

## Effects of natural and artificial pollination on fruit and offspring quality

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### Abstract

Worldwide, many crops rely on insect pollination. Insufficient pollination can reduce fruit and seed set by directly reducing pollen deposition, and can also affect offspring quality, such as growth rate and resistance to herbivores, by limiting outcrossing opportunities. Both effects are important in fruit agroecosystems where fruit size and the quality of seeds for re-planting are dependent on sufficient pollination. We experimentally manipulated pollination of the cape gooseberry, *Physalis peruviana* L. (Solanaceae), to test the effects of honey and bumble bee pollination compared to manual outcrossing and autonomous self-pollination on fruit and offspring characteristics. Compared to manual and self-pollination, bee pollination increased fruit size, seed set and germination rates, supporting the hypothesis that sufficient pollination increases plant fitness. Interestingly, plant growth rate and herbivore resistance were significantly and marginally greater in manually outcrossed plants compared to self-pollinated offspring, suggesting that inbreeding reduces offspring quality. Herbivore resistance and plant growth did not differ between one honeybee visit and self-pollination suggesting that multiple pollinator visits are needed to prevent inbreeding events. Our data suggest that the quantity and quality of pollen deposited by bee visitation can significantly alter ecologically and economically relevant traits in this agroecosystem.

### Zusammenfassung

Verschiedene Nutzpflanzen weltweit brauchen Insektenbestäubung, um hohe Erträge zu erzielen. Ungenügende Bestäubung kann, vermittelt durch eine geringe Pollenablage, die Grösse von Früchten und Samen reduzieren oder die Qualität der Nachkommen durch Inzuchtdepression beeinflussen. Beide Effekte sind wichtig in Obst-Agrarökosystemen, wo Fruchtgröße und die Qualität der Samen für die Wiederbepflanzung eine grosse Rolle spielen und von ausreichender Bestäubung abhängig sind. Wir haben experimentell die Bestäubung von *Physalis*-Pflanzen (*Physalis peruviana*) manipuliert, um die Auswirkungen von Honigbienen- und Hummel-Bestäubung im Vergleich zu manueller Fremdbestäubung und Selbstbestäubung auf Frucht- und Nachkommeneigenschaften zu testen. Im Vergleich zur manuellen und Selbstbestäubung, erhöhte Bienenbestäubung sowohl Samenansatz und Fruchtgröße, als auch die Keimungsrate. Unsere Ergebnisse unterstützen die Hypothese, dass Insektenbestäubung die Fitness der Pflanze erhöht und auch von Interesse für die Produzenten von *Physalis* sein sollte. Beachtlich war, dass Nachkommen von manuell fremdbestäubten Früchten eine marginal höhere Resistenz gegen Herbivorie aufwiesen

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als die von selbstbestäubten Früchten, was darauf hindeutet, dass Inzucht-Effekte die Qualität der Nachkommen negative beeinflussen können. Unsere Daten legen nahe, dass die Anzahl und die Qualität von Pollen, die von Bestäubern übertragen werden, ökologisch und ökonomisch relevante Eigenschaften in diesem Agrarökosystem beeinflussen können.

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## Introduction

Pollinators have been shown to be important for the production of more than 70% of 1330 tropical crops (Roubik 1995). While studies document the effects of insect pollinators' abundance on productivity (Morandin & Winston 2005; Klein et al. 2007; Klein 2009), there are few studies that have experimentally manipulated pollination and examined the consequences for offspring quality, specifically offspring resistance to herbivores. From an ecological point of view, pollinators can alter fruit, seed, and seedling quality by changing the quantity and/or quality of pollen grains deposited on the stigma (Mulcahy & Mulcahy 1987). Large pollen loads increase seed production (Bertin 1990; Richardson & Stephenson 1992; Németh & Smith-Huerta Nancy 2003), and fruit production (Bertin 1990; Brown & Kephart 1999), and may also influence traits related to offspring quality, including germination rate (Richardson & Stephenson 1992; Jóhannsson & Stephenson 1997; Brown & Kephart 1999), seedling biomass (Kalla & Ashman 2002), and plant phenology (Richardson & Stephenson 1992). The benefits of increased pollination can be offset in cases when resources limit a plant's ability to develop seeds, and increased seed set comes at the expense of individual seed quality (Wolf, Hainsworth, Mercier, & Benjamin 1986; Jakobsson & Eriksson 2000). However, for most crop species, the actual benefits of insect pollination remain untested.

