

NATIVE BEES POLLINATE TOMATO FLOWERS AND INCREASE FRUIT PRODUCTION

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Abstract—The tomato plant has a specific relationship with native pollinators because the form of its flowers is adapted to buzz pollination carried out by some pollen-gatherer bees that vibrate their indirect flight muscles to obtain that floral resource. The absence and the low density of these bees in tomato fields can lead to pollination deficits for crop. The aim of this study is to demonstrate that open tomato flowers, probably visited by native pollinator, have greater pollen load on their stigma than unvisited flowers. Another objective is to show that this great pollen load increases fruit production. We selected crops of the Italian tomato cultivar in areas of the State of Goiás, Brazil. Thirty seven plants of three crops each had one inflorescence bagged in the field. Bagged and non-bagged flowers had their stigmas collected and the amount of pollen on their surfaces was quantified. For the comparison of fruit production, we monitored bagged and not-bagged inflorescences and after 40 days, their fruits were counted, weighed, measured and had their seeds counted. The amount of pollen grains on the stigma of flowers available to pollinators was higher than that on the stigma of bagged flowers. On average, fruit production was larger in not-bagged inflorescences than in bagged inflorescences. In addition, not-bagged flowers produced heavier fruits than did bagged flowers. There was a significant difference in the number of seeds between treatments, with significantly more seeds in the non-bagged fruit. Our results show that native bees buzz-pollinate tomato flowers, increasing the pollen load on their stigma and consequently fruit production and quality.

Keywords: *Solanum lycopersicum* L., pollen load, pollination deficit

INTRODUCTION

Pollination is one of the most important ecological interactions and the first step for the sexual reproduction of most plant species (Murcia 1996). Pollination carried out by animals is considered an important ecosystem service with 35% of the plants cultivated in the world benefitting from this interaction (Klein et al. 2007). Bees are the main pollinators of most crops pollinated by animals (Free 1993; Delaplane & Mayer 2000; Klein et al. 2007). Many species of native bees contribute greatly to the pollination of crops such as coffee (*Coffea* spp.) (Klein et al. 2003; De Marco & Coelho 2004; Vergara & Fonseca-Buendía 2012), melon (*Citrullus lanatus*) (Winfrey et al. 2007), tomato (*Solanum lycopersicum*) (Greenleaf & Kremen 2006a; Macias-Macias et al. 2009; Vergara & Fonseca-Buendía 2012), sunflower (*Helianthus annuus*) (Greenleaf & Kremen 2006b), canola (*Brassica* spp.) (Morandin & Winston 2005) and blueberries (*Vaccinium* spp.) (Kevan et al. 1983), among others.

The tomato plant belongs to the genus *Solanum* of the family Solanaceae. This plant, formerly in the genus *Lycopersicon*, originated from the Andean regions. Today, it is widely cultivated throughout the world and adapted into many cultivars (Olmstead & Palmer 1997; Chetelat et al. 2009). Wild tomatoes are self-incompatible and feature a

close relationship with their pollinators for the formation of fruits (Chetelat et al. 2009). The cultivated plant is autogamous. However, one of the features of the genus is the poricidal opening of its anthers, which requires the agitation of the flowers by wind and/or the presence of pollinators that vibrate their indirect flight muscles for the release of pollen grains, even in cultivated varieties of tomatoes and especially in the still air of greenhouses (Kevan et al. 1991; Morandin et al. 2001a). Teppner (2005), while conducting studies on tomato plants in central Europe, observed that bees, such as *Bombus* and *Lasioglossum*, can be good pollinators of the flowers by vibrating their anthers easily. In respect of our study, we note that some families of bees from Brazil that perform buzz pollination are: Andrenidae, Apidae (except *Apis*), Colletidae, Halictidae, and Megachilidae (Harter et al. 2002).

Even though the importance of pollinators to tomato crops, especially in greenhouse production, is recognized, studies which demonstrate the direct relationship of pollinators to the pollen load on stigma and fruit production are scarce (Macias-Macias et al. 2009; Vergara & Fonseca-Buendía 2012). Tomato flowers in field crops in the State of Goiás, Brazil are visited by native bees, such as *Exomalopsis analis* (Apidae), *Augochloropsis* sp. (Halictidae) and *Centris tarsata* (Apidae) (Silva Neto et al., unpublished data). The visit frequency with which they visit the plants is high and apparently every flower is visited, sometimes more than once (Santos & Nascimento 2011; Silva Neto et al., unpublished data). This can be verified by the bruises on the anthers

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caused by the bees' mandibles as the grip the flower to buzz-pollinate (Morandin et al. 2001a; Silva Neto et al., unpublished data). Thus, native pollinators are assumed to be important to the pollen doses delivered to tomato flower stigmas and consequently to fruit production. To test this assumption, we proposed to quantify the difference between pollen doses transferred to the stigmas of open tomato flowers and those found on self-pollinated bagged flowers and then to quantify and compare fruit production in these two treatments. Here, we assume that almost all open non-bagged flowers were visited by native bees at least once as evidenced by the bruising on the anthers. Further, we checked for the main pollinator species of the tomato flowers in the study crops.

