The fate of pollen in two morphologically contrasting buzz-pollinated *Solanum* flowers

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Manuscript received _____; revision accepted _____

Running title: Pollen fates in buzz-pollinated *Solanum*

ABSTRACT

Study premise: Pollen transfer efficiency (PTE) and pollen deposition patterns on a pollinator's body significantly influence plant reproductive success. However, studies on pollen fates (i.e., the destination of pollen grains after being released) in animal-pollinated species, particularly those offering pollen as the sole reward, are limited. Here, we investigated pollen fates in two nectarless, buzz-pollinated *Solanum* species with contrasting floral morphology.

Methods: We conducted experimental trials involving one pollen donor and four recipient flowers of *Solanum rostratum* and *S. dulcamara*, using captive *Bombus terrestris* as pollinator. After each trial, we assessed the amount of pollen remaining in the anthers, deposited on stigmas, placed on the pollinator, and falling to the ground. We then estimated pollen fates and PTE, and modelled their pollen deposition curves.

Key results: We found that *S. rostratum* produced more pollen but had a more restricted pollen dispensing schedule compared to *S. dulcamara*. Although PTE was similar between species (0.72% vs. 1.07%, for *S. rostratum* and *S. dulcamara,* respectively), pollen loss mainly occurred due to falling to the ground in *S. rostratum* and bee grooming in *S. dulcamara*, potentially explained by their different floral architectures. Both species exhibited a typical exponential decay pattern in pollen deposition, with the first visited flowers receiving the most outcross pollen.

Conclusions: Our results suggest that PTE in pollen-rewarding, buzz-pollinated species is quantitatively similar to that in nectar-rewarding flowers with single pollen units (PTE = 1-2%), and that different buzz-pollinated flower architectures achieve similar PTE although through different pollen-loss pathways.

Keywords: Anther cone; buzz pollination; floral morphology; heteranthery; pollen carryover; pollen transfer and deposition; *Solanum* (Solanaceae)

INTRODUCTION

Pollen transfer from anthers to stigmas is a major determinant of plant fitness (Minnaar et al., 2019; Christopher et al., 2020). Plants produce a large amount of pollen per flower (Cruden, 2000), yet very few pollen grains produced in anthers ever make it to their destination. For instance, it is estimated that 2.4% of pollen grains produced by animal-pollinated plants with pollen grains released as single units (monads) reach the stigma of conspecific individuals (Harder and Thomson, 1989; Johnson and Harder, 2023). Pollen fates can be decomposed in three phases: pollen production and presentation, pollen

transfer, and pollen germination (Minnaar et al., 2019). Among these, pollen transfer, the pathway between pollen release and pollen deposition, is highly stochastic and mediated by the behavior of pollinators in animal-pollinated plants (Thomson and Plowright, 1980; Richards et al., 2009) (Figure 1). However, despite its importance for plant reproductive success, studies of pollen transfer are scarce and often focused on species with pollen packed in pollinia such as in orchids (Minnaar et al., 2019). The pathway followed by pollen is especially hazardous for plants giving pollen as the only reward, as floral visitors actively collect pollen grains during floral visitation and might further reduce the pool of pollen remaining for fertilizing other flowers (Luo et al., 2008; Vallejo-Marín et al., 2009).

Pollen transfer efficiency (PTE) measures the proportion of pollen removed from the anthers that successfully reaches conspecific stigmas and is therefore available for potential ovule fertilization (Johnson and Harder, 2023). In animal-pollinated species, PTE usually decreases as the amount of pollen released per visit increases (Harder and Thomson, 1989; Harder, 1990), giving plants with gradual pollen release or pollen packaging strategies a fitness advantage. The Pollen Presentation Theory (Thomson, 2006) suggests that natural selection via male fitness should favor traits that regulate pollen dispensing and/or restrict pollen removed per visit (Thomson, 2006).

In addition to PTE, a key characteristic affecting the fate of pollen and its consequences for plant fitness is the amount of pollen deposited on each stigma in a consecutive sequence of recipient flowers, i.e., the shape of its pollen deposition curve (pollen carryover curve; Thomson and Plowright 1980). Pollen deposition curves tend to show an exponential decay pattern, but most studies have found longer tails than expected under an exponential model (i.e., leptokurtic distribution) due to long dispersal events (Lertzman, 1981; Price and Waser, 1982; Morris et al., 1994; Santa-Martinez et al., 2021). The slope of the curve varies with pollinator type (Castellanos et al., 2003; Santa-Martinez et al., 2021), type of flower, and reward availability in the recipient flower (Thomson and Plowright, 1980). Deposition curves have been studied in hummingbird pollinated *Ipomopsis* and *Castilleja* (e.g., Lertzman, 1981; Price and Waser, 1982; Morris et al., 1994), and in bee-pollinated species (e.g., Thomson and Plowright, 1980, Castellanos et al., 2003; Santa-Martinez et al., 2021). These studies have shown that grooming behavior plays a crucial role in determining the steepness of the curve. However, pollen deposition curves have rarely been estimated for nectarless plants that use pollen as the only reward to floral visitors. Given that in these plants pollen serves both as a vehicle for male gametes and simultaneously as a reward to potential pollinators (Vallejo-Marín et al., 2009), pollen deposition curves could have a particularly steep slope if pollinators are efficient at grooming pollen following a visit.

Here, we characterize and compare the removal, dispersal, and transfer efficiency of pollen (pollen fates) in two species of nectarless, bee-pollinated species of *Solanum* (Solanaceae). As most of the other ~1,200 described *Solanum* species (Hilgenhof et al., 2023), the species studied here are buzz pollinated, i.e., they are pollinated by pollen-foraging bees that use vibrations to harvest pollen from their tube-like poricidal anthers (Buchmann, 1983; Vallejo-Marín and Russell, 2024). To characterise pollen fates in these two species, we exposed experimental flowers to visits by a generalist buzz-pollinating bee, *Bombus terrestris* (Apidae). The specific objectives of our research were: (1) To quantify pollen fates and pollen transfer efficiency of two *Solanum* species with contrasting size and floral architecture (*Solanum rostratum* and *S. dulcamara*), and (2) to estimate the pollen deposition curves for both species. Our study provides a starting point for establishing pollen fates in nectarless, buzz pollinated species, and to investigate how floral architecture may influence pollen removal and transfer efficiency.

MATERIALS AND METHODS

Plants

As most other *Solanum* species, the ones studied here have bisexual and nectarless flowers, with five poricidal stamens (anthers that open through small apical pores). However, the species studied here differ markedly in their stamen shape and architecture (i.e., the shape and arrangement of anthers within the flower). *Solanum dulcamara* is a perennial vine native to Eurasia and introduced into North America (Knapp et al., 2013). Its flowers are actinomorphic, relatively small (1.5-2 cm diameter) and have monomorphic anthers that are fused into an anther cone that surrounds the centrally-placed pistil (Figure 2A) (Glover et al., 2004). In Europe, *S. dulcamara* is visited by different species of bumblebees (Free, 1970), it is self-compatible, but its mating system is unknown. Conversely, *S. rostratum* is an annual herb native to Mexico and perhaps southern USA, and introduced in disturbed habitats around the world (Zhao et al., 2013). Flowers are medium-sized (2.3-3.5 cm diameter) and have two morphologically and functionally distinct sets of stamens in the same flower (i.e., heteranthery): one set is composed of four smaller, bright yellow ‘feeding’ anthers centrally located, and the other set includes a single larger, yellow to brown ‘pollinating’ anther that is laterally displaced, opposite to the style (Figure 2B) (Vallejo-Marín et al., 2009). *Solanum rostratum* is also enantiostylous, i.e., each individual produces flowers with the style deflected either to the right- or left-hand side; this type of enantiostyly

is thought to increase pollen export between flowers of reciprocal morphs (Jesson and Barrett, 2002). In its native and introduced range, *S. rostratum* is visited by different types of bees, including bumblebees (*Bombus spp.*) (Bowers, 1975; Solís-Montero et al., 2015). Genetic estimation of outcrossing rates, indicates that *S. rostratum* is predominantly outcrossing (t = 0.70) in both native and introduced ranges (Vallejo-Marín et al., 2013; Zhang et al., 2017). For our study, plants of *S. rostratum and S. dulcamara* were grown from field-collected seeds at the glasshouses in Uppsala University. For *S. rostratum,* we used seeds from accessions 10s71-5, 10s74, 10s77, 10s79, and 10s80 (San Miguel de Allende, Guanajuato, Mexico), and 11s182, 11s188, 11s191, 11s205, and 11s207 (Dolores, Guanajuato, Mexico). For *S. dulcamara,* we used seeds from accessions 22-UPP-1 and 22-UPP-2 collected at Uppsala. Seeds were germinated as described in Vallejo-Marín et al. (2014), grown at 16h/20°C light and 8h/16°C dark cycles, and fertilised with general purpose liquid fertiliser.

Pollinators

For evaluating pollen transfer, we used *Bombus terrestris*, a generalist, buzz-pollinating bumblebee that is widely distributed in Europe and which is commonly used to supplement pollination of soft-fruit crops. *Bombus terrestris* visits *S. dulcamara* in the native range and is similar in size and behaviour to other bumblebees visiting *S. rostratum* in the Americas (Bowers, 1975; M. Vallejo-Marín, Uppsala University, personal observation). We used individuals from two commercial colonies (Koppert Biological Systems, Helsingborg, Sweden; and Biobest Group, Westerlo, Belgium). Colonies were maintained on 2 M solution of sucrose available from artificial feeders within enclosed foraging arenas (82×60×60 cm), follow Russell et al. (2017). Twice a week, bees were also provided with approximately 10g of “pollen dough” made of pulverised honeybee-collected pollen (Koppert Biological Systems) mixed with sucrose solution and placed inside the colony. The foraging arenas were kept at room temperature and illuminated with an LED ceiling panel set to a 14 h:10 h light:dark cycle. Before the experiment, we conditioned the bees to visit *Solanum* flowers by allowing them to forage freely on flowers placed in the foraging arena. This conditioning phase was never done on the same day of the experimental trials to avoid bees carrying over pollen grain collected during the conditioning phase to the experimental trials.