Pollen quality (e.g., pollen source) can also affect fruit set, seed set, and offspring characteristics (Mulcahy & Mulcahy 1987; Bertin 1990; Winsor, Peretz, & Stephenson 2000). For example, deposition of self-pollen or pollen from relatives can lead to inbred progeny that experience inbreeding depression of fitness traits, including germination rate, growth and survival, in comparison to outcrossed offspring (Schemske 1983; Lande & Schemske 1985; Husband & Schemske 1996). In addition to effects on growth and reproduction, inbreeding may also affect ecological interactions with herbivores (Ivey, Carr, & Eubanks 2004; Delphia, De Moraes, Stephenson, & Mescher 2009; Bello-Bedoy & Núñez-Farfán 2010) by altering plant secondary metabolism (Campbell et al. unpublished data). Inbreeding can also reduce flower or inflorescence size, which can in turn reduce pollination in inbred progeny (Husband & Schemske 1996). As a result of physiological and ecological inbreeding depression, many plant species possess mechanisms for discriminating against self-pollen and reducing investment in seeds (and hence fruits) of lower future fitness value (de Nettancourt 1997; Takayama & Isogai 2005). Thus, inadequate deposition of

unrelated pollen can also have immediate, negative effects on fruit and seed production. The complex interaction between pollination services, mating system, and ecological interactions has considerable importance for many natural systems, yet few studies have explicitly examined the consequences of pollinator variation for plant offspring quantity and quality (Wolf et al. 1986). These interactions are also relevant in agricultural systems, where reduced pollination can lower marketable yield by reducing fruit size and number. Moreover, in crop species where a portion of the crop is withheld for re-planting, pollination may affect subsequent crops by altering offspring quality, particularly if this extended to interactions with pest species. However, no study has examined the consequences of crop pollination across generations.

The purpose of this study was to elucidate the diverse effects of pollination variation on fruit characteristics and offspring quality in a cultivated, wild fruit crop, cape gooseberry (*Physalis peruviana*). *P. peruviana* is an excellent model system for both ecological and agronomic studies of pollination. It is a self-compatible plant, but may nevertheless benefit from pollinator visitation, as has been demonstrated for other self-compatible crops (Klein, Steffan-Dewenter, & Tschardt 2003; Aldana, Cure, Almanza, Vecil, & Rodríguez 2007; Kodad & Socias 2008). In cape gooseberry fields, the presence of potential pollinators such as *Bombus* spp. *Xylocopa* spp. and *Apis mellifera* has been reported (Rodríguez 2006) but their importance for fruit production remains unknown. Moreover, farmers currently retain a portion of their crop for replanting purposes. Limited pollination services could negatively impact subsequent crops if the replanted progeny were the result of inbreeding, and suffered from inbreeding depression. We used a combination of experiments to first address whether fruit characteristics differ under pollination by honeybees (*A. mellifera*, Hymenoptera: Apidae) and bumblebees (*Bombus impatiens*, Hymenoptera: Apidae), in comparison to controlled self-fertilization and outcrossing. We then examined the progeny for differences in seed characteristics, growth and resistance to herbivory, to determine the longer-term consequences of these different types of pollination.

## Methods

### Study system

*P. peruviana* L. (Solanaceae) is a perennial herbaceous plant native to tropical and subtropical South America with

an altitudinal distribution from 1800 to 2800 m (Benavidez & Mora 2005), although it has also been reported to be grown as high as 3300 m (Puente, Pinto-Muñoz, Castro, & Cortés 2001). *P. peruviana* presents bell-shaped flowers that open every morning and close in the afternoon for 5–7 days (Lagos, Vallejo, Criollo, & Muñoz 2008) and which are pollinated by a variety of insects and also wind (National Research Council 1989). Self-pollination also appears to be a common mode of reproduction (Gupta & Roy 1981; Lagos et al. 2008), giving *S. peruviana* an effectively mixed mating system (Lagos et al. 2008). *P. peruviana* has high percent fruit set under natural conditions (Gupta & Roy 1981), and farmers in Colombia, Ecuador, Peru and South Africa, India, New Zealand and Hawaii among others have established commercial crops of cape gooseberry (National Research Council 1989; Salazar, Jones, Chaves, & Cooman 2008).

