

SHORT RESEARCH PAPER

# Assessment of pollen reward and pollen availability in *Solanum stramoniifolium* and *Solanum paniculatum* for buzz-pollinating carpenter bees

A. Burkart<sup>1</sup>, C. Schlindwein<sup>2</sup> & K. Lunau<sup>1</sup>

<sup>1</sup> Department Biology, Institute of Sensory Ecology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

<sup>2</sup> Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

## Keywords

Buzzing; carpenter bee; pollen; pollination; poricidal anther; *Solanum paniculatum*; *Solanum stramoniifolium*.

## Correspondence

K. Lunau, Department Biology, Institute of Sensory Ecology, Heinrich-Heine-University Düsseldorf, 40225 Düsseldorf, Germany.  
E-mail: lunau@uni-duesseldorf.de

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## ABSTRACT

The two widespread tropical *Solanum* species *S. paniculatum* and *S. stramoniifolium* are highly dependent on the visits of large bees that pollinate the flowers while buzzing them. Both *Solanum* species do not offer nectar reward; the rewarding of bees is thus solely dependent on the availability of pollen. Flower visitors are unable to visually assess the amount of pollen, because the pollen is hidden in poricidal anthers. In this study we ask whether and how the amount of pollen determines the attractiveness of flowers for bees. The number of pollen grains in anthers of *S. stramoniifolium* was seven times higher than in *S. paniculatum*. By contrast, the handling time per five flowers for carpenter bees visiting *S. paniculatum* was 3.5 times shorter than of those visiting *S. stramoniifolium*. As a result foraging carpenter bees collected a similar number of pollen grains per unit time on flowers of both species. Experimental manipulation of pollen availability by gluing the anther pores showed that the carpenter bees were unable to detect the availability of pollen by means of chemical cues before landing and without buzzing. Our study shows that the efficiency of pollen collecting on *S. paniculatum* is based on large inflorescences with short between-flower search times and short handling time of individual flowers, whereas that of *S. stramoniifolium* relies on a large amount of pollen per flower. Interestingly, large carpenter bees are able to adjust their foraging behaviour to drastically different strategies of pollen reward in otherwise very similar plant species.

## INTRODUCTION

The Solanaceae comprise more than 2500 species, of which some have economic importance (Knapp 2002). *Solanum* is the most speciose genus within the family, with about 1500 species, and has flowers that are buzz-pollinated due to their typical poricidal anthers that hide the pollen inside. The release of pollen from the stiff anthers requires manipulation of the stamens (Thorp 2000; Smith & Knapp 2002). Only some bees are capable of vibrating the flowers (buzzing) in order to collect the pollen (Michener 1962; Wille 1963; Buchmann 1983). *Solanum* flowers do not offer nectar as a reward and thus are dependent of buzz-pollinating bees. The signalling strategies of *Solanum* species are uniform with respect to five yellow anthers contrasting against the corolla (Lunau 2007), but vary considerably with respect to the number of flowers grouped into an inflorescence, and size and colour of the flower petals (Diggle & Miller 2004). It is largely unknown how the amount of pollen reward shapes the foraging strategy of buzz-pollinating bees. In this study, we ask how pollen reward impacts the attractiveness to pollinators of two syntopic *Solanum* species with different floral signalling strategies, *S. stramoniifolium* Jacq. and *S. paniculatum* L.

Both *S. paniculatum* and *S. stramoniifolium* belong to the subgenus *Leptostemonum* (Levin *et al.* 2006) and are common in rural areas of the province of Pernambuco on the eastern

coast of Brazil. Several authors have studied the floral biology of these species (Forni-Martins *et al.* 1998; Bezerra & Machado 2003; Silva *et al.* 2004) and found a typical buzz-pollination syndrome including roundish, small pollen grains and a pollinator spectrum mainly capable of buzzing. Silva *et al.* (2004) found a large amount of pollen (about 1,800,000 pollen grains per flower) in *S. stramoniifolium*, which decreased during the day because of visiting bees. The amount of pollen in *S. paniculatum* is not known.

