

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Flora

journal homepage: [www.elsevier.de/flora](http://www.elsevier.de/flora)



# Fruit characteristics, seed production and pollen tube growth in the wild chilli pepper *Capsicum flexuosum*

Carolina Carrizo García\*

Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), CC 495, CP 5000, Córdoba, Argentina

## ARTICLE INFO

### Article history:

Received 23 February 2010

Accepted 20 May 2010

### Keywords:

*Capsicum flexuosum*

Wild chilli pepper

Seedless fruits

Parthenocarpy

Pollen tube impairment

Empty seeds

## ABSTRACT

Fruits formed after different pollination regimes (flowers hand pollinated, unpollinated, and open pollinated) and the seeds obtained were characterized in the wild chilli pepper *Capsicum flexuosum* Sendtn. Pollen tube development *in vivo* and ovary growth were also analyzed. Seedless fruits and empty seeds were abundant among the fruits from hand pollinated and open pollinated flowers, while no more than one seed with embryo was found in a low percentage of fruits from such pollination treatments. Parthenocarpic fruits were formed from unpollinated flowers. Pollen tube growth was arrested in the upper third of the style for almost all pollen tubes except for a single one that may continue elongating occasionally. The ovary size increased continuously after pollination, even without fertilization. The sum of the evidence registered may help to explain the low number of seeds with embryo harvested, the abundance of seedless fruits formed from pollinated flowers (possibly parthenocarpic), and the high rate of parthenocarpic fruits formed from unpollinated flowers.

© 2010 Elsevier GmbH. All rights reserved.

## Introduction

Sweet and hot chilli peppers (*Capsicum* spp.) are vegetables and spices consumed worldwide. The most important species, with a large number of cultivars, belong to the *Capsicum annuum* complex (*C. annuum* L., *C. chinense* Jacq. and *C. frutescens* L.), and there are two other species (*C. pubescens* Ruiz & Pav. and *C. baccatum* L.) cultivated predominantly in Latin America (Pickersgill, 1997). Given that the fruit is the part of the plant consumed, the processes of pollination and fructification, and the factors that may affect them, have been studied in *C. annuum* (e.g. Aleemullah et al., 2000; De Ruijter et al., 1991; Ercan and Onus, 2003; Heuvelink and Körner, 2001; Kubišová and Háslbachová, 1991; Marcelis and Baan Hofman-Eijer, 1997; Raw, 2000; Roldán Serrano and Guerra-Sanz, 2006). In contrast, these aspects are almost unknown for the other species of the genus, either wild, semi-domesticated or domesticated.

*Capsicum flexuosum* Sendtn. is a wild chilli pepper that grows in shadow sites in forests in the north-eastern extreme of Argentina, central and south-eastern Paraguay, and south-western Brazil (Hunziker, 1998; Jarvis et al., 2005). This species has been considered a rare and threatened crop relative (Jarvis et al., 2005), because plants are difficult to find and their natural environment suffers from anthropogenic destruction (Barboza, pers. comm.; Jarvis et al., 2005). The plants are small shrubs that fructify profusely, producing

red, spherical-depressed berries (Hunziker, 1998; pers. obs.), but viable seeds have been difficult to recover (Jarvis et al., 2005; pers. obs.). A previous study of pollen tube growth in different conditions and regimes of pollination in this species suggested a possible process of generalized pollen tubes arrestment in the style (Carrizo García, 2007), which seems contradictory to the fact that numerous fruits are formed. In cases of compatible pollinations, different forms of failure in the pollen tube growth have been related with low seed sets and also with the development of parthenocarpic fruits. However, these features have been observed in particular mutants (e.g. Mazzucato et al., 2003) and genotypes (e.g. Weiss et al., 1993), or after treatments that impaired pollen functioning (e.g. Mesejo et al., 2006; Rao et al., 1992). In *C. flexuosum*, the pollen tube behavior referred above was observed in natural conditions. Therefore, a series of assays were planned in order to characterize more accurately the production of fruits and seeds in this species, trying to understand better the relationship with pollination and pollen tube growth, and to determine if there is parthenocarpy (meaning the production of fruits without fertilization).

## Materials and methods

### Plant material

Plants of *C. flexuosum* were grown in pots in a greenhouse or in the open field in Córdoba Province (central Argentina), but they came from three populations of Misiones Province (north-eastern Argentina). The eight plants used in this study were either grown

\* Corresponding author. Tel.: +54 351 4331056; fax: +54 351 4332104.  
E-mail address: [ccarrizo@imbiv.unc.edu.ar](mailto:ccarrizo@imbiv.unc.edu.ar)

from seeds obtained in natural populations or directly transplanted from their native location. The peak of flowering occurred in summer (December–March) and the peak of fructification was a little later, reaching the beginning of autumn (February–April/May). The observations were made in the summer seasons 2005/6 and 2006/7.

#### Hand pollinations

The structure of the flower follows the basic pattern described for *Capsicum* (Quagliotti, 1979). The flower lives approx. three days; the corolla (with the stamens) falls down between the second and third day of the flower life, while the gynoecium and the calyx fall four–five days later if a fruit was not set. Hand pollinations were made with pollen from the first day of anthesis (the moment of maximum pollen viability in this species; Carrizo García, 2007) on pistils of the same age, when the stigma was covered with a sticky exudate. Mixed pollen from different donor plants was used to pollinate. Closed anthers from at least five flowers of each plant were detached, put all together into a tube and kept in the open air until the pollen was shed (ca. 2 h later). Anther debris were removed with tweezers and the pollen was mixed. Pollinations were made with small amounts of pollen collected with a toothpick and gently applied on the stigma; the operation was repeated until the stigma was covered with pollen grains. Pollen viability and germinability were tested regularly to favor successful pollinations (viability higher than 80%, germinability over 25%). The fluorochromatic reaction test (Heslop-Harrison and Heslop-Harrison, 1970) was used to assess pollen viability while pollen germinability was tested using the culture medium recommended by Mercado et al. (1994), after 3 h of germination.