Experimental trials

Before each trial, fresh flowers were collected from the greenhouse each morning before experiments and carefully transported in floral foam (OASIS Floral Products, Washington, UK) within a closed plastic

146 box. Each trial consisted of one donor flower from which pollen could be removed by the bee, and four
147 recipients (R1-R4) that were experimentally prevented from releasing pollen by sealing the anther pores
148 using a small drop of PVA glue (Elmer's Products, Ohio, USA) under a stereoscopic microscope. A similar
149 amount of glue was applied just below the anther pore of the donor flowers as a sham treatment. After
150 the glue dried, we mounted each flower on a bamboo stick with Blu-Tack adhesive gum (Bostik,
151 Staffordshire, UK) and placed it in a plastic tube containing floral foam. A black plate was placed under
152 the donor flower to recover pollen falling from the flower (dislodged pollen). To control for differences
153 in pollen transfer within and between morphs of enantiostylous *S. rostratum*, we set all recipients to
154 have the reciprocal mirror-image morph of the donor flower (i.e., the left-hand side morph for all
155 recipients if the donor was a right-hand flower, and vice versa).
156 For each trial, one donor flower was placed in the centre of the foraging arena and a single bee
157 was allowed to enter the arena. Immediately after the bee landed on the donor flower, we placed the
158 first recipient flower (R1) next to it in the arena and removed the donor flower from the arena as soon
159 as the bee departed from it. We proceeded like so with the consecutive recipients (R2, R3 and R4). If
160 after 10 minutes the bee had not landed on the donor flower, we ended the trial and removed the bee.
161 All trials were video recorded using a camera (Canon EOS M50, Tokyo, Japan) placed right outside of the
162 arena. Recordings were used to calculate the duration of each flower visit. Temperature, humidity of the
163 arena and time of the day were recorded.

164 **Pollen sampling and counting**

165 After each trial, the stigmas of the four recipients were individually cut and placed on fuchsin jelly cubes
166 (32 mm3) (Kearns and Inouye, 1993). Dislodged pollen was collected from the black plate using 1-2
167 cubes of jelly, and anthers from the donor flower were cut and preserved in 1ml of 70% ethanol. Under
168 a stereoscopic microscope, we removed the pollen in the corbiculae of the bee and placed it on a jelly
169 cube and collected pollen on the body of the bee with a separate jelly cube. Each sample was stored in
170 individual 1.5 mL Eppendorf tubes. Each fuchsin jelly cube containing a stigma sample was mounted on a
171 slide and examined under a light microscope at 400x magnification for pollen grain counting. To process
172 the remaining pollen samples, 500 µL of 70% ethanol was added to tubes containing pollen from the
173 ground, corbiculae, and body. Donor anthers were subjected to sonication using an Elmasonic S40H
174 ultrasonic cleaner (Elma Schmidbauer GmbH, Singen, Germany), followed by staining pollen grains with
175 200 µL of lactoglycerol-aniline blue (Kearns and Inouye, 1993). For pollen grain counting, an aliquot was
176 taken from each tube and placed onto a hemocytometer chamber until 200 pollen grains were counted.

To estimate the number of pollen grains, we considered the combined volumes of ethanol, jelly, and aniline used in their respective cases. After the trial concluded, we estimated: (C) the number of pollen grains remaining of the donor anthers, (D) number of pollen grains that fell on the ground, (F) the number of pollen grains on the bee's corbiculae and (G) on the bee's body, and (H) the number of pollen grains deposited on the stigma of each recipient. From these variables we also calculated the following derived variables: total pollen production (A = D + F + G + H), pollen released (B = F + G + H + D), pollen collected (E = F + G + H), and pollen lost from the pollination process (I = D + F).

Statistical analyses

Proportion of pollen grains deposited on the body or the stigma were calculated from the total pollen production and from the amount of pollen released. To determine if the proportions of pollen grains deposited on body and stigmas varied between species, we first verified the assumptions of normality and homoscedasticity using the Shapiro-Wilk and Levene's tests from *stats* (R Core Team, 2023) and *car* (Fox and Weisberg, 2019) packages, respectively. These assumptions were tested using absolute values and the log transformations from both species separately. In all cases, at least one of the assumptions was not met. Thus, we compared each variable using a non-parametric Wilcoxon test (R Core Team, 2023).

Pollen deposition curves (Thomson and Plowright, 1980) were analyzed using Generalized Linear Mixed-effects Models (GLMM) with a Poisson error (Log-link) using the *glmer* function (*lme4*) (Bates et al., 2014). First, we built a model using the number of pollen grains deposited in each recipient flower was the response variable, recipient order (R1-R4), species, and visit time were considered as fixed effects, and trial number as a random effect. Second, we analyzed a similar GLMM model as above but adding the number of removed pollen grains as an offset variable. This allowed us to compare pollen deposition curves independently of the absolute number of pollen grains removed from the donor. Back transformation and visualization were conducted using the *sjPlot* package (Lüdecke et al., 2018). We assessed overdispersion and zero inflation using the *DHARMa* package (Hartig, 2018). All statistical analyses were done in R v.4.1.0 (R Core Team, 2021) using *RStudio* (RStudio Team, 2021).

RESULTS

Pollen fates and pollen transfer efficiency

The absolute numbers of pollen grains in each stage of pollen transfer are presented in Figure 3. We found that *Solanum rostratum* flowers produce on average 27,59% more pollen grains per flower than *S. dulcamara* flowers (Figure 4). However, after a single visit, a bee removed on average 37.5% of the flower's total production of pollen grains in *S. dulcamara*, and only 8.91% in a *S. rostratum*. This is equivalent to the removal of about four times as many pollen grains in *S. dulcamara* than in *S. rostratum* in absolute numbers (Table 1). From the pollen released by the flowers, between 19.12% (*S. dulcamara*) and 67.93% (*S. rostratum*) fell to the ground. In addition to the pollen wasted on the ground, bees collected in their corbiculae, on average, 65.82% of released pollen grains for *S. dulcamara* and 24.03% for *S. rostratum*. The stigmas of recipient flowers received 1 860 $\pm$ 370 (mean $\pm$ se; *S. dulcamara*) and 356 $\pm$ 93 (*S. rostratum*) pollen grains. Thus, only approximately 8.03% (*S. rostratum*) to 15.06% (*S. dulcamara*) of released pollen grains remain available for pollination (pollen on stigmas and body) (Table 1).

The pollen transfer efficiency (PTE; i.e., the percentage of released pollen grains that reach recipient's stigmas) was 1.07% for *S. dulcamara* and 0.72% for *S. rostratum* (Figure 5). The percentage of pollen remaining on the body was not significantly different between the two species N=19, U=44, P = 0.968). Most of the pollen was lost on the ground (67.93% in *S. rostratum*) or in the corbiculae (65.82% in *S. dulcamara*) (Figure 5). There was a significant difference in the percentage of pollen lost in the corbiculae and the dislodged pollen between species (N=19, U = 77, P = 0.008; N=19, U = 5, P < 0.001, respectively). However, the percentage of all the pollen lost (from released pollen) was not significantly different between both species (N=19, U = 43, P = 0.9).

Pollen deposition curves

As expected, most pollen from the donor was deposited in the first recipient flower visited, strongly decreasing with the increase of recipient order (Figure 6 and 7B; Table 2, Z = -43.19, P < 0.001 and Z = -42.53, P < 0.001, for analyses with and without using pollen released as an offset, respectively). We found a statistically significant difference in the average number of pollen grains per recipient stigma between *Solanum* species with fewer pollen grains deposited on *S. rostratum* than in *S. dulcamara* (Figure 6; Table 2, Z = -3.65, P < 0.001). However, our analysis of the relative rates of pollen deposition

232 (using the number of pollen grains released as an offset variable) suggests that both species deposit
233 approximately the same proportion of pollen grains (Figure 7B; Table 2, Z = 0.01, P = 0.996). The
234 relationship between recipient order and pollen deposited differed between species, with *S. rostratum*
235 exhibiting a steeper pollen deposition curve, as indicated by the statistically significant interaction
236 between recipient order and flower species (Table 2, Z = -7.23, P < 0.001 and Z = -7.89, P < 0.001, for
237 analyses with and without using pollen released as an offset, respectively). We also found that longer
238 visits resulted in more pollen deposited per stigma (Figure 7A; Table 2, Z = 30.24, P < 0.001 and Z =
239 30.02, P < 0.001, with and without pollen released as an offset, respectively).

240 DISCUSSION

241 Our study's main findings can be summarised as: (1) Both buzz-pollinated species of *Solanum* studied
242 here have a similar or lower values of pollen transfer efficiency (PTE) as those reported for other non-
243 buzz pollinated plants that disperse pollen in individual pollen grain units (monads), (2) pollen
244 deposition curves follow a classic exponential decay pattern, as in other animal-pollinated plants, and
245 (3) although both *Solanum* species studied showed a similar percentage of pollen loss, the pathway to
246 this loss differs and is either lost on the ground (*S. rostratum*) or in the bees' corbiculae (*S. dulcamara*),
247 potentially reflecting the functional effects of different flower architectures.

248 Pollen transfer efficiency in buzz-pollinated flowers

249 Buzz pollinated species are thought to produce large numbers of pollen grains compared to nectar-
250 producing relatives as a mechanism to offset the costs of active pollen collection by floral visitors (Vogel,
251 1978; De Luca and Vallejo-Marín, 2013; Vallejo-Marín and Russell, 2024). The average pollen production
252 per flower is indeed high in *S. dulcamara* (>600,000) and *S. rostratum* (>1,000,000), and comparable to
253 other nectarless plant species (Vallejo-Marín et al., 2009; Vallejo-Marín et al., 2014; Vallejo-Marín et al.,
254 2022). In our experiment, visitation by a generalist, buzz-pollinating bumblebee resulted in a pollen
255 transfer efficiency (PTE) of between 0.72 and 1.07%, which is towards the lower end of the 1-2% PTE
256 range reported for other species with monads (Johnson and Harder, 2023). Pollen transfer efficiency has
257 only been rarely quantified for buzz pollinated plants, but low PTE characterizes two previously studied
258 species. *Rhexia virginica* (Melastomataceae), an herbaceous species distributed in eastern North
259 America, has a PTE of 0.92% (Supplementary material from Johnson and Harder, 2023), while
260 *Senna reticulata* a neotropical tree, has a much lower PTE (0.05%) (Supplementary material from

261 Johnson and Harder, 2023). These relatively low values of PTE in buzz pollinated species might suggest
262 that active pollen collection indeed impacts the realized efficiency of pollen transfer, and that higher
263 rates of total pollen production are selected for to ensure delivery of sufficiently high numbers of pollen
264 grains to fertilize all ovules. However, further work comparing total pollen production and PTE in buzz
265 pollinated taxa and their closest non-buzz pollinated relatives are required to establish if this is a general
266 pattern. Furthermore, PTE can vary among different species of floral visitors, and this is an area that
267 requires additional study.