Both *S. paniculatum* and *S. stramoniifolium* are visited by a number of bee species, particular two species of carpenter bee (Xylocopini, Apidae), *Xylocopa* (*Neoxylocopa*) *frontalis* and *X. (N.) suspecta*, which are among the largest bees found in eastern Brazil (Gerling *et al.* 1989). Both species nest in dead wood and collect pollen from plants of various families (Gerling *et al.* 1989; Bernardino & Gaglianone 2007; Marchi & Melo 2010). Bees of the tribe Xylocopini are capable to buzzing flowers for pollen collection. While buzzing a flower, the bees keep their body close to the anthers in order to transfer vibratory force to the flower. Buzzing behaviour was described in Buchmann (1983) and King *et al.* (1996) as vibration of the thorax by the flight musculature with decoupled wings. This buzzing behaviour produces a high buzzing sound (Burkart *et al.* 2011) and, beside pollen collection, it is used as a warning signal in nest defence and to compress soil during nest-building (King & Buchmann 2003). The clearly audible sound of the buzzing of

big bees is easily perceived by human observers, even at distances up to 5 m. The display of poricidal anthers, which release pollen when vibrated, is seen as a plant strategy to control the rate of pollen removal from flowers (Harder & Barclay 1994; De Luca & Vallejo-Marín 2013). In addition, nectarless, buzz-pollinated flowers restrict floral reward to bees capable of buzzing (King *et al.* 1996).

In order to investigate the foraging behaviour of buzz-pollination by carpenter bees in patches of *S. paniculatum* and *S. stramonifolium* we tested the capacity of the carpenter bees to sense the amount of pollen released from poricidal anthers before landing and while buzzing; we quantified the amount of collected pollen per bee visit in both species of *Solanum* and measured the amount of collected pollen in relation to presentation of the flowers.

## MATERIAL AND METHODS

### Plant habitat and morphology

The observations and recordings were performed in the municipality of Camaragibe, near Recife, Pernambuco. According to the meteorological station at the international airport in Recife the temperature during the observation period from November 2010 to January 2011 was almost constant, with an average maximum temperature of  $29.6 \pm 0.7$  °C and an average minimum night temperature of  $25.2 \pm 1.1$  °C. The relative humidity was constant, with an average of  $69 \pm 5\%$ , but increased to 85% due to heavy rainfall during the last weeks of January.

The two observed *Solanum* species *S. paniculatum* and *S. stramonifolium* species are regionally known as 'Jurubeba', which generally represents different *Solanum* species with spiked stems or leaves. *S. paniculatum* is an invasive plant up to 2 m tall with a spiked stem. The main stem is usually unbranched and carries an apical inflorescence with light purple to whitish pentamerous flowers with yellow anthers. The plant was observed on former cropland (07°58.230'S, 35°00.179'W), where it occurred within an area of 100 m to 200 m as the dominant flowering plant. *S. stramonifolium* is a shrub covered in spines, with a circumference up to 2 m, and grows to 2 m in height (Silva *et al.* 2004). Unlike *S. paniculatum*, the inflorescences of *S. stramonifolium* are axillary over the whole plant and usually carry only one or two white flowers at the same time. The study site for this plant was fallow land close to the forest in the Condomínio Parque Cedros (07°58.650'S, 35°00.102'W). Here, *S. stramonifolium* was scattered within an area of about 100 m along the forest edge. Positioning of flowers and growth of the stem is schematically shown for both *Solanum* species (Fig. 1). The distance between the two habitats is about 800 m.

The architecture of the flowers was analysed by measuring overall size, size of the petals and anthers using callipers, and diameter of anther pores using a Zeiss Axiolab light microscope with an ocular scale (Carl Zeiss, Oberkochen, Germany).

### Pollen amounts

The pollen quantities of both plant species were counted from unvisited flowers or buds. Pollen counting was performed with a Neubauer chamber using a slightly modified method after Lloyd (1972). The anthers were removed and placed in an

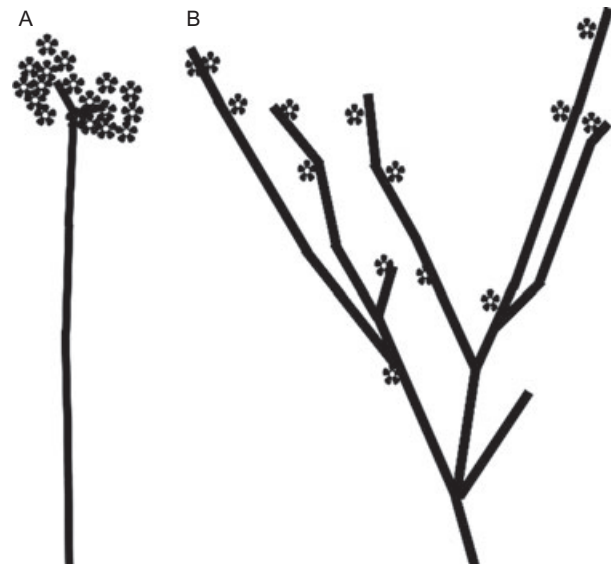


Fig. 1. Habit of typical plants of *Solanum paniculatum* (A) and *S. stramonifolium* (B), showing the position of the flowers (drawn from a photo).