### Experimental set-up

To evaluate the effect of two different pollinators on fruit quality, we conducted experiments in two separate locations. One experiment was performed under field conditions in a commercial field in Colombia to assess the effects of natural pollination by *A. mellifera* in comparison to artificial pollination, and another experiment evaluated pollination by *B. impatiens* in potted *P. peruviana* plants at Cornell University, USA.

### Effect of artificial and natural *Apis* pollination on fruit quality

This study was conducted between March and May 2010 in a commercial cape gooseberry field located in the municipality of Tena (Cundinamarca, Colombia) at an altitude of 2000 m. The 2.4-ha field was established in September 2009, and comprises 1300 plants planted 3 m apart.

To evaluate the effect of *A. mellifera* visitation on fruit quality, four treatments were imposed: autonomous self-pollination (selfing), cross-pollination by hand (manual outcrossing), *A. mellifera* pollination (one visit by *A. mellifera*), and natural, open pollination. We randomly chose 10 plants and on each plant 10 flower buds were covered with a gauze bag to avoid visitation before the start of the experiment. On each plant, two flowers were left bagged to serve as the self-pollination treatment. Four flowers were unbagged immediately following anthesis and allowed one visit by a single *A. mellifera*, before being re-bagged. Two flowers were unbagged and received the combined pollen of flowers from ten separate plants on their stigmas with a fine brush before being rebagged, to create the manual outcrossed treatment. Stigma receptivity was experimentally confirmed at anthesis by the hydrogen peroxide test (Dafni 1992) in 10 flowers on separate cape gooseberry plants. Two flowers were left

unbagged for the open-pollination treatment. While bagging itself can affect fruit traits, overall similar responses in open and *Apis*-pollinated treatments suggest minimal effects of bagging in these experiments (see Results). Approximately two months after the start of the experiment and when the fruits were ripe, we harvested the fruits of all treatments following the method of Galvis, Fischer, and Gordillo (2005). Fruits were taken to the laboratory where the following fruit quality variables were measured: fresh mass, equatorial diameter, number of seeds per fruit, sugar content and average seed mass per fruit. Sugar content was estimated in % Brix using an HI 96803 sugar refractometer (Hanna Instruments®, Rhode Island). Based on observations and the local abundance of *A. mellifera*, flowers in the open pollination treatment likely received more than one *Apis* visit and could also have received visits by meliponine bees and vespids, which were observed visiting other flowers during the experiment.

### Effect of artificial and natural *Apis* pollination on offspring quality

To determine if the type of pollination affects the offspring quality at both the seed and plant stage, we performed a greenhouse experiment at Cornell University with the seeds generated from the *Apis* pollination experiment in the field and measured: germination rate, plant growth (height, number of leaves per plant) and plant resistance to herbivory. To measure germination rates, 120 seeds were drawn randomly from each treatment, and left to germinate in 12 four-inch (355 mL) pots (each with 10 seeds) filled with moist Metro-Mix 360 all-purpose potting soil (Scotts-Sierra Horticultural Products, Marysville, OH). Every week for a period of three weeks the number of germinated seeds per pot was counted. Germinated seedlings were then transplanted to individual four-inch pots, and grown under a 16:8 LD schedule, watered *ad libitum* and fertilized weekly (21–5–20 NPK, 150 ppm). Forty-five days after the germination experiment was started (ca. 1 month of plant growth) we measured plant height and number of leaves per plant on 30 plants from each pollination treatment (120 total). Plant resistance to herbivory was measured on 92 of these plants, excluding very small plants to assess resistance independent of ontogenetic variation among treatments. Half the plants in each pollination treatment were randomly selected to receive damage by three newly hatched *Manduca sexta* (Lepidoptera: Sphingidae) larvae, with half serving as control plants ( $N \approx 12$  plants per combination of pollination and damage treatment). All larvae were initially placed on the first fully expanded leaf. Larvae were removed after 3 days of feeding, at which point they had consumed ca. 20–25% of each leaf. Resistance to herbivory was measured using the growth of *M. sexta* neonates allowed to feed for 36 h on a 3.1 cm<sup>2</sup> leaf disc taken from a damaged leaf on each damaged plant, and a similarly positioned, fully expanded leaf from each control plant.