268 Although both *Solanum* species studied here achieve a similar PTE, the pathways of pollen
269 production, release, and loss differ (Figure 5). Percentage of pollen released in other buzz pollinated
270 species ranges widely from 10-60% (*Rhexia virginica*: 10–43%; Larson and Barrett, 1999;
271 *Senna reticulata*: 25%; Snow and Roubik, 1987; *Erica tetralix* and *Vaccinum myrtillus:* 28–60%; Moquet
272 et al., 2017), and is similar or lower than in non-buzz-pollinated species such as *Aconitum septentrionale*
273 (44-61%; Thøstesen and Olesen, 1996), *Malus domestica* and *Prunus dulcis* (40-50%; Thomson and
274 Goodell, 2001), and *Calluna vulgaris* (≤ 88%; Moquet et al., 2017). The small-flowered *S. dulcamara*
275 produces fewer pollen grains than *S. rostratum* but releases a higher number (and percentage) of pollen
276 grains after a single visit by a bumblebee. If all pollen produced in the flower is available for removal,
277 about 11 visits would be required to empty a flower of *S. rostratum* (8.9% of total pollen removed per
278 visit), while in *S. dulcamara* fewer than three visits would be needed (37.5% pollen released per visit).
279 However, pollen inside the anther may not all be available to be removed at first, and it appears that
280 some buzz pollinated flowers present a staggered release of pollen as the flower ages (Harder and
281 Barclay, 1994; Kemp and Vallejo-Marín, 2021). The extent to which pollen becomes available
282 simultaneously or in a staggered fashion in buzz pollinated species is not well understood. Regardless of
283 the mechanism, the high rates of pollen release observed in *S. dulcamara,* characterize other small-
284 flowered, non-heterantherous taxa in *Solanum* Section *Androceras* and this has been interpreted as a
285 consequence to a shift of mating system towards more selfing (Kemp and Vallejo-Marín, 2021). The
286 mating system of *S. dulcamara* is presently unknown, but this self-compatible species sometimes sets
287 fruit autonomously (M. Vallejo-Marín, Uppsala University, personal observation). In contrast, although
288 *S. rostratum* is also self-compatible, it has a high outcrossing rate (0.70-0.90) (Vallejo-Marín et al., 2013;
289 Morra-Carrera et al., 2019) and rarely sets fruits autonomously, at least in the native range. Thus, the
290 difference in the proportion of pollen removed per visit could be associated to different floral
291 morphologies and mating systems in *S. dulcamara* and *S. rostratum*. For outcrossing species visited
292 regularly by pollinators, a more restricted pollen schedule might allow them to dispense pollen more

293 efficiently or to more flowers (Harder and Thomson, 1989; Harder and Wilson, 1994; Kemp and Vallejo-
294 Marín, 2021).
295 Although both species end up exporting similar proportion of pollen grains to stigmas, the
296 pathway to pollen loss differs: In *S. dulcamara*, pollen is mostly loss to bees collecting it and placing it in
297 their corbiculae, while in *S. rostratum,* pollen is mostly loss by falling on to the ground, meaning that this
298 removed pollen does not contribute to the fitness of either bee or plant. What is the reason for these
299 contrasting pollen fates in these two species? With only two species studied, it is difficult to generalize,
300 but in *S. dulcamara* the bees can curl their body in close proximity of the anther pores while buzzing the
301 flower. The fused anther cone of *S. dulcamara* then works as a single pollen dispensing unit that places
302 pollen grains in the ventral region of the bee where it can be groomed and placed in the corbiculae
303 (Nevard and Vallejo-Marín, 2022). In contrast, in heterantherous *S. rostratum*, the bee cannot work
304 simultaneously both feeding and pollinating anthers, and it usually focuses its attention on the feeding
305 anthers. The feeding anthers contact the bee on the ventral side, while the pollinating anthers contact
306 the bee on the dorsal side of the abdomen, where it is harder to groom by the bee. The body of the bee
307 often contacts the terminal pores of the feeding anthers, but the distance between the bee's body and
308 the terminal pore of the pollinating anther depends both on the positioning of the bee and on the
309 relative size of bee and flower. Because approximately 50% of the total pollen production in these
310 flowers resides on the single pollinating anther (Vallejo-Marín et al., 2009; Vallejo-Marín et al., 2014),
311 flower handling and bee-flower size matching can then affect significantly whether the pollen reaches
312 the bee's body or falls to the ground. Although we did not quantify this in our experiment, we expect
313 that much of the pollen loss occurring during buzz pollination in *S. rostratum* is due to mismatches
314 between the size of the bee and the flower. All else being equal, a bee of similar characteristics to the
315 ones studied here might benefit more from a single visit to a species like *S. dulcamara* than one like
316 *S. rostratum.* Of course, differences in total pollen production, and number of flowers per plant might
317 help offset the increased difficulty of gathering pollen from flowers with a more complex morphology as
318 in *S. rostratum*.

319 **Pollen deposition curves**

320 Our results demonstrate that the pollen deposition curves (i.e., the pattern of pollen deposition on the
321 stigmas of consecutive recipients) for the buzz-pollinated *Solanum* species studied follow a typical
322 exponential decay pattern, as reported for other species (Thomson and Plowright, 1980; Mitchell et al.,
323 2013; Santa-Martinez et al., 2021). Specifically, the amount of pollen deposited on stigmas decreased by

27.6% and 36.9% with each successive recipient for *S. dulcamara* and *S. rostratum*, respectively. These percentages are on the higher end of the spectrum for pollen carryover curves, which ranges from 50% to 0.5% (see Table 1 in Robertson, 1992). This indicates a relatively steep decline in deposited pollen with each successive recipient. Generally, grooming pollinators such as bees exhibit steeper deposition curves, indicating less extensive pollen carryover, compared to pollinators like hummingbirds and hawkmoths (Robertson, 1992; Castellanos, 2003; Mitchell et al., 2013). However, this pattern has not been studied in other buzz-pollinated plants, highlighting a gap in our understanding of these systems.

Pollen deposition curves are influenced by both the amount of pollen that lands on the pollinator's body and the intensity of its grooming before visiting the next flower. Buzz-pollinated *Solanum* flowers, which produce and gradually release large amounts of pollen as a reward for pollinators, tend to encourage more intense grooming behavior compared to nectar-producing, non-buzz-pollinated flowers (Harder, 1990; Johnson and Harder, 2023). This intense grooming results in steeper pollen deposition curves (Holmquist et al., 2012), potentially influencing gene flow patterns (Santa-Martinez et al., 2021). Consequently, if buzz-pollinated flowers generally exhibit steeper pollen deposition curves, they might experience more restricted gene flow compared to non-buzz-pollinated species offering other rewards, such as nectar. While insect-pollinated species often display higher levels of population genetic structure (Dellinger et al., 2022; Gamba and Muchhala, 2020; Gamba and Muchhala, 2022), the specific impact of buzz pollination on genetic structure remains underexplored

CONCLUSIONS

Empirical studies of pollen fates, although experimentally complex and time-consuming, provide an opportunity to illuminate a key process affecting the reproductive success of plants. Our study found significant differences in pollen fates between *S. dulcamara* and S*. rostratum*, highlighting the impact of floral architecture on pollen transfer, even among species visited by the same buzz-pollinating bee. Specifically, we observed distinct pollen deposition curves and pathways of pollen loss between species. *S. rostratum* primarily loses pollen through dislodging due to its restricted dispensing schedule, while *S. dulcamara* experiences significant pollen loss from bee grooming. Despite these differences, both species achieve comparable pollen transfer efficiency (PTE). These findings highlight the role of floral morphology on pollen transfer dynamics and suggest that different floral architectures can lead to similar reproductive outcomes. Future research should encompass a wider range of species with diverse

morphological traits and reward types and explore how different pollen pathways affect realized female and male fitness.

ACKNOWLEDGMENTS

We thank D. Scaccabarozzi and L. van Kolfschoten for their technical support and advice throughout the development of this work. We are also grateful to the Research Experience for Peruvian Undergraduates (REPU) Program and the University of Lausanne exchange program for enabling A.V.C., E.M., and B.G.S. to carry out this project at Uppsala University. This work was partly supported by start-up funds from the Plant Ecology and Evolution program at Uppsala University, awarded to M.V.M.

AUTHOR CONTRIBUTIONS

A.V.C., E.M., B.G.S., and M.V.M conceived the study. A.V.C., E.M. and B.G.S. conducted the experiments, collected the data and performed the analyses. A.V.C., E.M., B.G.S., and M.V.M wrote and revised the manuscript. M.V.M supervised the study and acquired funding.

DATA AVAILABILITY STATEMENT

Pollen transfer data used for the analyses and the R script will be made available upon acceptance.

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512 # TABLES

513 **Table 1**. Estimated number of pollen grains (mean ± se) at different stages of the pollination process in
514 *Solanum dulcamara* and *S. rostratum*.

	S. dulcamara		***S. rostratum***	
	N	**Number of pollen grains**	**N**	**Number of pollen grains**
Total pollen production (A)	9	641 780 ± 92 979	10	1 065 712 ± 116 142
Pollen release phase				
Pollen released (B)	9	243 323 ± 49 331	10	86 732 ± 21 423
Remaining pollen (C)	10	378 642 ± 65 596	10	1 000 891 ± 121 491
Pollen transfer phase				
Pollen on ground/dislodged (D)	9	41 177 + 19 734	10	53 561 + 17 369
Pollen collected (E)	9	202 146 + 51 589	10	29 358 + 9 222
Pollen on the pollinator				
Pollen in corbiculae (F)	9	188 601 ± 53 757	10	24 121 ± 8 634
Pollen on body (G)	9	11 685 + 4466	10	4 892 + 2198
Pollen deposition phase				
Pollen deposited on stigmas (H)	9	1860 + 370	10	356 + 93
Pollen loss				
Pollen on corbiculae and on the ground (I)	9	229 779 + 51 778	10	81 495 + 21 477

515

516 **Table 2.** Statistical analysis of the effect of recipient order, flower species (*S. dulcamara, S. rostratum*)
517 and duration of visit (s) on the number of pollen grains deposited on stigmas. Model estimates were
518 obtained from GLMMs with trial number as a random effect and recipient order, flower species and
519 duration of visit as fixed effects. (A) N = 73, residual df = 67; (B) N = 66, residual df = 60.