#### Production of fruits and seeds

The fruit set was evaluated for two controlled conditions: hand pollinated and unpollinated flowers. Flowers were emasculated when the corolla started to open. Pollen prepared as explained above was used to hand pollinate the emasculated flowers. In the case of the flowers that should remain unpollinated, they were bagged immediately after emasculation. A total of 65 flowers were hand pollinated, while 30 were isolated unpollinated. The fruit/flower ratio was calculated considering ripe fruits formed. Since the berries are spherical-depressed, the longest transversal axis was used to define the fruit sizes, which were categorized in intervals of 1 mm. The fruits were measured and separated accordingly. Histograms were plotted to show the sizes frequencies.

The number of seeds per fruit was counted, and the average number per fruit as well as the seed/ovule ratio were calculated. To this end, the average number of ovules per ovary was calculated over 20 ovaries dissected from recently open flowers. All seeds were blackish, and many of them were considerably flattened. To establish if the seeds had an embryo, they were treated with 50% sodium hypochlorite in water (adapted from Carrizo García, 2002). The treatment turned the seeds translucent, allowing the recognition of the embryo under a stereomicroscope. Two types of seeds were found: with and without embryo. The seeds without embryo were those flattened, always being smaller and papery. These latter will be referred in the following as 'empty seeds'.

A random harvest of 150 fruits was done simultaneously; these fruits produced by open pollinated flowers were analyzed with respect to the already mentioned variables.

#### Pollen tube growth

Pollen tube growth was studied *in vivo* in pollinated flowers. These latter were numbered when the corolla started to open, and immediately emasculated. The pistils were hand pollinated as pre-

viously described. Post-pollination stages (P) were defined every 24 h from the moment of pollination, and were named P1–P7, corresponding with 1 day = 24 h until 7 days = 168 h after pollination. Ten pistils per stage were fixed in FAA and transferred to 70% ethanol after 48 h. The method proposed by Martin (1959), with some modifications, was followed to detect the pollen tubes. Stigmas/styles were washed with water, submerged in 8 N sodium hydroxide for 2 h, washed with water, submerged in 50% sodium hypochlorite for ca. 30 min, washed with water, and finally stained with 0.1% Aniline Blue in water. The stigmas/styles were squashed onto a slide and the pollen tubes were detected under UV light ( $\lambda$  365 nm). Digital photographs were taken to measure the length of pollen tubes. According to the observed pattern of pollen tube growth, the longest one from the population of pollen tubes was measured in each style. The mean and standard deviation were calculated for every stage. Isolated much longer pollen tubes were observed in some cases, which were measured independently.

The ovary growth was also determined from P1 to P7 in the same flowers. The longest transversal diameter of the ovaries was measured. The percentage of increase of the ovary size was assessed according to the average ovary size at the moment of corolla opening, calculated from 20 ovaries measured.

The data of pollen tube length and ovary diameter were statistically analyzed using ANOVA ( $P=0.05$ ). Every possible pair of data was compared following a Tukey test, from P1 to P3 for pollen tubes and from P1 to P7 for ovaries.

## Results

#### Production of fruits and seeds from hand pollinated and unpollinated flowers

Thirty eight fruits were formed from the 65 flowers hand pollinated, which is a fruit/flower ratio of 0.585. The fruit sizes ranged from ca. 3 to ca. 8 mm, distributed in all the intervals defined from  $\leq 3.9$  mm to  $\geq 8$  mm (Fig. 1A). Half of the fruits were in the interval ' $\leq 3.9$  mm' (Fig. 1A), while much lower quantities of fruits were found in all the others (Fig. 1A).