Leaf discs were mounted on pins over moist filter paper in Petri dishes, to allow larvae to feed on the underside of the leaves. A few larvae that fell off the discs and died without initiating feeding were accordingly excluded prior to analysis. Constitutive (undamaged) and induced (damaged) resistance was measured as larval performance (mass gain). Thus low larval performance corresponds to higher plant resistance.

### Effect of artificial and natural *Bombus* pollination on fruit quality

We evaluated the effects of an additional pollinator, *B. impatiens*, using potted plants growing on the rooftop (4th floor) of a building at Cornell University. Plants were grown from seeds as in the offspring quality experiment for 12 weeks before being transplanted to 4 L pots and placed outside. Depending on flower availability per plant, on each of 60 plants, one to six flowers were marked and bagged as in the previous experiment, and assigned to one of four treatments: visitation by a single *B. impatiens* (assigned first, based on the visit, due to low visitation rates), selfing, manual cross-pollination, and open pollination. Treatments were applied following the protocol outlined in the *Apis* experiment. Visitation rates were lower at this rooftop location than in the field, and at the end just the 29 plants that received at least one *Bombus* visitation were included in the analysis, with 1–5 flowers assigned to a treatment per plant. If two flowers per plant received the same treatment, the average of both fruits was included in the analysis.

### Statistical analysis

In order to determine the overall effect of pollination type on fruit and seed quality, all dependent variables for the field *Apis* and rooftop *Bombus* experiments were analyzed using a multivariate analysis of variance (MANOVA). If significant, separate ANOVAs were performed for each dependent variable (Scheiner & Gurevitch 2001), with pollination treatment as the main fixed effect and plant identity as a random block effect. Growth (number of leaves) and plant height were similarly evaluated by univariate ANOVA, while germination rate was evaluated with repeated measures ANOVA with treatment as the main factor and the three-week period as the repeated measure (time). Resistance to herbivory was analyzed by two-factor ANOVA with the breeding treatment as one factor and the prior herbivory treatment as a second factor. Sugar concentrations in the *A. mellifera* field experiment were  $\log_{10}$ -transformed prior to analysis to correct for heteroscedasticity. Following all ANOVAs, treatment means were compared by the Ryan–Einot–Gabriel–Welsch multiple comparison test (REGWQ) (Day & Quinn 1989), with an experiment wise  $\alpha=0.05$ . All analyses were conducted in SAS v.9.0 using the GLM procedure, except for

the MANOVA and repeated measures analyses, which were conducted in Statistica v. 6.0.

## Results

### Effects of artificial and natural *Apis* pollination on fruit quality

Pollination type and block (plant identity) affected fruit quality (MANOVA:  $F_{12,137.87}=2.8$ ,  $P=0.001$ ;  $F_{36,196.6}=1.94$ ,  $P=0.002$ , respectively). Fruit mass differed among treatments ( $F_{3,55}=6.10$ ,  $P<0.001$ ), with *Apis*- and open-pollinated fruits weighing more than the fruits of the self-pollination treatment (Fig. 1A). Pollination treatment also affected fruit diameter ( $F_{3,55}=4.9$ ,  $P=0.004$ ), the number of seeds per fruit ( $F_{3,55}=5.045$ ,  $P=0.003$ ) and seed mass ( $F_{3,22}=4.74$ ,  $P=0.01$ ) but had no effect on fruit sugar concentration ( $F_{3,55}=1.59$ ,  $P=0.2$ ). Open-pollinated fruit diameter was significantly greater than manually outcrossed and self-pollinated fruit diameter (Fig. 1B). Seed number was significantly higher in open-pollinated flowers in comparison to self-pollination and manually outcrossed flowers (Fig. 1C). Similarly, seed mass was significantly increased under open pollination in comparison to self-pollination (Fig. 1D).