Eppendorf tube with lactic acid diluted to 40–50%. The volume of the lactic acid depended on the expected amount of pollen grains. The solution was then vigorously shaken for 5 min and a reference volume pipetted into the Neubauer counting chamber. Using a Zeiss Axiolab microscope with scale ocular, the number and the size of pollen grains were determined. The number of pollen grains per anther was then estimated from the number in the reference volume in the Neubauer counting chamber.

Flowers of *S. paniculatum* and *S. stramonifolium* have five similarly shaped anthers. As presented below, the standard deviation of pollen amounts in anthers of the same flower is low. For calculations we thus assume that anthers of the same flower have a similar pollen amount. Using this assumption, we were able to measure the pollen load collected by a single bee during one flower visit with the following steps. On the day before the experiment, the buds were coated to prevent bees from foraging before the start of the observation. On the next day and immediately before presenting the flowers to the bees, one anther was removed from the unvisited flower, which was used as reference for the pollen amount of the fresh flower. After the first bee visit, another anther was removed, representing the pollen left in a flower after one visit. This procedure was repeated with the remaining anthers of the flower, *i.e.* after the next bee visit another anther was removed, representing the pollen left in a flower after one more visit. The pollen was then counted using the same procedure as in unvisited flowers (Lloyd 1972).

### Analyses of foraging, choice and buzzing behaviour of carpenter bees

Comparative analyses of the handling time on the two plant species were performed using visual observations of the bees and measuring the foraging time using a stopwatch. The following observation pattern was applied to the visitors of both plant species. When a big carpenter bee was identified in the field, its foraging flight was followed and the stopwatch was

started when it contacted the first flower. When the bee continued the foraging flight, the number of visited flowers was counted. After leaving the fifth flower, the time was recorded. In a second approach, the number of buzzings performed in the field during a single flower visit of freely available flowers of both *Solanum* species was counted.

In order to analyse the capacity of bees to assess whether the poricidal anthers of *S. stramonifolium* would release pollen or not, the pores were glued to prevent the release of pollen (Buchmann & Cane 1989). Commercially available superglue was used to close the pores of all anthers of ten unvisited flowers. As control, we put droplets of glue on the petals of ten other unvisited flowers with open anthers, and chose a number of unvisited flowers, which were not treated with glue, as a reference. The bees' choice of flowers for these two treatments was recorded. At each visit of a flower, the number of buzzings of the bees was counted using visual and acoustic observations. Buzzing counting experiments were performed in the morning between 06:30 h and 10:30 h on several days in December.

### Statistical analyses

All data processing and statistical tests were performed in Microsoft Excel 2002 (Microsoft, Redmond, WA, USA) and Graphpad Prism 4.0 (GraphPad Software, La Jolla, CA, USA). Significant statistical differences were assessed using Student's *t*-test. Differences between two datasets were regarded as significant at  $P < 0.05$ . All data are shown as mean  $\pm$  SD. For graphical work, Paint Shop Pro 9 (Jasc Software, Eden Prairie, MN, USA) was used.

## RESULTS

### Plants morphology

The comparison of *S. paniculatum* and *S. stramonifolium* revealed no significant differences in the size of the flowers and their components, only in the size of the anthers (Table 1). The length of the anthers is about 7 mm in both species. The diameter of anthers of *S. paniculatum* is about 1 mm, whereas the diameter of anthers of *S. stramonifolium* is twice as large (exemplary measurements). In both species, the diameter of the anther pore is 0.25 mm (Table 1). *S. paniculatum* has about

**Table 1.** Floral size parameters in *Solanum stramonifolium* and *S. paniculatum*. Data are presented as mean  $\pm$  SD. The measurements of flowers and anthers were done on ten flowers of *S. stramonifolium* and ten flowers of *S. paniculatum* collected from ten different plants. The diameter of anther pores was determined on each species (*S. paniculatum*  $n = 30$  anthers from different flowers, *S. stramonifolium*  $n = 26$  anthers from seven different flowers) with anthers of flowers in all flowering stages from bud to wilted.