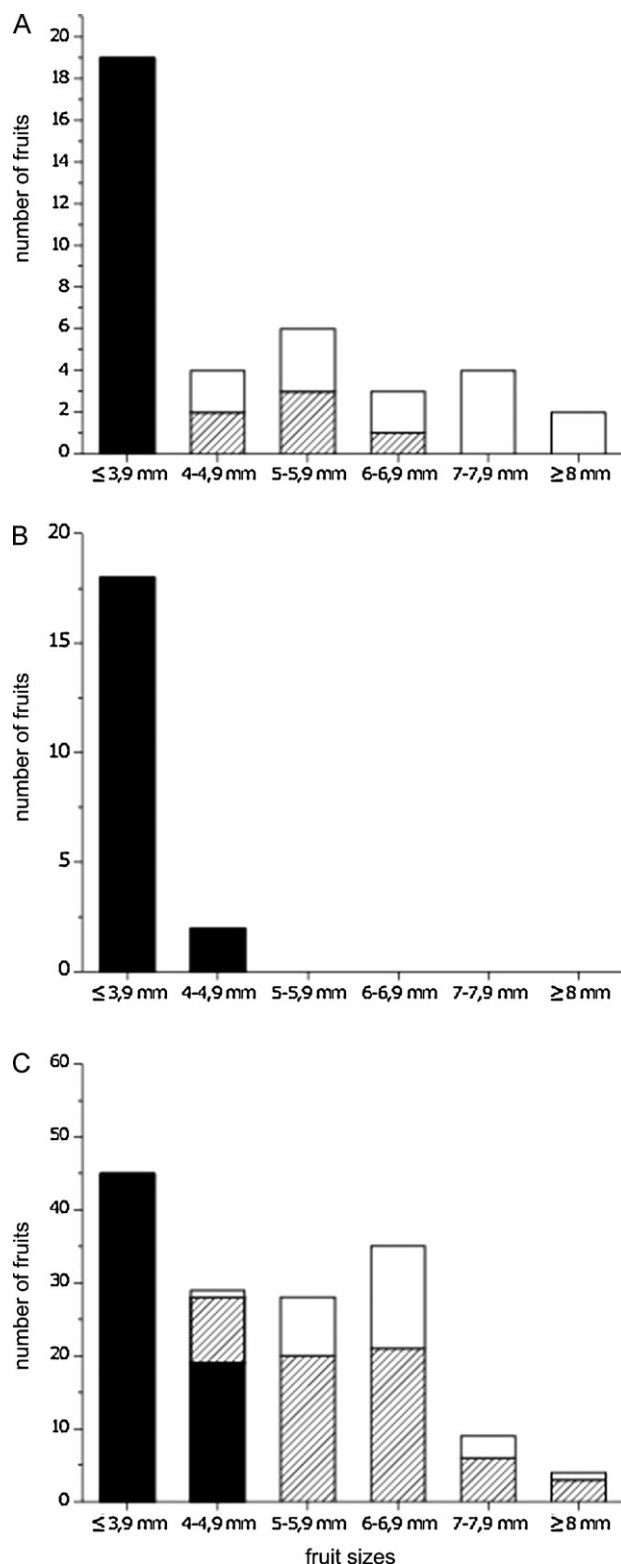
Twenty fruits matured from the 30 unpollinated flowers, which represents a fruit/flower ratio of 0.667. The size of these fruits ranged from ca. 3 to ca. 5 mm, i.e. only the two smallest intervals were found (Fig. 1B). Most of fruits fell in the interval ' $\leq 3.9$  mm' (Fig. 1B), and the remaining in the interval '4–4.9 mm' (Fig. 1B).

In the fruits formed from hand pollinated flowers, all the fruits belonging to the interval ' $\leq 3.9$  mm' were seedless, while all were seeded in the other intervals (Fig. 1A). A total of 85 seeds were harvested. The average of seeds per fruit increased with the increment of the fruit size, from ca. 2 to ca. 11 (Fig. 2A). The general average of seeds per fruit was 2.24. The average of ovules per ovary was  $14.05 \pm 2.40$ , and consequently, the general seed/ovule ratio was 0.16. Among all the seeds harvested, 72 were of the empty type (84.7%), which represented a high proportion of seeds in each size interval of seeded fruits (Fig. 2B). Only empty seeds were found in 6 of the 19 seeded fruits (31.6% of them), which belong to the three smaller intervals of size among the seeded fruits (Fig. 1A). Only 13 of the total (15.3%) were seeds with embryo; the average per fruit was 0.34, and 0.024 the embryo/ovule ratio. In no one of the seeded fruits was more than one seed with embryo.

All fruits formed from unpollinated flowers were seedless (Fig. 1B).

#### Fruits and seeds from open pollination

The fruits randomly collected from open pollinated flowers appeared irregularly distributed among all the size intervals



**Fig. 1.** Fruit and seed production in *C. flexuosum*. The height of the bars represents the total number of fruits in each interval. Within each bar, the amount of seedless (black) and seeded fruits (white background) are discriminated. Among the seeded fruits, those containing only empty seeds are distinguished with diagonal lines. (A) Fruits from hand pollinated flowers. (B) Fruits from unpollinated flowers. (C) Randomly collected fruits from open pollinated flowers.

(Fig. 1C). There were two higher peaks, one in the middle of the range (6–6.9 mm) and the other in the lower extreme (≤3.9 mm), which was the highest (45 fruits, Fig. 1C). The two intervals between both of them included most of the remaining fruits, while a few were found in the two bigger size intervals (Fig. 1C).

All the fruits of the interval '≤ 3.9 mm' were seedless, while more than a half were seedless in the interval '4–4.9 mm' (Fig. 1C). The average number of seeds per fruit ranged from less than 1 to ca. 5 according to the increasing fruit sizes (Fig. 2C). The general average of seeds per fruit was 0.93, and the seed/ovule ratio was 0.066. A total of 140 seeds were harvested, 110 of them belonged to the empty type (78.6%). High numbers of empty seeds were found in fruit of all sizes (Fig. 2D). More than the half of the seeded fruits (66.3%) contained only empty seeds, representing a high proportion in each size interval where seeded fruits were recorded (Fig. 1C). 30 seeds of the total (21.4%), i.e. 0.20 per fruit, contained an embryo; this represents embryo/ovule ratio of 0.014. No one seeded fruit had more than one seed with embryo.