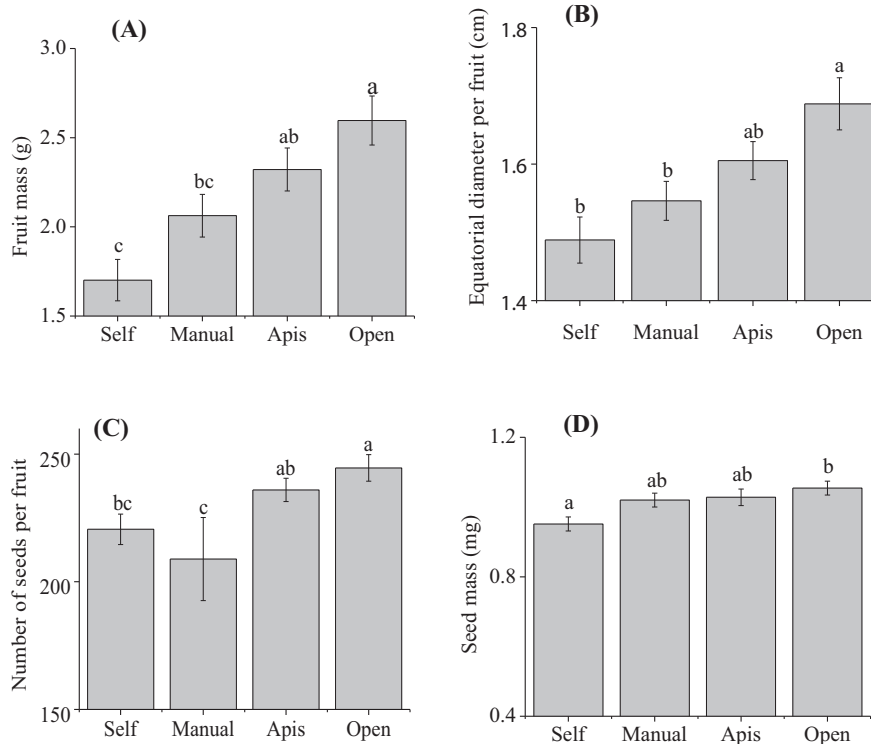
### Effect of artificial and natural *Apis* pollination on offspring quality

Germination rate differed among pollination treatments ( $F_{3,44}=7.70$ ,  $P=0.0003$ ) and over time ( $F_{2,88}=19.19$ ,  $P<0.0001$ ). Germination rate was highest in seeds from open- or *Apis*-pollinated fruits in comparison with manual cross pollination (Fig. 2). Pollination type had no effect on plant height ( $F_{3,39}=0.538$ ,  $P=0.65$ ), but influenced leaf number ( $F_{3,39}=3.08$ ,  $P=0.0384$ ). Manually cross-pollinated plants had a higher number of leaves in comparison to self- and open-pollinated plants (Fig. 3). Resistance to herbivory, as estimated by *M. sexta* larval growth, was significantly greater in plants that had experienced prior caterpillar damage ( $F_{1,80}=11.32$ ,  $P=0.0012$ , Fig. 4), but was only marginally different among breeding treatments ( $F_{3,80}=2.16$ ,  $P=0.0991$ ). There was no statistical evidence for an interaction between breeding and damage treatment ( $F_{3,80}=1.40$ ,  $P=0.2486$ ).