Fixed effects	**Coefficient**	**Standard error**	**Effect size (Z)**	**p-value**
(A) Pollen deposition model				
Intercept	5.801	± 0.205	28.33	<0.001
Recipient order	-0.303	± 0.007	-42.53	<0.001
Flower species (*S. rostratum*)	-1.053	± 0.289	-3.65	<0.001
Log (duration of visit (s))	0.324	± 0.011	30.02	<0.001
Recipient order: flower species (*S. rostratum*)	-0.147	± 0.019	-7.89	<0.001
(B) Pollen deposition model with offset variable				
Intercept	-6.248	± 0.317	-19.67	<0.001
Recipient order	-0.323	± 0.007	-43.19	<0.001
Flower species (*S. rostratum)*	0.002	± 0.449	0.01	0.996
Log (duration of visit (s))	0.332	± 0.011	30.24	<0.001
Recipient order: flower species (*S. rostratum*)	-0.138	± 0.019	-7.24	<0.002

520

521 # FIGURE LEGENDS

522 **Figure 1**. Pathways depicting the fates of pollen in a bee pollinated species such as the ones studied
523 here. The diagram shows pollen contained inside the anthers (yellow); pollen released that can be
524 potentially transferred to conspecific stigmas (blue); pollen lost from the fertilization process, including
525 dislodged pollen that falls to the ground and pollen stored by the bee (e.g., in corbiculae) and thus
526 unable to contribute to fertilization (red); and pollen deposited on stigmas (green).
527 **Figure 2**. (A) Flower of *S. dulcamara*, arrow indicates fused anthers (anther cone). (B) Flower of *S.*
528 *rostratum*, arrows indicate the feeding (above) and pollinating anthers (below). Photo credits: M.
529 Vallejo-Marín.
530 **Figure 3**. Diagram showing the fates of pollen of (A) *S. rostratum* and (B) *S. dulcamara*. The number of
531 pollen grains for each stage of the pollen dispersal process is specified (mean ± SE).
532 **Figure 4.** Percentage (from the total pollen production) of pollen in different paths after one visit on a
533 donor flower in *S. dulcamara* and *S. rostratum*.
534 **Figure 5.** Percentage (from the amount of pollen released from the anthers) in different paths after one
535 visit on a donor flower in *S. dulcamara* and *S. rostratum*.
536 **Figure 6.** Number of pollen grains deposited on recipient flower's stigmas as influenced by flower
537 species and recipient order. Plotted lines indicate estimated means and shaded regions indicate
538 standard errors.
539 **Figure 7.** Number of pollen grains deposited on recipient flower stigmas as influenced by (A) duration of
540 the visit in seconds, and (B) flower species and recipient order with released pollen as offset variable.
541 Plotted lines indicate estimated means and shaded regions indicate standard

FIGURES

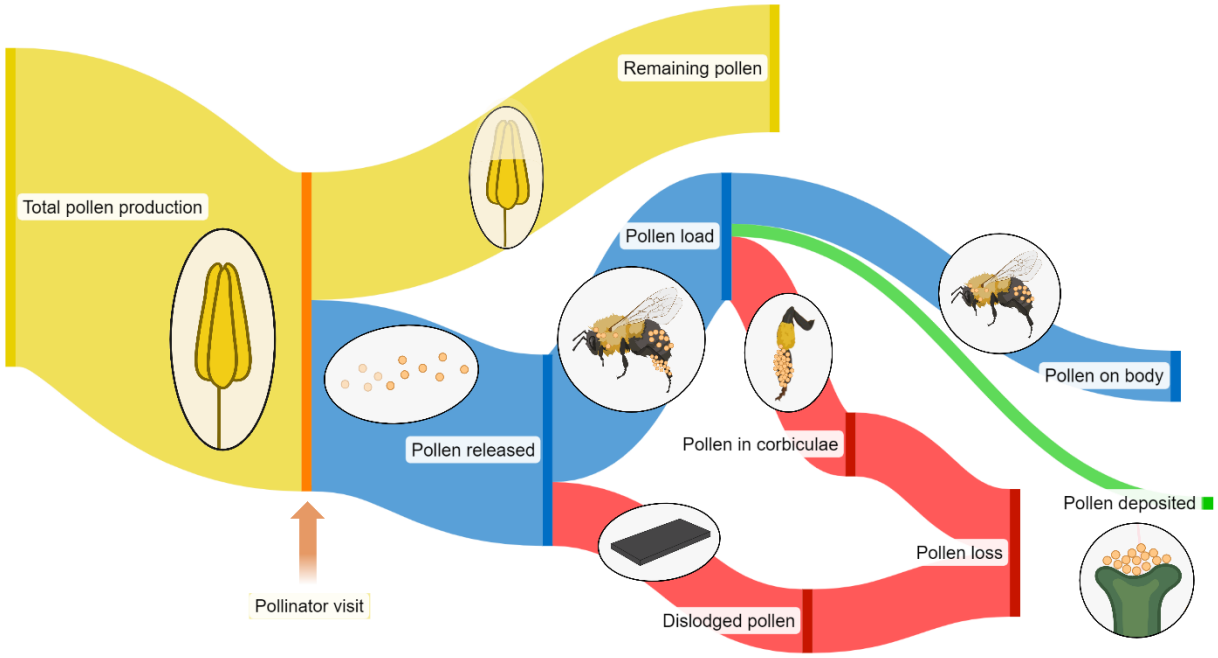

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Figure 1. Pathways depicting the fates of pollen in a bee pollinated species such as the ones studied here. The diagram shows pollen contained inside the anthers (yellow); pollen released that can be potentially transferred to conspecific stigmas (blue); pollen lost from the fertilization process, including dislodged pollen that falls to the ground and pollen stored by the bee (e.g., in corbiculae) and thus unable to contribute to fertilization (red); and pollen deposited on stigmas (green).

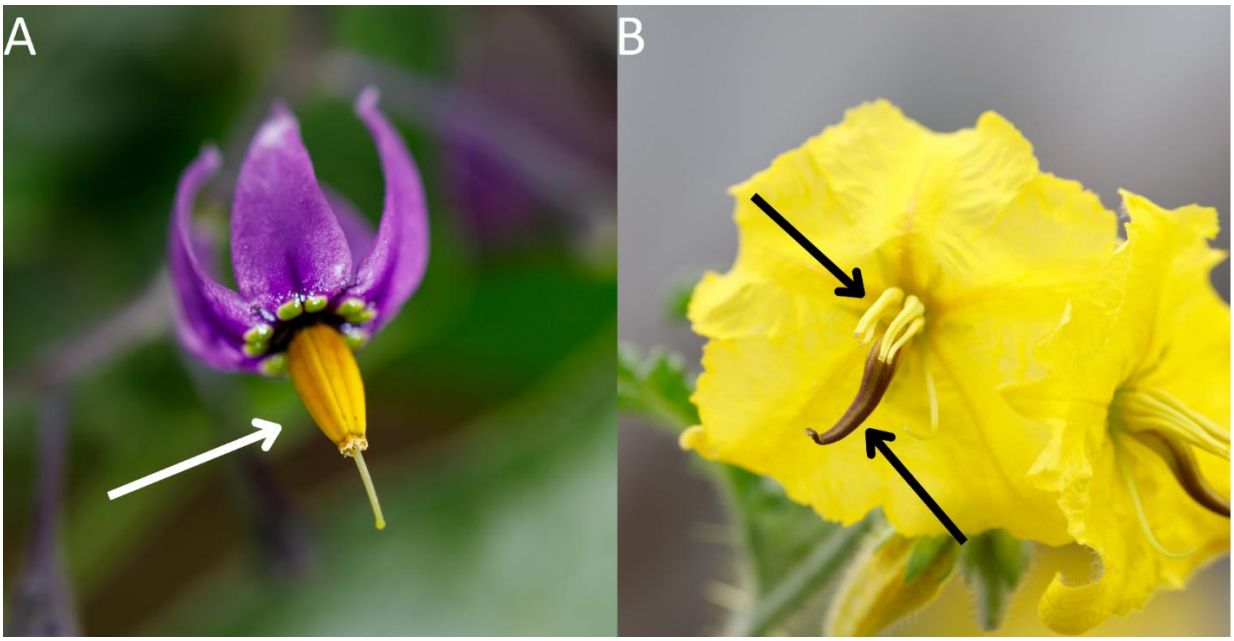

Figure 2. (A) Flower of *S. dulcamara*, arrow indicates fused anthers (anther cone). (B) Flower of *S. rostratum*, arrows indicate the feeding (above) and pollinating anthers (below). Photo credits: M. Vallejo-Marín.