#### *In vivo pollen tube growth*

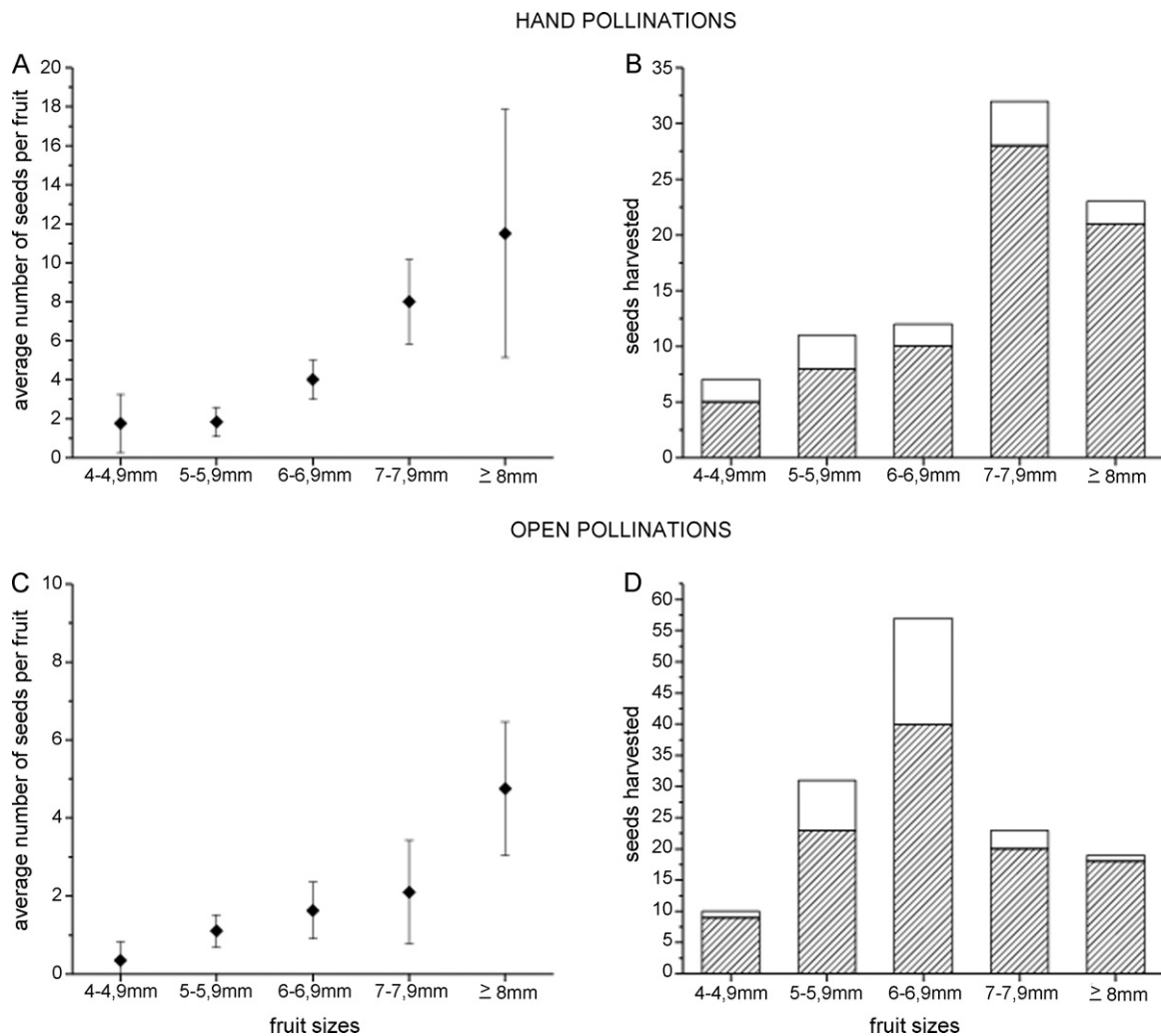
In general, pollen tubes grew almost all together and they extended through approximately the apical third of the style at P1 (Fig. 3A). Isolated pollen tubes grew beyond that zone only in some pistils analyzed, elongating over 40–50% of the style length (Fig. 3A). Nevertheless, this phenomenon was infrequent (see below). Almost the entire population of pollen tubes lengthened to a maximum at P2 (Fig. 3B); pollen tube growth was apparently stopped around this stage. Shorter pollen tubes were seen from the stages P3 and P4 (Fig. 3B), being only fragmented pollen tubes in most cases of P4 (Fig. 3C). However, there were no significant differences between any stages from P1 to P3. From P3 to P4 onwards, pollen tubes appeared twisted, sometimes with thicker callose walls (Fig. 3C). At the same time, the stylar tissues began to become necrotic from the basal end upwards. At the stages P6 and P7 only fragments of the pollen tubes were detectable in the apical end of the style, while most of the style length had a necrotic appearance (dark colored and shrunken).

Isolated longer pollen tubes were measured separately from the rest; it was always a single pollen tube per style whose length difference with the rest was conspicuous (Fig. 3A). So, it did not represent the general behavior of most pollen tubes. In only two pistils fixed at P1, a single pollen tube reached the base of the style and entered the ovary. Excluding these cases, among the 70 pistils analyzed from P1 to P7, only 9 isolated pollen tubes reached the basal third of the style, all of them found at the stages P1–P3.

#### *Ovary size and pollen tube development*

The mean transversal diameter of the ovary was  $1.26 \pm 0.10$  mm (Fig. 4A). The ovaries started to enlarge from P1 (Fig. 4), even though the size of some ovaries at this stage can fall in the range of those measured at the moment of corolla opening (e.g. a diameter of 1.30 mm). The average diameter of the ovary increased almost continuously until P7, with an increasing level of variation (Fig. 4). Only the average diameter of the ovary at P7 was significantly different from all the other stages. A substantial ovary growth was observed at P7, in which the ovaries' sizes overlap with the range of sizes recorded for the mature fruits, although with the smallest (e.g. 3.5 mm; Fig. 4). Relating the length of pollen tubes and the ovary diameter in each pistil, it was observed that the ovaries started to enlarge even though the pollen tubes were arrested, and yet degenerated, in the apical part of the style (i.e. fertilization did not occurred).