### Effect of artificial and natural *Bombus* pollination on fruit quality

Final fruit mass differed among pollination treatments ( $F_{3,30}=3.74$ ;  $P=0.0023$ ). Fruits visited once by *B. impatiens* weighed more than the fruits of the self-pollination and manual pollination treatments (Fig. 5A), with open-pollinated fruits being statistically intermediate. Fruit diameter also





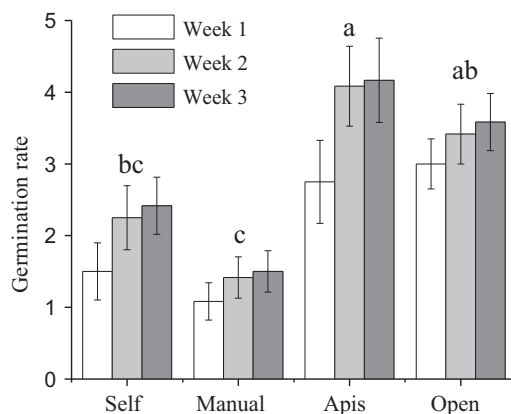
**Fig. 1.** Cape gooseberry fresh fruit mass (A), diameter (B), number of seeds per fruit (C), and mean individual seed mass (D) (mean  $\pm$  SE) in pollination treatments. Treatments are: self-pollination (Self), manual cross-pollination (Manual), single *A. mellifera* visitation (*Apis*) and natural pollination (Open). Treatments with a common letter are not significantly different (REGWQ test,  $P > 0.05$ ).

differed among treatments ( $F_{3,30} = 3.07$ ,  $P = 0.0430$ ): fruit diameter was significantly greater in *Bombus*- than in self-pollinated fruits (Fig. 5B), and open-pollinated fruits were of intermediate size. We did not observe any treatment differences in seed mass ( $F_{3,22} = 2.61$ ,  $P = 0.0772$ ), sugar

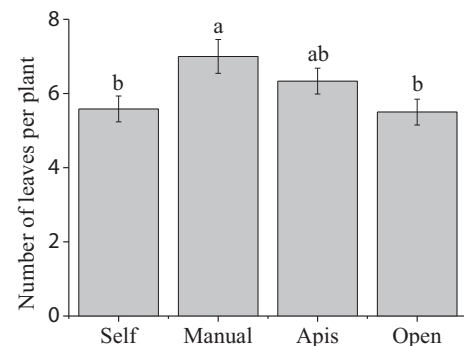
concentration ( $F_{3,30} = 1.78$ ,  $P = 0.1730$ ), or seed number ( $F_{3,30} = 0.24$ ,  $P = 0.8678$ ).

## Discussion

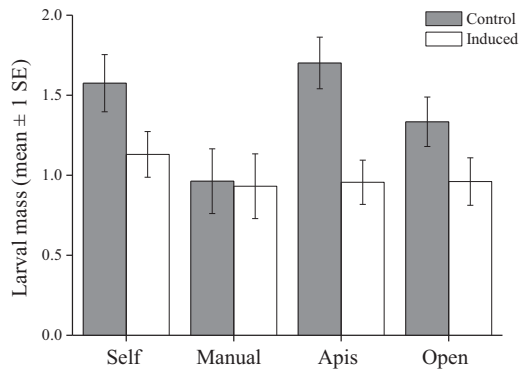
Of nine fruit and offspring traits measured in response to pollination treatments in the field experiment with *A. mellifera*, seven traits were affected by insect pollination, while



**Fig. 2.** Germination rates (mean  $\pm$  SE), measured as number of seeds germinated out of 10 seeds, from each pollination treatment over a three week period. Treatments are: self-pollination (Self), manual cross-pollination (Manual), single *A. mellifera* visitation (*Apis*) and natural pollination (Open). Pollination treatments with a common letter are not significantly different across all time periods (REGWQ test,  $P > 0.05$ ).



**Fig. 3.** Number of leaves (mean  $\pm$  SE) of cape gooseberry plants from different pollination treatments. Treatments are: self-pollination (Self), manual cross-pollination (Manual), single *A. mellifera* visitation (*Apis*) and natural pollination (Open). Treatments with a common letter are not significantly different (REGWQ test,  $P > 0.05$ ).