| flower part                      | <i>S. paniculatum</i> (n) | <i>S. stramonifolium</i> (n) | P-value (t-test) |
|----------------------------------|---------------------------|------------------------------|------------------|
| flower diameter (mm)             | 25.5 $\pm$ 2.5 (10)       | 23.7 $\pm$ 3.5 (10)          | 0.205            |
| petal length (mm)                | 12.9 $\pm$ 1.5 (10)       | 12.5 $\pm$ 2.3 (10)          | 0.650            |
| anther length (mm)               | 6.7 $\pm$ 0.6 (30)        | 7.0 $\pm$ 0.6 (30)           | 0.027            |
| anther pore diameter ( $\mu$ m)  | 254.7 $\pm$ 51.1 (30)     | 249.2 $\pm$ 47.9 (26)        | 0.565            |
| pollen grain diameter ( $\mu$ m) | 28.6 $\pm$ 3.5 (50)       | 29.0 $\pm$ 2.3 (25)          | 0.610            |

four times more open flowers per plant than *S. stramonifolium* (Figs 1 and 2A).

### Pollen amounts

The pollen counts of unvisited flowers revealed seven-fold fewer pollen grains per flower ( $P < 0.0001$ , *t*-test) in *S. paniculatum* (187,070  $\pm$  26,274 pollen grains per flower estimated from counting four out of five anthers per flower,  $n = 8$ ) than in *S. stramonifolium* (1,354,427  $\pm$  156,487,  $n = 6$ ; Fig. 2B). In *S. paniculatum* the mean number of pollen grains per anther was 37,414 ( $n = 8$ ; Fig. 2B). The variation in number of pollen grains per anther (average  $\pm$  SD = 8519) was considerably lower in *S. paniculatum* than that in *S. stramonifolium* ( $\pm$ SD = 15,410). In *S. stramonifolium* the mean number of pollen grains per anther was 270,885 ( $n = 6$ ; Fig. 2B). The variation in number of pollen grains within flowers (average  $\pm$  SD = 25,605) was lower than that between flowers ( $\pm$ SD = 31,298).

### Analyses of foraging and buzzing behaviour of carpenter bees

Bees needed more than three-fold less handling time for five flowers of *S. paniculatum* (15.75  $\pm$  3.67 s) than for five flowers of *S. stramonifolium* (54.47  $\pm$  7.13 s; Fig. 2C). The carpenter bees collected about six times less pollen (12,953  $\pm$  4747) per flower visit and anther on *S. paniculatum* than on *S. stramonifolium* (73,411  $\pm$  53,865; Fig. 2D). When visiting flowers of *S. stramonifolium* and *S. paniculatum* the body of the carpenter bee adopts a C-shaped position, where all legs are attached to the flower and the mandibles grasp the anthers. Flowers of *S. paniculatum* are buzzed only once (1.02  $\pm$  0.14), whereas flowers of *S. stramonifolium* are buzzed several times (3.13  $\pm$  1.54), and the bees regularly change their radial position between different buzzings (Fig. 3).

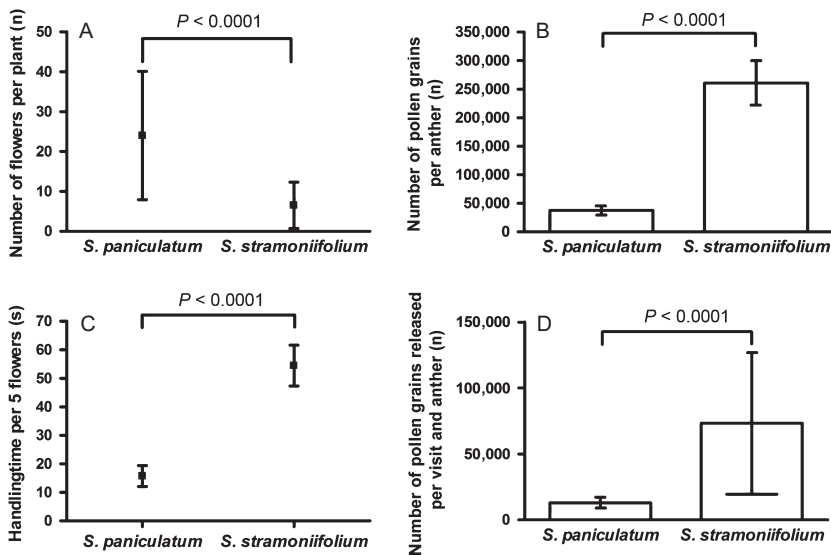
*Xylocopa* bees visiting pairs of manipulated flowers did not exhibit a preference for flowers with glued petals (14 visits to ten flowers) over flowers with glued anthers petals (16 visits to ten flowers). Flowers of *S. stramonifolium* with glued anthers were buzzed less often (1.46  $\pm$  0.98) than control flowers with glued petals (2.91  $\pm$  0.83) (Fig. 3). During personal observations, no noticeable behavioural difference were seen in the floral handling of individual carpenter bees of the species *X. frontalis* and *X. suspecta*. Other large bee species (*Centris flavifrons*, *Eulaema nigrita* and *Eulaema cingulata*) showed similar handling times and buzzing counts.

