (standard error) and sample sizes for pollen grains, pollen tubes reaching the ovary, fruit set and seed set for the three study cultivars of grapefruit (*Citrus paradisi* Macf.).

Dependent variable	Emasculated		Spontaneously self-pollinated		Self-pollinated		Cross-pollinated		Open-pollinated	
	Ls [†] (SE)	N ^{‡§}	Ls (SE)	N	Ls (SE)	N	Ls (SE)	N	Ls (SE)	N
Pollen grains										
Río Red	7.74 (8.20)	75	21.50 (8.39)	97	90.53 (8.59)	73	81.56 (8.68)	58	48.82 (8.38)	78
Rouge La Toma	14.67 (10.44)	31	18.57 (10.04)	38	58.61 (9.56)	51	81.91 (11.66)	21	62.26 (10.75)	23
Foster Seedless	5.89 (11.79)	23	14.18 (11.04)	38	52.79 (11.14)	34	63.46 (11.36)	31	57.48 (13.66)	19
Pollen tubes										
Río Red	1.09 (1.26)	75	2.36 (1.25)	97	6.92 (1.30)	73	8.95 (1.34)	58	6.19 (1.28)	78
Rouge La Toma	2.52 (1.65)	31	2.54 (1.56)	38	9.83 (1.48)	51	15.55 (1.87)	21	12.77 (1.75)	23
Foster Seedless	0.54 (1.86)	23	1.12 (1.68)	38	5.54 (1.71)	34	7.35 (1.76)	31	10.40 (2.12)	19
Fruit set [¶]										
Río Red	0.012 (0.019)	34 (247)	0.027 (0.019)	33 (405)	0.037 (0.018)	43 (304)	0.032 (0.019)	33 (195)	0.021 (0.019)	34 (216)
Rouge La Toma	0.014 (0.018)	36 (302)	0.003 (0.018)	37 (557)	0.027 (0.017)	52 (433)	0.051 (0.019)	36 (217)	0.042 (0.018)	37 (367)
Foster Seedless	0.016 (0.030)	10 (75)	0.016 (0.029)	11 (138)	0.032 (0.030)	10 (110)	0.035 (0.029)	11 (88)	0.078 (0.030)	10 (59)
Seed set										
Río Red			1.52 (0.67)	5	1.98 (0.73)	9	2.23 (1.01)	4	2.46 (1.18)	3
Rouge La Toma	3.17 (1.39)	5	3.24 (1.18)	6	2.75 (0.97)	9	2.33 (0.85)	8	4.24 (1.31)	12
Foster Seedless					2.66 (1.38)	3	4.44 (2.26)	2	2.78 (1.36)	3

[†]Ls, least-square means.

[‡]Sample size (N) for pollen grains, pollen tubes and fruit set represents the total number of flowers and for seed set the total number of fruits examined.

[§]The total number of flowers considered for each treatment is also given for fruit set.

[¶]Least-square means and standard errors for each treatment were back transformed following Sokal and Rohlf (1995).

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