**Fig. 4.** Growth rate of neonate *Manduca sexta* larvae feeding on undamaged control plants (Control), and plants that had been previously damaged by *M. sexta* herbivory larvae (Induced). Plants were derived from the following pollination treatments: self-pollination (Self), manual cross-pollination (Manual), single *A. mellifera* visitation (*Apis*) and natural pollination (Open). The induction effect (Control vs. Induced) was the only significant effect, while the breeding treatment had just marginal effects (see results for more details).

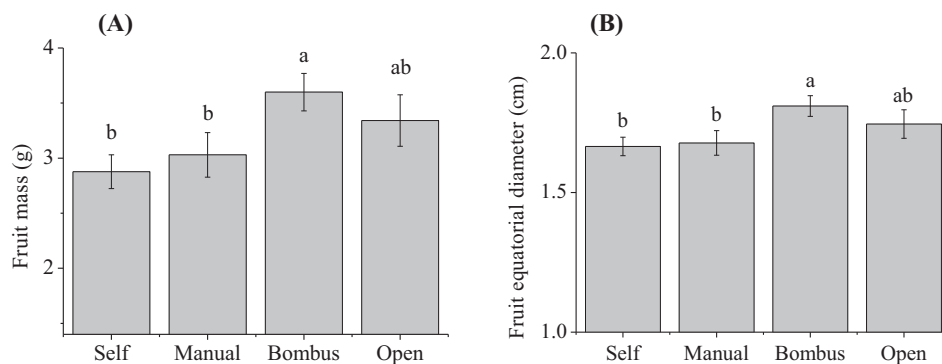
two of five fruit and seed traits were affected by pollination in the *B. impatiens* experiment. Our results support the hypothesis that pollen quantity and quality are important factors affecting not only fruit and seed set but also progeny growth and marginally resistance to herbivores. From both ecological and agricultural perspectives, pollination by *A. mellifera* and *B. impatiens* enhances fruit quality, suggesting that adequate insect pollination should be considered in management strategies for *P. peruviana*.

In our study, *A. mellifera* increased equatorial diameter of the fruits of cape gooseberry by 13.3%, fruit mass by 30.3%, the number of seeds by 7% and seed mass by 8.4% in comparison with the self-pollination treatment. The increase in fruit size could be the result of increased pollen deposition, as well as pollen competition (Free 1970; Roldán Serrano & Guerra-Sanz 2006), favoring selection of higher quality pollen grains (Mulcahy & Mulcahy 1975, 1987; Winsor et al. 2000) and hence increased seed set (Cowan,

Marshall, & Michaelson-Yeates 2000; Winsor et al. 2000; Al-Ghzawi, Zaitoun, Freihat, & Alqudah 2009) and fruit growth (Cruz, Freitas, Silva, Silva, & Bomfim 2005; Lovett-Doust & Lovett-Doust 2005). While the pollen competition hypothesis is supported in part by the increased seed mass in the open-pollinated treatment, the most probable explanation for our results is greater, or more effective, pollen deposition by bees. While not statistically significant, the ca. 25–30% increase in fruit size in open compared to *Apis*-pollinated plants (Fig. 1) supports this hypothesis, since open-pollinated flowers had the potential for receiving more than a single visit. In contrast to previous studies on pollination and fruit nutrition (Shin, Park, & Kim 2007; Freihat, Al-Ghzawi, Zaitoun, & Alqudah 2008) our data suggest that pollination in *P. peruviana* does not affect fruit quality in terms of sugar content.

Similarly, our roof top experiment revealed that *B. impatiens* was also an effective pollinator, as measured by the increased fruit mass and diameter in comparison to the self-pollination and manual pollination treatments, and was likely the result of either increased or more effective pollen deposition (Free 1970). Although common farming practices rely on the fact that self-pollination is sufficient for *P. peruviana* to produce fruits and previous studies show that self-pollination might be more effective than open pollination (Lagos et al. 2008), our data suggest that the size and mass of fruits are significantly increased by the presence of two different pollinators. These results suggest that farmers should be cognizant of bee presence and abundance when monitoring their crops for sufficient fruit set. Moreover, future agronomic studies of *P. peruviana* management could consider artificially augmenting bee abundance to test for increased productivity in sites with low pollination.