**Fig. 2.** Seed production in *C. flexuosum*. (A, C) Average number of seeds per fruit according to the fruit sizes. (B, D) Total seeds harvested distributed among the fruits sizes. The height of the bars represents the total amount of seeds in each size interval, and the empty seeds are distinguished with diagonal lines within each bar (A, B: Fruits from hand pollinated flowers; C, D: randomly collected fruits from open pollinated flowers).

## Discussion

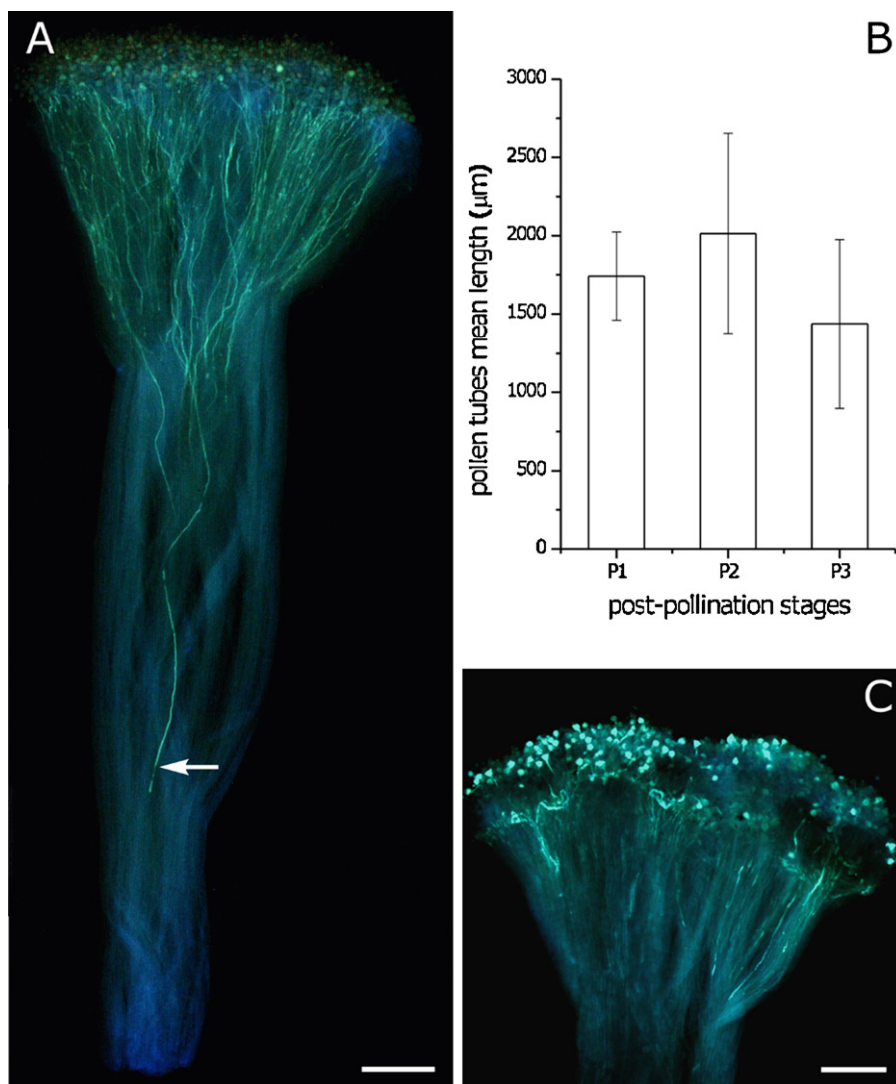
### Fruit characteristics and seed production

The range of fruit sizes in *C. flexuosum* is wide, considering that the biggest can have twice the diameter size of the smallest fruits. The number of seeds per fruit was also a highly variable feature, particularly among those obtained from hand pollinated flowers, and seedless fruits were abundant. The number of seeds per fruit increased in parallel to the fruit size in both, the fruits from open pollination and those from hand pollinated flowers. This trend was also observed in some cultivated peppers (Aleemullah et al., 2000; Ercan and Onus, 2003; Marcelis and Baan Hofman-Eijer, 1997; Roldán Serrano and Guerra-Sanz, 2006), even though the fruit sizes and the number of seeds were much bigger in those cultivars. However, the increase in the number of seeds per fruit in *C. flexuosum* was due to the presence of more empty seeds, since no one fruit had more than one seed with embryo. Therefore, higher seed numbers do not represent a reproductive advantage.

There were two interesting differences between the fruits from hand pollinated flowers and those from open pollination. On the one hand, seedless fruits were present only in the interval '≤3.9mm' among the fruits from hand pollinated flowers, while they were found in the intervals '≤3.9mm' and '4–4.9mm' in the

case of fruits from open pollination. On the other hand, the average number of seeds per fruit was higher in fruits from hand pollinated flowers, considering all the fruits together and also discriminated by sizes. Intuitively these features, in particular the numbers of seeds per fruit, may point the attention on the pollen load on the stigma (as for the pollen quantity and the eventual development of a proportional number of pollen tubes), because it is likely that it could be different between the open pollinations and the hand pollinations made. Actually, bigger fruits and more seeds were produced in *C. annuum* when higher pollen loads were used (Marcelis and Baan Hofman-Eijer, 1997; Roldán Serrano and Guerra-Sanz, 2006). However, considering the general behavior of the pollen tubes, the fact that in just a few cases only a single pollen tube could have achieved fertilization, and that most seeds formed were empty, the number of seeds per fruit does not seem related with the amount of pollen used to pollinate. Nevertheless, it can be wondered if a bigger pollen load could enhance the probability of the development of those longer pollen tubes that eventually fertilize.