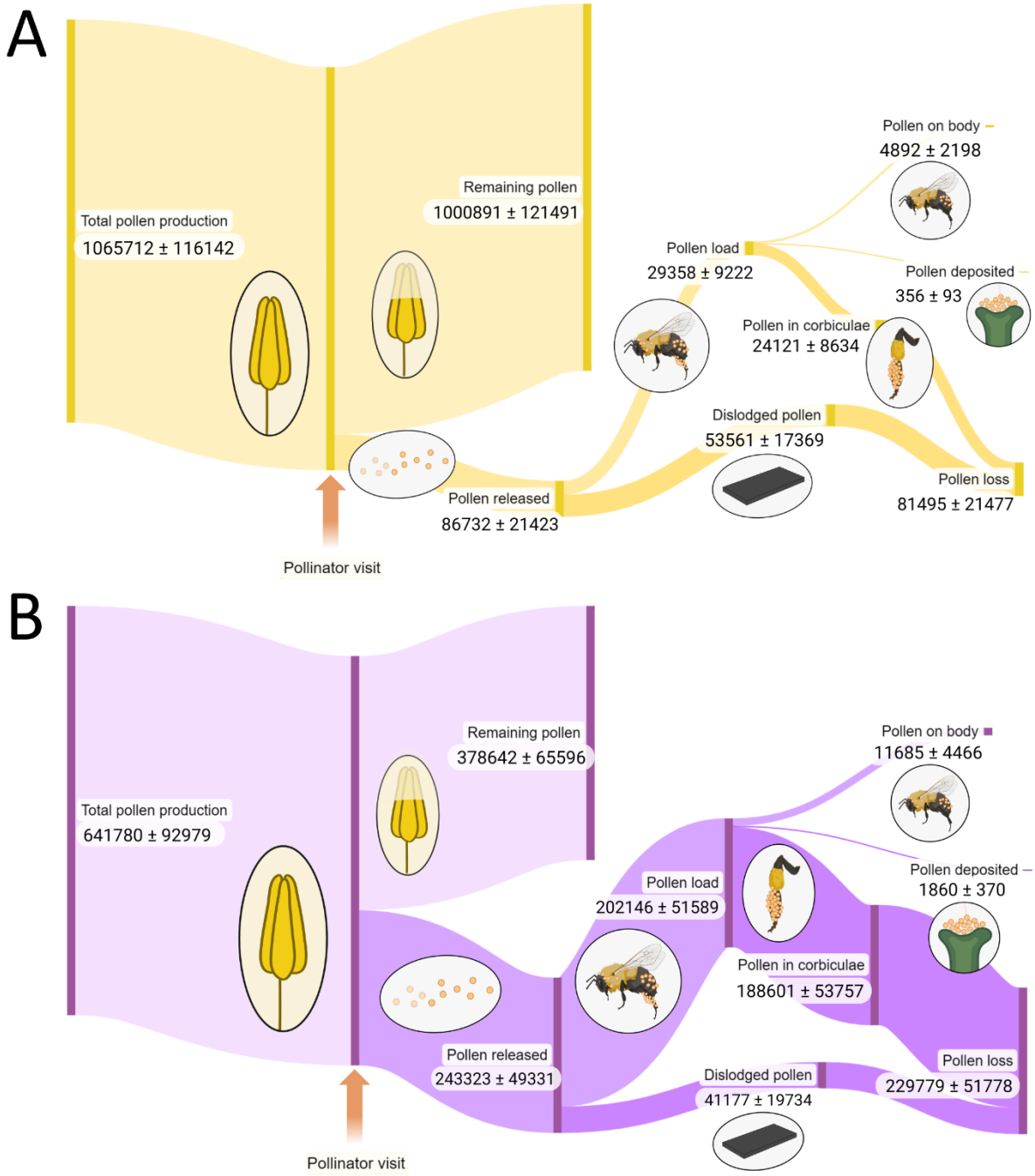

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Figure 3. Diagram showing the fates of pollen of (A) *S. rostratum* and (B) *S. dulcamara*. The number of pollen grains for each stage of the pollen dispersal process is specified (mean ± SE).

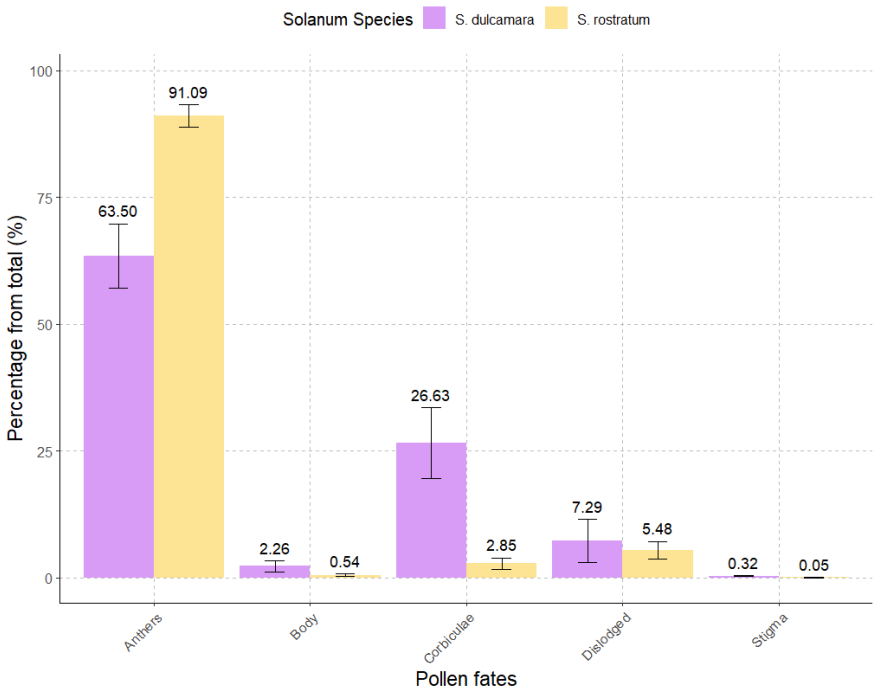


Figure 4. Percentage (from the total pollen production) of pollen in different paths after one visit on a donor flower in *S. dulcamara* and *S. rostratum*.

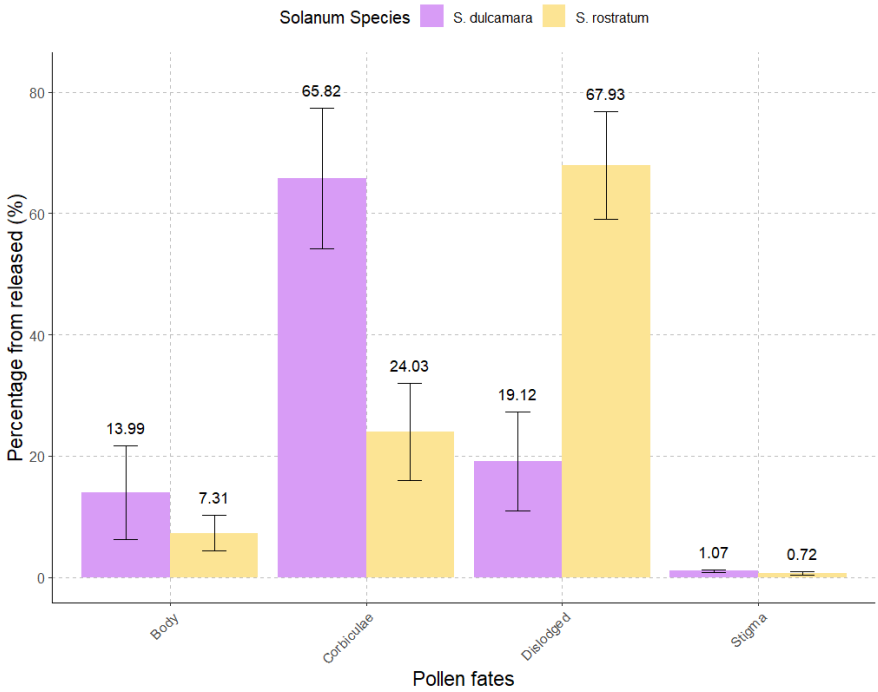


Figure 5. Percentage (from the amount of pollen released from the anthers) in different paths after one visit on a donor flower in *S. dulcamara* and *S. rostratum*.

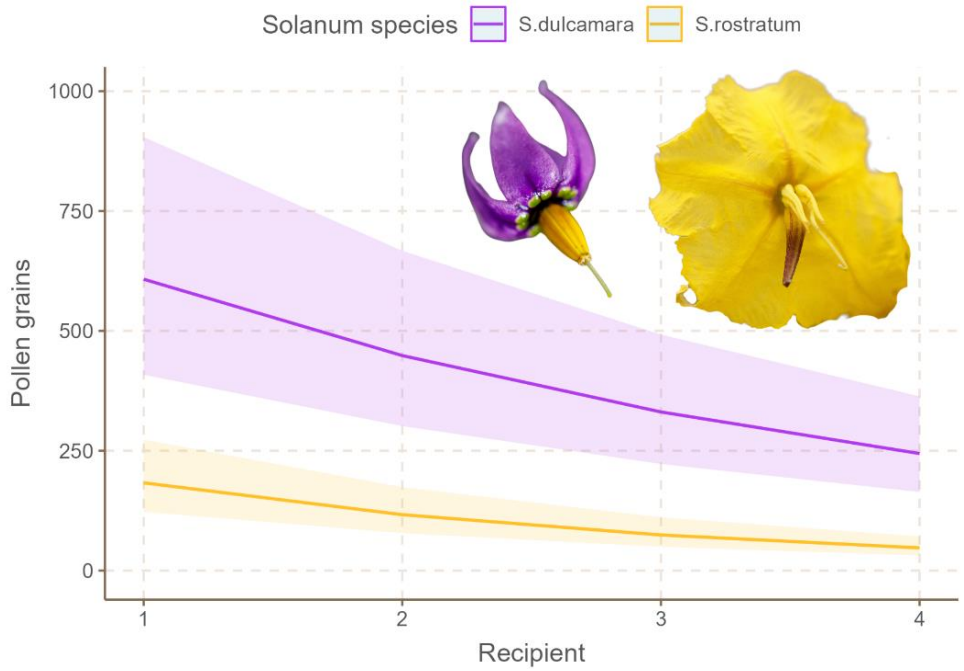


Figure 6. Number of pollen grains deposited on recipient flower stigmas as influenced by flower species and recipient order. Plotted lines indicate estimated means and shaded regions indicate standard errors.