Our data also support our hypothesis that pollination type can influence components of offspring quality and performance. This is both ecologically and economically relevant, since crops such as cape gooseberry are planted from a portion of the previous harvest. The germination rates of *Apis*- and open-pollinated seeds were substantially greater than selfed and manually outcrossed seeds (Fig. 2), suggesting



**Fig. 5.** Cape gooseberry fruit mass (A) and equatorial diameter (B) (mean ± SE). Treatments are: self-pollination (Self), manual cross-pollination (Manual), single *A. mellifera* visitation (*Apis*) and natural pollination (Open). Treatments with a common letter are not significantly different (REGWQ test,  $P > 0.05$ ).

an additional, long-term benefit of adequate insect pollination. Several studies have found that germination can be higher for seeds from high, compared to low, pollen loads (Richardson & Stephenson 1992; Jóhannsson & Stephenson 1997; Brown & Kephart 1999), potentially as a result of pollen competition (Mulcahy & Mulcahy 1975). In contrast to the results on fruit quality and germination rate, manual outcrossing significantly increased a component of offspring growth (leaf number). Since the data suggest that manual pollination did not increase pollen deposition, the most plausible interpretation for this result is that manual outcrossing improved plant vigor compared to self-pollination, either as a result of reduced inbreeding depression or heterosis (Charkesworth & Charkesworth 1999; Waser & Williams 2001). Indeed, inbreeding depression for vigor is common in many plant species (Husband & Schemske 1996). Although just marginally significant we found support for the hypothesis of inbreeding depression, where manually outcrossed offspring were 43% and 39% more constitutively resistant to herbivory in comparison to offspring derived from *Apis*- and self-pollinated breeding treatments, respectively (Fig. 4). Only a handful of studies have examined the effects of mating system variation on resistance, and these studies suggest that inbreeding has the potential to increase susceptibility to herbivores (Hayes et al., 2004; Ivey et al. 2004; Delphia et al. 2009; Bello-Bedoy & Núñez-Farfán 2010), most likely as a result of deleterious effects on defence-related secondary metabolite production (Campbell et al. unpublished data). Our data add to this small, but growing body of work highlighting the significance of plant mating history (and pollinator-mediated mating) for plant–herbivore interactions, including crop–pest interactions. If this hypothesis is true, the results for the *Apis* and open pollinated offspring would suggest that some pollen deposition by bees is self-pollen, potentially as a result of more movement by bees among flowers on the same plant (geitonogamy) than among plants (Chautá & Poveda personal observations).

Our results allow us to speculate on at least two processes that are likely to underlie these results. First, the amount of, and/or manner in which, pollen was deposited on stigmas by bees may have increased the number of successfully fertilized ovules, and led to higher seed set and concomitant changes to fruit investment. Second, the genetic background of the pollen (self vs. outcross) may have contributed in part to the observed differences, particularly in offspring quality. Our treatments do not allow us to explicitly infer the relative contribution of each mechanism, because we did not control for the amount of pollen deposited by bees compared to manual or self pollination, and we did not use the same methodology to self-fertilize and manually outcross the different treatments. Nevertheless, our results suggest both mechanisms: the consistently higher fruit trait values in open and bee-pollinated plants compared to selfed and manually outcrossed plants suggests that visitation (which generally increases pollen deposition) is a strong driver of

fruit quality. However, the marginal differences between bagged and manually outcrossed treatments in seed mass and constitutive resistance suggest that there may also be inbreeding depression in some ecologically relevant traits.

We conclude that sufficient pollinator visitation should be a concern for growers of *P. peruviana* since our results show that autonomous self-fertilization should reduce the number of export-quality fruits by reducing fruit diameter. Growers replant *P. peruviana* using in-field progeny. Given that our results show that inbred offspring may be less vigorous and resistant to pests, we also tentatively suggest replanting with numerous different genotypes of *P. peruviana*, which would buffer against the potential for mating among relatives and the consequences of inbreeding depression. However we would urge a cost-benefit analysis prior to recommending such a practice to farmers.

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