Although the number of seeds per fruits is usually low in the wild species of *Capsicum* (Barboza, pers. comm.; Barboza and De Bem Bianchetti, 2005), the general average values registered in *C. flexuosum* were markedly low. The high number of seedless fruits has negatively affected these values. These averages are even lower if only the seeds with embryo are considered, which can be rec-



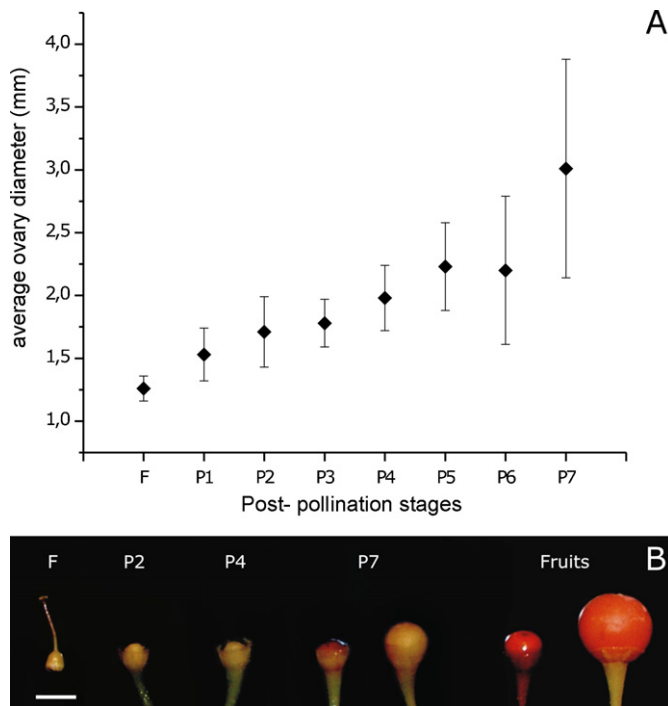
**Fig. 3.** *In vivo* pollen tube growth in *C. flexuosum*. (A) At the stage P1 (24 h after pollination); note a single faster pollen tube (arrow). (B) Mean lengths and standard deviations of pollen tubes at successive post-pollination stages (P1–P3, defined every 24 h). (C) At the stage P4 (96 h after pollination); observe the segmented and twisted pollen tubes restricted to the apical part of the style. Scale bars = 250 μm.

ognized as the 'true seeds', with the potentiality to continue the species. Under this regard the difficulty mentioned by Jarvis et al. (2005) to obtain viable seeds can be understood. This underlines the status of *C. flexuosum* as a threatened species, as it was mentioned by these authors.

#### Pattern of pollen tube growth

The growth of most pollen tubes seemed to be stopped at some moment around 48 h after pollination. It seems that the pollen tubes degenerate after they have stopped growing. It can be stated that the performance of the pollen tubes in *C. flexuosum* is poor with respect to their ability to arrive at the ovary and fertilize the ovules, because they collectively fail, except for sporadic, isolated pollen tubes. This pattern appears to be a regular feature in this species. The direct consequence may be the scarce number of seeds with embryo found among the fruits dissected, even if this may not be the only cause. Some of those isolated pollen tubes that could grow beyond the group of arrested pollen tubes should be able to successfully fertilize. This is consistent with the formation of not more than one seed with embryo per fruit (when formed), at least in the conditions that were given here.

The causes for the pollen tube arrestment and later degeneration are not clear. Though a possible occurrence of incompatibility in *C. flexuosum* was suggested (Jarvis et al., 2005), it was assumed that the species was self-compatible, as are other wild species of the genus [except *Capsicum cardenasii* Heiser & Smith (Onus and Pickersgill, 2004) and *C. pubescens* (Saborío and Da Costa, 1992)], and self-pollinating, as are the cultivated peppers (Bosland and Votava, 2000). This assumption resulted from two previous observations. First, according to a preliminary study, there were no significant differences in the mean pollen tubes lengths until 48 h after pollination between self-pollinated, cross-pollinated pistils, and those pollinated with mixed pollen from several plants (Carrizo García, 2007). Second, plants kept isolated were able to set fruits with embryo-containing seeds that could germinate (pers. obs.). If an incompatibility reaction were taking place in the present pollination investigations, compatible pollen tubes must have continued growing, unlikely only one in just a few cases, since mixed pollen from different donor plants was used. An incompatible reaction does not seem likely because the arrestment of the pollen tube growth occurred evenly for self and foreign pollen. On this regard, it could be suggested that the pistilar support for the pollen tubes may not be unrestricted in compatible pollinations. A reduction in the number of pollen tubes growing along the style in compatible



**Fig. 4.** Ovary size and post-pollination growth in *C. flexuosum*. (A) Average ovary diameter from the flower (F) to different post-pollination stages (P1–P7, defined every 24 h). (B) Ovaries at flowering (F, the entire pistil is shown) and several post-pollination stages (P), and fruits from contrasting sizes. In none ovary shown there was fertilization. Observe that the ovary size at P7 can overlap with the range of the fruit sizes. Scale bar = 3 mm.

pollinations has been reported for many species [see Hormaza and Herrero (1996) for examples and a discussion on the subject]. The relevance of the female factor on this phenomenon and a possible male selection has been supported (Hormaza and Herrero, 1996). This could be the case also in *C. flexuosum*, in which the style may produce a high selective pressure on the developing pollen tubes, and in consequence only sporadically a single pollen tube per style can reach the ovary and fertilize. However, the possibility also of a male control of the pollen tube growth cannot be disregarded until accurate tests are performed.