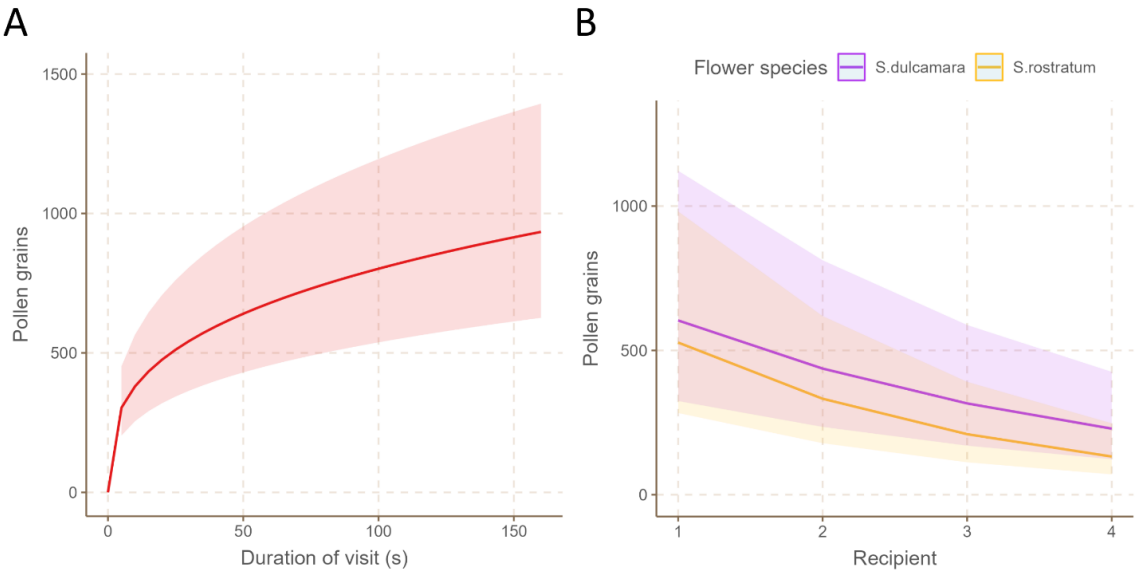
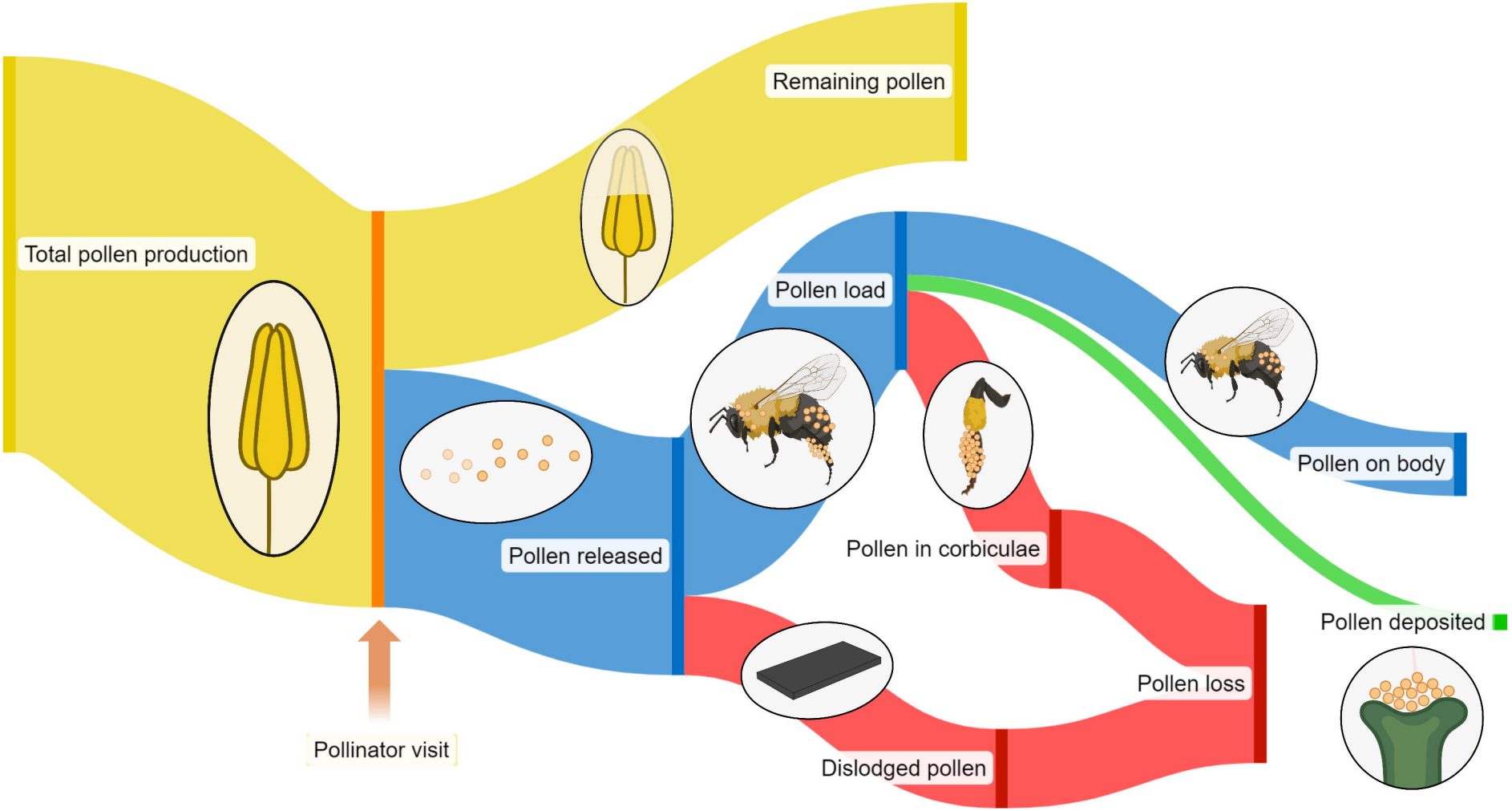
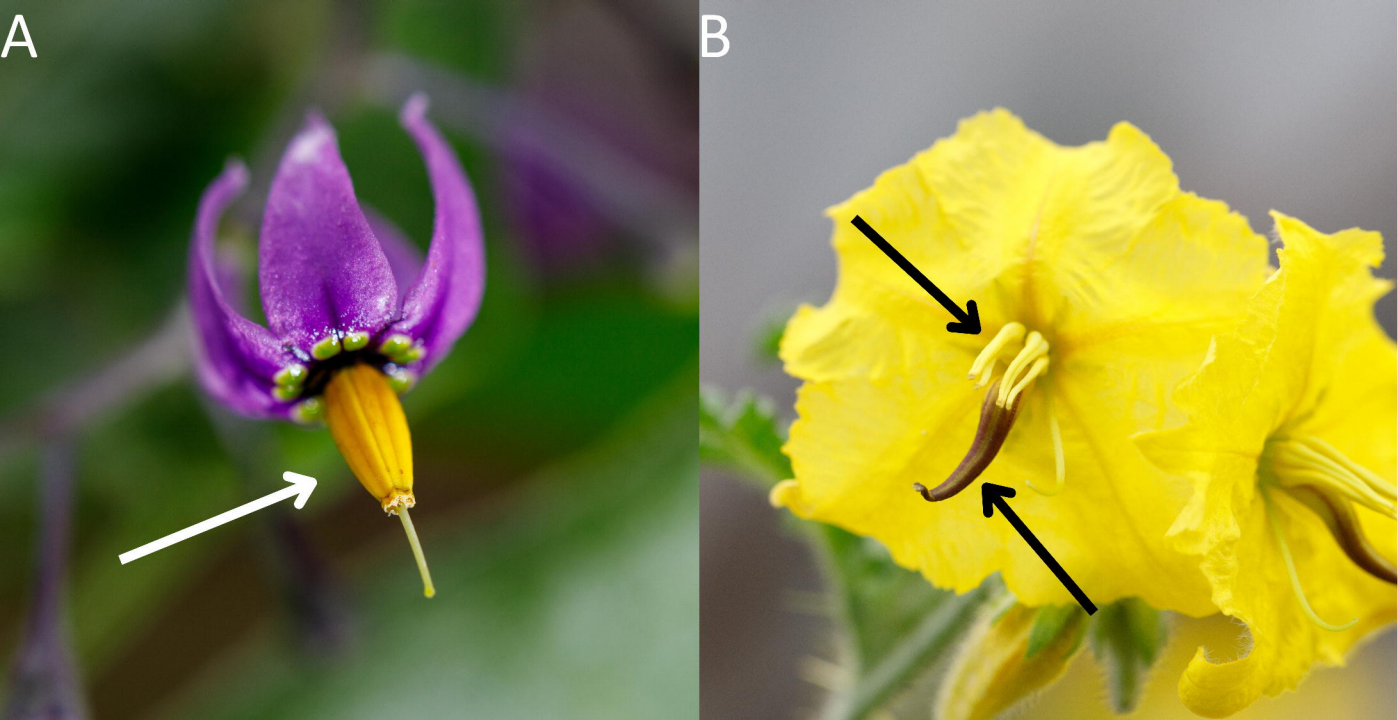
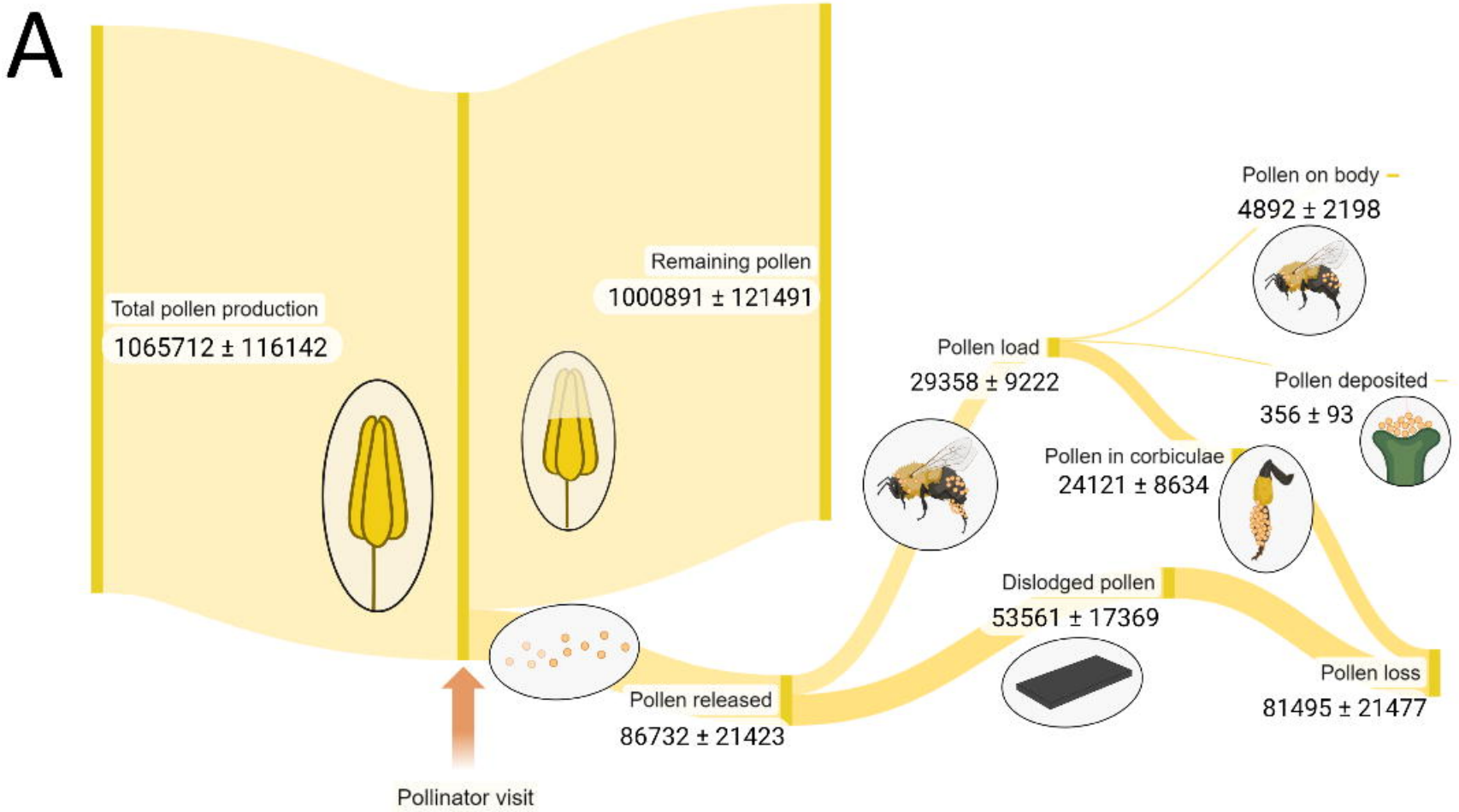
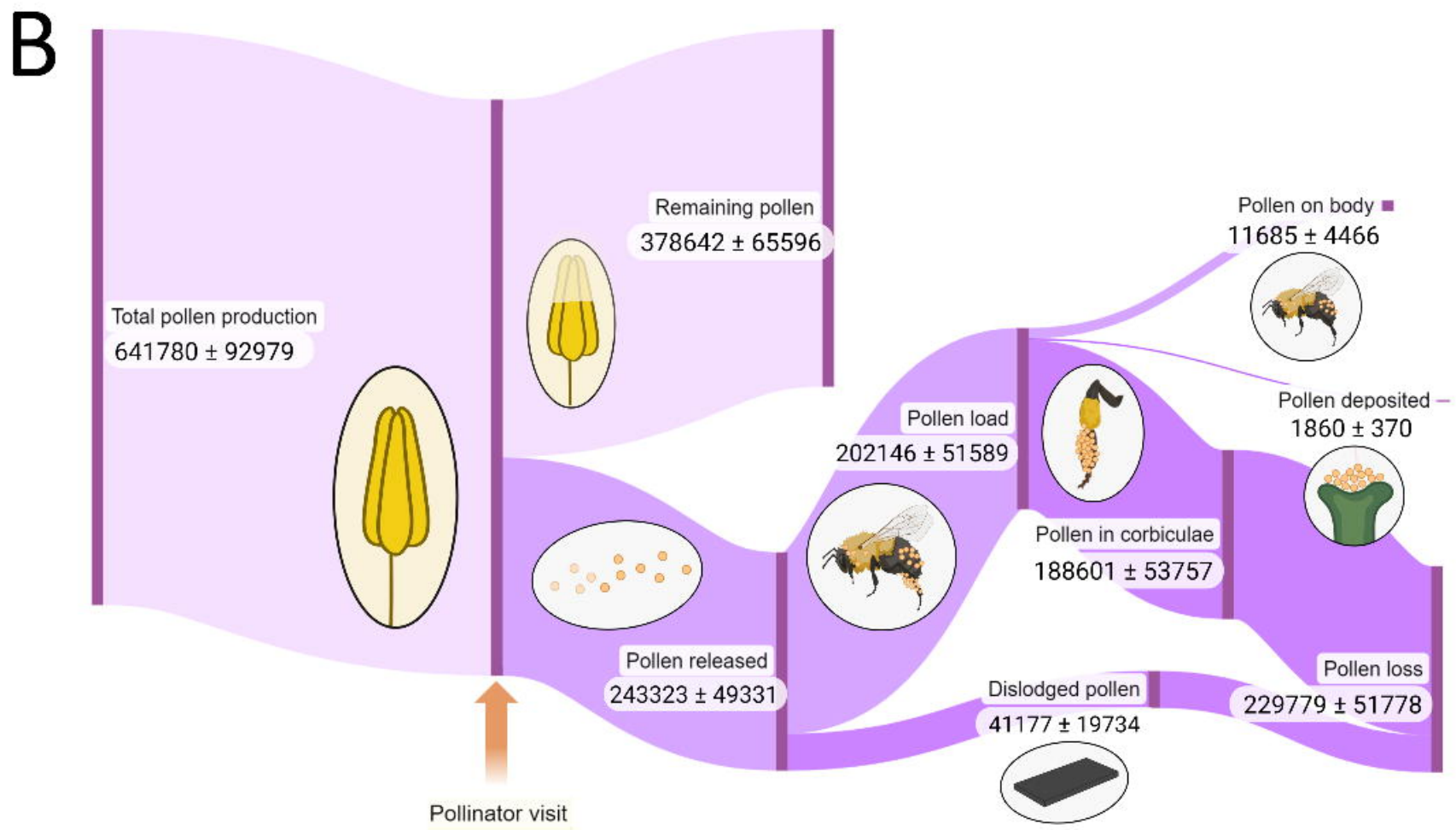