### Foraging efficiency calculation

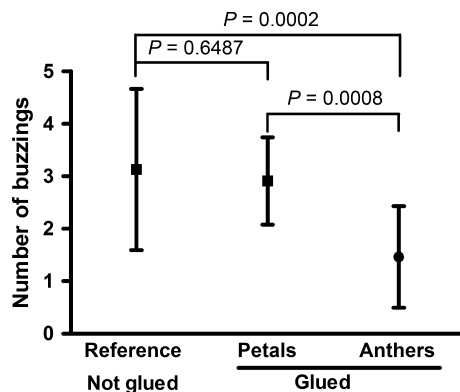
Using the data on handling time and pollen collected per anther, the amount of pollen collected per time unit was estimated for carpenter bees foraging on the two *Solanum* species. When foraging on *S. paniculatum*, carpenter bees visited 19.1 flowers  $\text{min}^{-1}$ , buzzed 19.4 times  $\text{min}^{-1}$  and collected about 250,000 pollen grains  $\text{min}^{-1}$ ; while foraging on *S. stramonifolium*, they visited 5.5 flowers  $\text{min}^{-1}$ , buzzed 17.4 times  $\text{min}^{-1}$  and collected about 400,000 pollen grains  $\text{min}^{-1}$ .

## DISCUSSION

As obvious from botanical descriptions of *S. stramonifolium* and *S. paniculatum* (Whalen & Costich 1986) and underlined



**Fig. 2.** Comparison of pollen reward in *S. stramonifolium* and *S. paniculatum* and of flower handling by carpenter bees. (A) Number of open flowers per plant in *S. paniculatum* (n = 36) and *S. stramonifolium* (n = 42). (B) Number of pollen grains per anther in previously unvisited flowers of *S. stramonifolium* (n = 44) and *S. paniculatum* (n = 32). (C) Handling time for five consecutively visited flowers of *S. paniculatum* (n = 40) and *S. stramonifolium* (n = 18) by *Xylocopa frontalis* or *X. suspecta*. (D) Number of pollen grains released per anther and visit to *S. paniculatum* (n = 18) and *S. stramonifolium* (n = 10) by *X. frontalis* or *X. suspecta*. All data are mean  $\pm$  SD. P-values refer to Student's t-test.



**Fig. 3.** Number of buzzings per flower and visit of *Xylocopa* bees on *S. stramonifolium* flowers without glue (n = 96), with glued petals (n = 14) and with glued anthers (n = 16). The data show a significant difference between flowers with glued anthers and flowers with glued petals ( $P = 0.0008$ ), but no significant difference between the non-manipulated flowers and flowers with glued petals (Student's t-test;  $P = 0.6487$ ).

by our observations, the flowers of both *Solanum* species have five poricidal anthers, are nectarless and are similar in colour and size. The anthers in *S. stramonifolium* surpassed those in *S. paniculatum* in size and contain seven-fold the number of pollen grains. The size of pollen grains is similar in the two species. Not only the amount of pollen per flower, but also the amount of collected pollen per flower visit differs among these species. While there are many investigations on optimal nectar foraging in bees (Thomson *et al.* 1982; Harder 1986; Raine & Chittka 2003), there are few studies on pollen collecting in bees (Dobson *et al.* 1999). In this study, we analysed the pollen foraging behaviour of carpenter bees on flowers that completely hide their pollen within poricidal anthers. The focus of our study is on the complex behaviour patterns of bees needed to estimate the pollen reward while buzzing the flowers.