In relation to the female part, a possible negative effect on the developing pollen tubes induced by the growing ovaries may also be discarded. This is because the ovaries did not grow in pollinated flowers cut and kept in water for 7 days (pers. obs.), as it did happen in the flowers analyzed here, while the pattern of pollen tube growth and arrestment was similar in both cases (Carrizo García, 2007). The increase of the ovary size registered in the first week after pollination, while the pollen tubes have not reached the ovary (i.e. there was no fertilization), is an interesting phenomenon. The rapid growth of the ovary is in part responsible for the development of parthenocarpic fruits in a tomato variety (Lin et al., 1983). Taking into account the general trend of pollen tube arrestment, the ovary growth without fertilization, and the high percentage of seedless fruits obtained from hand pollinated flowers, it can be speculated about a possible parthenocarpic origin of the seedless fruits obtained in this case.

#### Parthenocarpic fruits

Parthenocarpic fruits were developed from unpollinated, isolated flowers, in a fruit/flower rate of 0.67, which evidences that this type of fruit is not uncommon. Indeed, this rate was higher than the

rate of fruits formed from hand pollinated flowers. The spontaneous formation of parthenocarpic fruits differs from other cases recorded in cultivated *Capsicum*, since this phenomenon usually had to be induced in different ways. Parthenocarpic fruits were developed in genetically controlled plants or crosses (Curtis and Scarchuk, 1948; Malhova, 1977; Pathak et al., 1983; Shifriss and Eidelman, 1986), by the application of hormones or chemicals (Bisaria and Prakash, 1978; Heuvelink and Körner, 2001; Jayakaran, 1973; Wong, 1941), or after treatments with low temperatures (Polowick and Sawhney, 1985; Rylski, 1973). In the case of *C. flexuosum*, parthenocarpic and seed-containing fruits appeared at the same time in all the studied plants, at any moment of the period of fructification, and the formation of parthenocarpic fruits could not be related strictly to a particular genotype or an environmental condition. Regarding the fruit quality, in other such cases in *Capsicum* the parthenocarpic fruits were usually smaller than the seeded fruits (Pathak et al., 1983; Polowick and Sawhney, 1985), and in general they had deformations (Bosland and Votava, 2000). The parthenocarpic fruits in *C. flexuosum* were also smaller, but they did not show signs of deformation, and they were even histologically similar to those with seeds (pers. obs.).

It was previously hypothesized that the seedless fruits obtained from hand pollinated flowers could also be parthenocarpic. Therefore, if all the seedless fruits were parthenocarpic, no matter the pollination regime, the amount of this type of fruit registered here would evidence its relevance in the total fruit production of *C. flexuosum*.

#### Conclusions

The fruit characteristics and the seed production in *C. flexuosum* show some interesting particularities, which may be related, at least in part, with the presence of pollination and/or pollen tube development. The features registered have some important implications. On the one hand, a possible cause for the low proportion of seeds with embryos was found, the arrested elongation growth of pollen tubes. This can be taken as an embryological peculiarity of interest even beyond the specific flower biology of *Capsicum*. If formation of viable seeds is pursued, some aspects still deserve further attention (e.g. the performance of different plants as male donors or female receptors). On the other hand, the observations direct the attention to a trait that could be of interest for *Capsicum* breeding, the occurrence of spontaneous parthenocarpy as an apparently regular phenomenon.

#### Acknowledgements

The author thanks R. Carrizo and I. García for their assistance with the field work, Drs. J. Daviña and M. Grabele for their help to obtain seeds and plants from natural populations, and Dr. G. Barboza for the constant support and for sharing her knowledge on *Capsicum* species. This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Fondo para la Investigación Científica y Tecnológica (FONCYT).

#### References

- Aleemullah, M., Haigh, A.M., Holford, P., 2000. Anthesis, anther dehiscence, pistil receptivity and fruit development in the Longum group of *Capsicum annum*. Aust. J. Exp. Agric. 40, 755–762.
- Barboza, G.E., De Bem Bianchetti, L., 2005. Three new species of *Capsicum* (Solanaceae) and a key to the wild species from Brazil. Syst. Bot. 30, 863–871.
- Bisaria, A.K., Prakash, U., 1978. Growth, sex expression, pollen germination and yield in pepper as affected by chlorflurenol. Sci. Hort. 9, 15–20.
- Bosland, P.W., Votava, E.J., 2000. Peppers: Vegetable and Spice Capsicums. CABI Publishing, Oxon.
- Carrizo García, C., 2002. An approach to the diversity of endothelial thickenings in Solanaceae. Flora 197, 214–223.