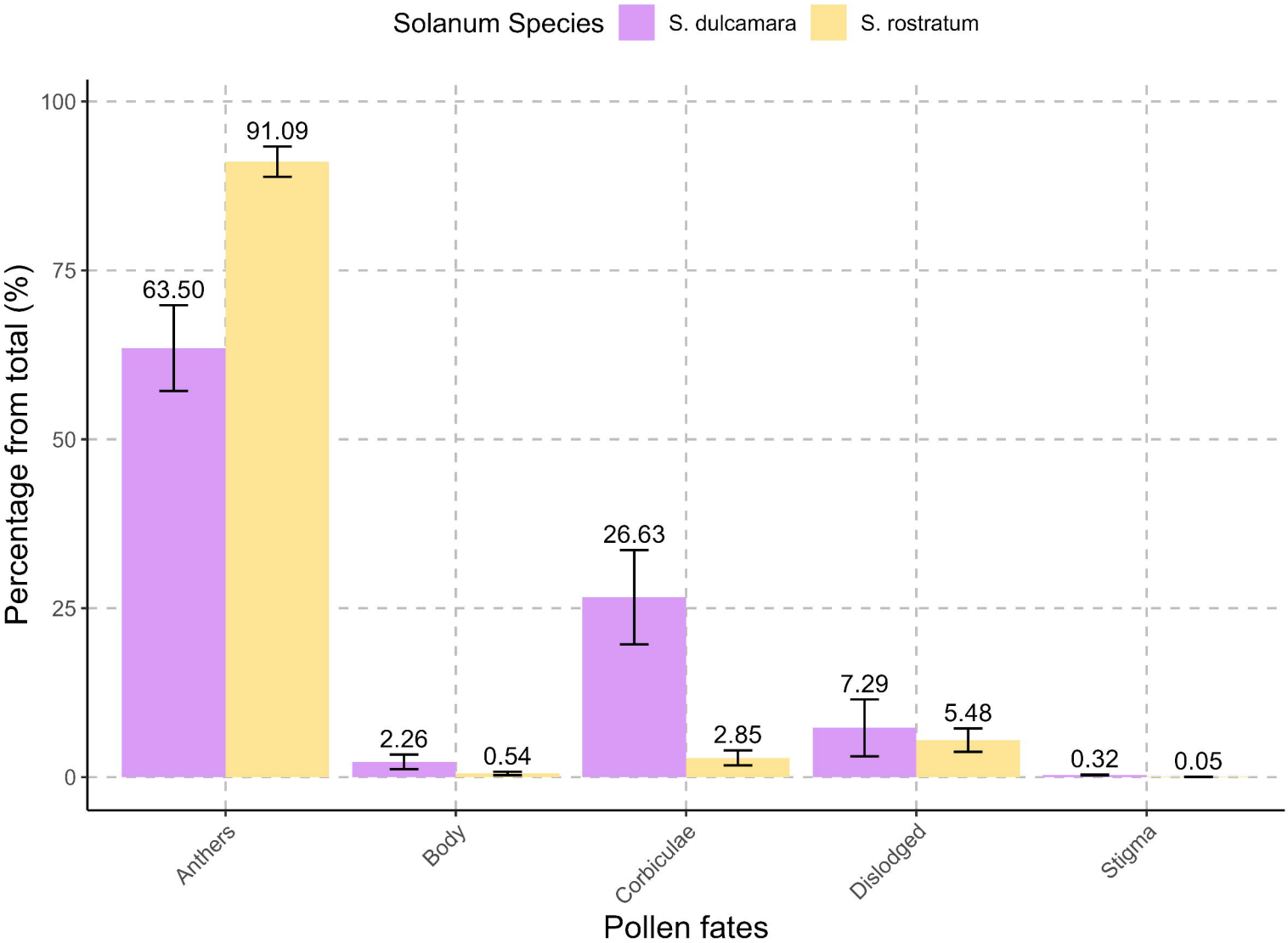
Figure 7. Number of pollen grains deposited on recipient flower stigmas as influenced by (A) duration of the visit in seconds, and (B) flower species and recipient order with released pollen as offset variable. Plotted lines indicate estimated means and shaded regions indicate standard errors.