Our data show that carpenter bees do not avoid visits to flowers with glued anthers. This finding indicates that pollen odour, which is known to be a suitable indicator of the amount of pollen in *Rosa rugosa* flowers (Dobson *et al.* 1999), plays no

role in the choice of the *Solanum* flower in the tested carpenter bees. These results do not exclude odour emission from pollen, but exclude the influence of pollen odour on the behaviour choice of the carpenter bees. Prior to landing on the flowers, the carpenter bees were obviously unable to assess the presence of harvestable pollen. Only when buzzing the flowers were the bees able to assess if further pollen was available. The bees buzz all flowers they visit and a reduction of the number of pollen grains per visit indicates that during each visit only a small portion of the totally available pollen is extractable through buzzing. While several authors have shown that flowers that are buzz-pollinated portion the pollen reward to more than one bee visit (Snow & Roubik 1987; Harder 1990a,b; Harder & Barclay 1994; King & Ferguson 1994), although the mechanism providing this pollen portioning is still not fully understood. In this study, we found no evidence for sequential pollen release from the five stamens; instead, our results suggest that the anthers ripen simultaneously and that buzzing ejects pollen from all five anthers at once. The progressive desiccation of pollen is thus a plausible mechanism for regulation of the discharge and retention of pollen grains. We never observed bees harvesting all or almost all pollen from the anthers of a flower during a single visit. The amount of pollen released from a previously unvisited flower during a single visit of a bee ranged from 20% to 42% in *S. paniculatum* and from 8% to 48% in *S. stramonifolium*. The number of buzzings during a single flower visit differed among *S. stramonifolium* and *S. paniculatum*; carpenter bees buzzed *S. stramonifolium* flowers more than three times per visit, whereas they buzz *S. paniculatum* only once. The single buzz of *S. paniculatum* flowers indicates that the bees have learned that only a portion of pollen could be released and that further buzzings would not lead to further release of pollen, whereas the multiple buzzings of *S. stramonifolium* flowers are consistent with the assumption that the release of pollen during buzzing induces further buzzings.

Our experiment with glued anthers in *S. stramonifolium* showed that carpenter bees buzz all flowers at least once and are obviously able to determine whether the anther releases pollen or not. In *S. stramonifolium* flowers they continue buzzing if pollen is released and stop buzzing if it is not released.

From the plant's perspective, it is noteworthy that the flower's manipulation of the behaviour of the carpenter bees is independent of the presence or absence of available pollen, and thus completely independent of the availability of rewards.

From our results, the carpenter bees foraged on *S. stramonifolium* and *S. paniculatum* in nearby habitats at the same time. Whereas they earned more pollen from *S. stramonifolium* flowers during a single visit, the handling time in *S. paniculatum* was shorter due to higher within-plant flower density and to less buzzings per flower visit. Interestingly, the number of buzzings per unit time is very similar, and the overall number of pollen grains collected per unit time is also similar. We did not find that *S. stramonifolium* was able to outcompete *S. paniculatum* due to the much higher amount of pollen reward per flower. Flight, and particularly buzzing, probably consumes a large amount of energy (King & Buchmann 2003); in carpenter bees, flight leads to elevated oxygen consumption of about 70-fold above the resting rate (Gäde & Auerswald 1998). We conclude that *S. stramonifolium* and *S. paniculatum* use different strategies for adjusting their attractiveness to buzz-pollinating bees, characterised by the amount of pollen reward per flower and its portioning, the amount of buzzings needed to harvest one portion of pollen, and the amount of flowers grouped into an inflorescence. The carpenter bees probably adjust their

energy-consuming movements so that they return the most pollen per unit of expended energy (Harder 1990b). *S. stramonifolium* and *S. paniculatum* are both self-sterile, exhibit polymorphism in style length and have short-styled flowers that do not set fruits and thus only serve as pollen donors (Forni-Martins *et al.* 1998; Bezerra & Machado 2003). This indicates that either the attraction of potential pollinators through an increase in the number of rewarding flowers and/or the reduction of within-plant pollen transfer may play a role in the pollination strategies of these *Solanum* species. However, it is not known how the potentially high capacity for long flight distances in carpenter bees is involved in the pollination strategy, because plant densities of these *Solanum* species in natural habitats are not known. Further studies will elucidate which of these strategies has additional benefits in terms of plant outcrossing rate and long-distance pollen dispersal.

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