- Carrizo García, C., 2007. Crecimiento de los tubos polínicos de *Capsicum flexuosum*. Bol. Soc. Argent. Bot. 42 (Suppl.), 39.
- Curtis, L.C., Scarchuk, J., 1948. Seedless peppers: a single Mendelian recessive character. J. Hered. 39, 692–694.
- De Ruijter, A., van den Eijnde, J., van der Steen, J., 1991. Pollination of sweet pepper (*Capsicum annuum* L.) in greenhouses by honeybees. Acta Hort. 288, 623–636.
- Ercan, N., Onus, A.N., 2003. The effects of bumblebees (*Bombus terrestris* L.) on fruit quality and yield of pepper (*Capsicum annuum* L.) grown in an unheated greenhouse. Isr. J. Plant Sci. 51, 275–283.
- Heslop-Harrison, J.S., Heslop-Harrison, Y., 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. Stain Technol. 45, 115–120.
- Heuvelink, E., Körner, O., 2001. Parthenocarpic fruit growth reduces yield fluctuation and blossom-end rot in sweet pepper. Ann. Bot. 88, 69–74.
- Hormaza, J.I., Herrero, M., 1996. Dynamics of pollen tube growth under different competition regimes. Sex. Plant Reprod. 9, 153–160.
- Hunziker, A.T., 1998. Estudios sobre Solanaceae. XLVI. Los ajíes silvestres de Argentina (*Capsicum*). Darwiniana 36, 201–203.
- Jarvis, A., Williams, K., Williams, D., Guarino, L., Caballero, P.J., Mottram, G., 2005. Use of GIS for optimizing a collecting mission for a rare wild pepper (*Capsicum flexuosum* Sendtn.) in Paraguay. Genet. Resour. Crop Evol. 52, 671–682.
- Jayakaran, M., 1973. Parthenocarpic fruit development in *Capsicum* by morphactin. Sci. Cult. 39, 188–189.
- Kubišová, S., Háslbachová, H., 1991. Pollination of male-sterile green pepper line (*Capsicum annuum* L.) by honeybees. Acta Hort. 288, 364–370.
- Lin, S., Splittstoesser, S.E., George, W.L., 1983. Factors controlling the expression of parthenocarpy in 'severianin' tomato. Sci. Hort. 19, 45–53.
- Malhova, E., 1977. Cytoembryology du genre *Capsicum*. Eucarpia Capsicum 77, 191–197.
- Marcelis, L.F.M., Baan Hofman-Eijer, L.R., 1997. Effect of seed number on competition and dominance among fruits in *Capsicum annuum* L. Ann. Bot. 79, 687–693.
- Martin, F.W., 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol. 34, 125–128.
- Mazzucato, A., Olimpieri, I., Ciampolini, F., Cresti, M., Soressi, G.P., 2003. A defective pollen–pistil interaction contributes to hamper seed set in the *parthenocarpic* fruit tomato mutant. Sex. Plant Reprod. 16, 157–164.
- Mercado, J.A., Fernández-Muñoz, R., Quesada, M.A., 1994. In vitro germination of pepper pollen in liquid medium. Sci. Hort. 57, 273–281.
- Mesejo, C., Martínez-Fuentes, A., Reig, C., Rivas, F., Agustí, M., 2006. The inhibitory effect of CuSO<sub>4</sub> on *Citrus* pollen germination and pollen tube growth and its application for the production of seedless fruit. Plant Sci. 170, 37–43.
- Onus, A.N., Pickersgill, B., 2004. Unilateral incompatibility in *Capsicum* (Solanaceae): occurrence and taxonomic distribution. Ann. Bot. 94, 289–295.
- Pathak, C.S., Singh, D.P., Lleshpande, A.A., 1983. Parthenocarpy in chillies (*Capsicum annuum* L.). Capsicum Newsl. 2, 102–103.
- Pickersgill, B., 1997. Genetic resources and breeding of *Capsicum* spp. Euphytica 96, 129–133.
- Polowick, P.L., Sawhney, V.K., 1985. Temperature effects on male fertility and flower and fruit development in *Capsicum annuum* L. Sci. Hort. 25, 117–127.
- Quagliotti, L., 1979. Floral ecology of *Capsicum* and *Solanum melongena*. Linn. Soc. Symp. Ser. 7, 399–419.
- Rao, G.U., Jain, A., Shivanna, K.R., 1992. Effects of high temperature stress on *Brassica* pollen: viability, germination and ability to set fruits and seeds. Ann. Bot. 69, 193–198.
- Raw, A., 2000. Foraging behaviour of wild bees at hot pepper flowers (*Capsicum annuum*) and its possible influence on cross pollination. Ann. Bot. 85, 487–492.
- Roldán Serrano, A., Guerra-Sanz, J.M., 2006. Quality fruit improvement in sweet pepper culture by bumblebee pollination. Sci. Hort. 110, 160–166.
- Rylski, I., 1973. Effect of night temperature on shape and size of sweet pepper. J. Am. Soc. Hort. Sci. 98, 149–152.
- Saborío, M., Da Costa, C.P., 1992. Autoincompatibilidad en *Capsicum pubescens*. Agron. Costarric. 16, 279–286.
- Shifriss, C., Eidelman, E., 1986. An approach to parthenocarpy in peppers. HortScience 21, 1458–1459.
- Weiss, J., Nerd, A., Mizrahi, Y., 1993. Vegetative parthenocarpy in the cactus pear *Opuntia ficus-indica* (L.) Mill. Ann. Bot. 72, 521–526.
- Wong, C.Y., 1941. Chemically induced parthenocarpy in certain horticultural plants, with species reference to the watermelon. Bot. Gaz. 103, 64–86.