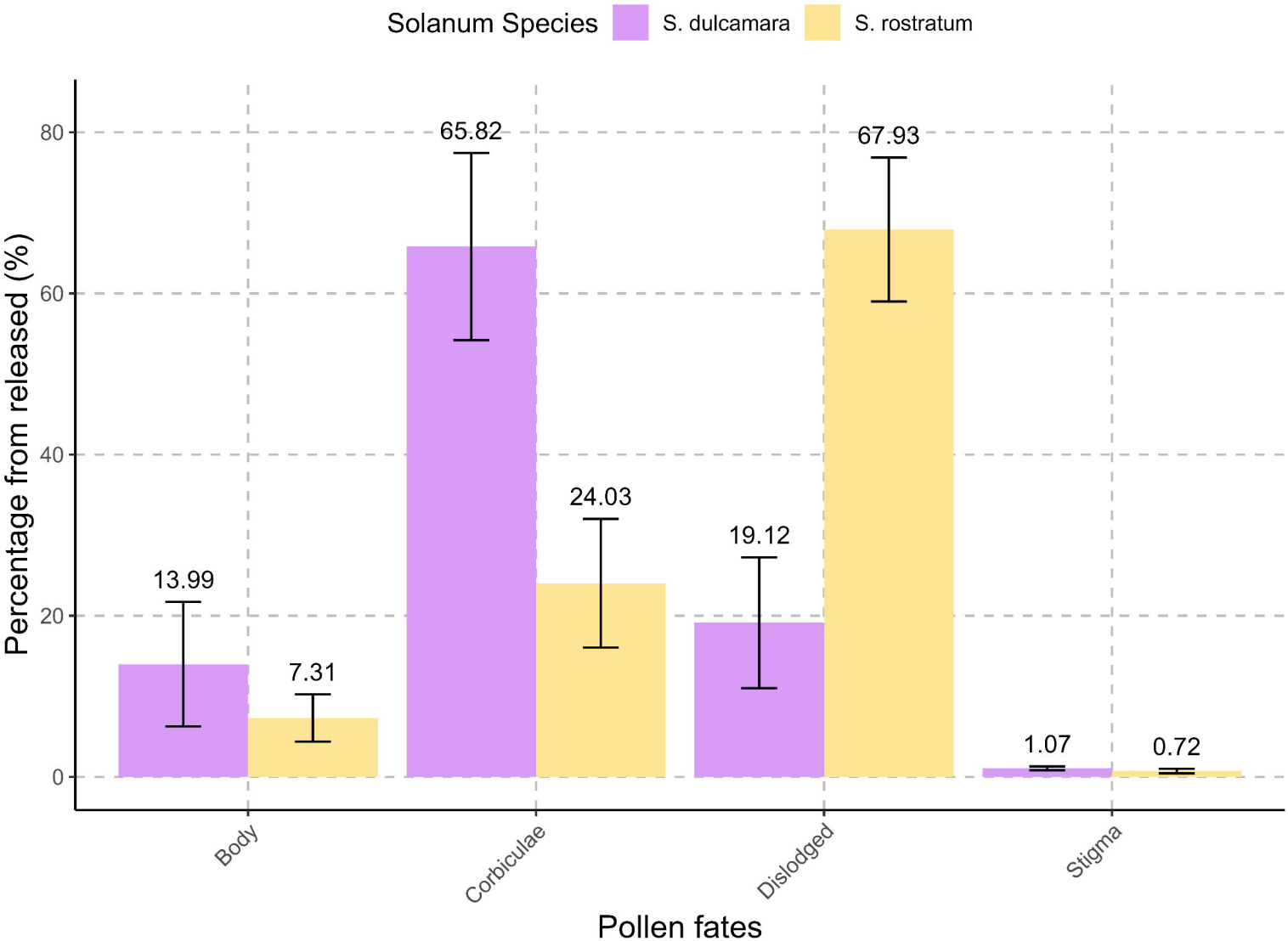

Remaining pollen
Total pollen production
Pollen load
Pollen on body
Pollen released
Pollen in corbiculae
Pollen deposited
Pollen loss
Pollinator visit
Dislodged pollen

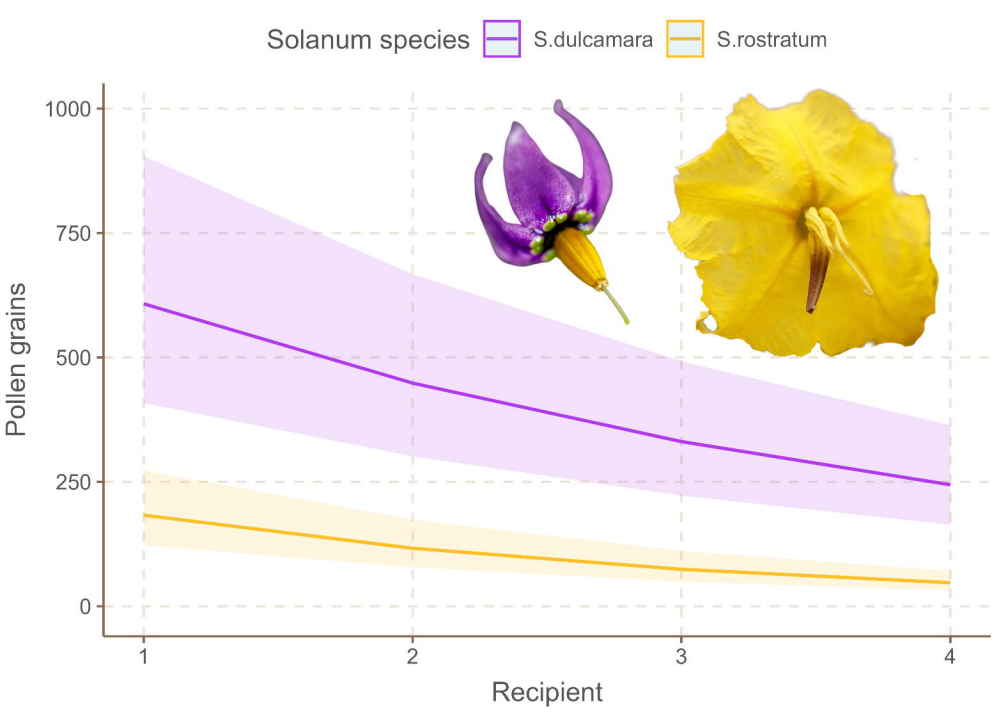

A
B

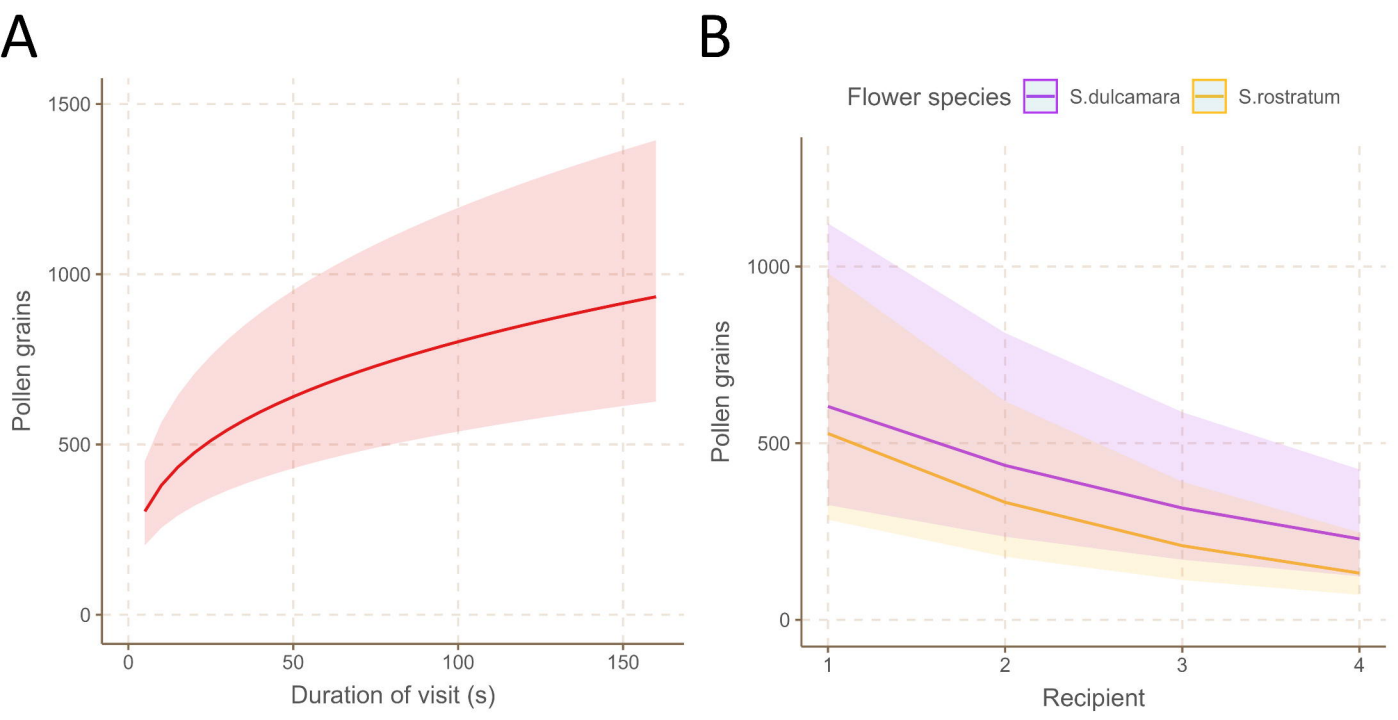

A
Total pollen production
1065712 ± 116142
Remaining pollen
1000891 ± 121491
Pollen released
86732 ± 21423
Pollinator visit
Pollen load
29358 ± 9222
Pollen on body
4892 ± 2198
Pollen deposited
356 ± 93
Pollen in corbiculae
24121 ± 8634
Dislodged pollen
53561 ± 17369
Pollen loss
81495 ± 21477


B
Total pollen production
641780 ± 92979
Remaining pollen
378642 ± 65596
Pollen released
243323 ± 49331
Pollinator visit
Pollen load
202146 ± 51589
Pollen on body
11685 ± 4466
Pollen in corbiculae
188601 ± 53757
Pollen deposited
1860 ± 370
Dislodged pollen
41177 ± 19734
Pollen loss
229779 ± 51778


Solanum Species
S. dulcamara
S. rostratum
Percentage from total (%)
100
75
50
25
0
91.09
63.50
26.63
2.26
0.54
2.85
7.29
5.48
0.32
0.05
Anthers
Body
Corbiculae
Dislodged
Stigma
Pollen fates


Solanum Species
S. dulcamara
S. rostratum
Percentage from released (%)
80
60
40
20
0
13.99
7.31
65.82
24.03
19.12
67.93
1.07
0.72
Body
Corbiculae
Dislodged
Stigma
Pollen fates


Solanum species
S.dulcamara
S.rostratum
1000
750
500
250
0
Pollen grains
1
2
3
4
Recipient


A
1500
1000
500
0
Pollen grains
0
50
100
150
Duration of visit (s)
B
Flower species
S.dulcamara
S.rostratum
1000
500
0
Pollen grains
1
2
3
4
Recipient