MATERIALS AND METHODS

Area of study

The State of Goiás is the largest producer of tomato in Brazil (CEASA/GO 2013) with large crops of industrial and fresh-market tomato. Our experiments were made on field crops located in the municipalities of Nerópolis and Goianópolis in Goiás (area of study: <https://docs.google.com/file/d/0ByQNaWHmeZ15YmpSQk4yZXZ6bTQ/edit>). The study area consisted of conventional field-grown and irrigated tomato crops (variety Italian). Trials were made from March to November, 2012.

To determine the main pollinators, we observed and collected bees that buzz pollinated the flowers. Four rows of about 120 plants were surveyed in each crop for 30 minutes and the presence of pollinators was recorded and quantified. The number of visits per pollinator species was recorded. Flower visitors were collected for identification. When collection was not possible, the identification to genus was made in the field. Those data were collected during two separate days in each property to make for statistically applicable sampling results. Our field studies took place between 09:00 and 12:00 (UTC/GMT – 3 hours), which is when previous data indicated peak floral visitation and greatest amount of pollen available for pollination in the anthers (Silva Neto, unpublished data).

Pollen load on stigmas

To compare the amount of pollen grains on stigmas of pollinated and not pollinated (bagged) flowers, 37 plants had flower buds from one inflorescence bagged in the field. After the opening and senescence of these bagged flowers, three of them were collected. At the same time, another three senescent but not-bagged flowers were collected. All were fixed in FAA 80% in the field and taken to the laboratory. There, the stigmas of these flowers were separated and softened in a solution of NaOH 9N for one hour, stained with acetic carmine and observed under an optical microscope. The pollen grains per stigma were counted in three visualization fields at 40 x magnification. The fields consisted of the two opposite ends of the stigma and its central part (Dafni et al. 2005).

Statistical comparison of pollen doses between bagged and non-bagged flowers used the Kolmogorov-Smirnov test

and paired Student's *t*-test with 95% significance (Malagodi-Braga & Kleinert 2007; Montemor & Souza 2009).

Fruit production

To assess the effect of pollination on tomato production, the flower buds bagged in the previous experiment and other buds tagged and not bagged were monitored on 37 different individual plants. Fruits were collected and taken to the laboratory forty days after the opening of their flowers. The fruits were weighed, measured (diagonal diameter) and their seeds counted in a similar manner to that described by Kevan et al. (1991). We chose to use only the first fruit produced by inflorescences because the remaining fruit had not fully developed over the sampling period.

Statistical comparisons between the number, mass and amount of seeds developed from bagged and not-bagged flowers used Kolmogorov-Smirnov test and subsequently through the paired Student's *t*-test with 95% significance. The relationship between mass and seeds was determined by simple linear regression (Spears 1983; Malagodi-Braga & Kleinert 2007; Montemor & Souza 2009).

RESULTS

The species of bees observed in the study crops were *Exomalopsis analis* Spinola (the most common), *Centris tarsata* Smith, *Bombus morio* Swederus, *Eulaema nigrita* Lepeletier and *Epicharis* sp. In three hours of observations, those bees were seen 47 times visiting tomato flowers (Table 1). The bees that performed buzz pollination approached the tomato flowers from the front, landing on the anther cones. They clung to the cone by their mandibles, vibrated the anthers and the pollen was expelled and adhered to the abdomen and other parts of their body. The same bees made circular motions on the anther, vibrating many times and over various anthers. When large amounts of pollen were deposited in their bodies, the bee stopped the vibration and cleaned themselves by collecting the pollen and putting it in its pollen basket (*Eulaema* sp. Complementary material filmed is available at: <http://www.youtube.com/watch?v=ovjI7wVdUmo>).

On average, the stigma of not-bagged flowers had on average 114 pollen grains ($t = 5.678$; $P = 0.0001$) (Figure 1A and Table 2) more than on the stigma of bagged flowers.

TABLE 1. Species of native bees that visit tomato flowers in three conventional plantations of the Italian variety in the State of Goiás – Brazil and their total number of visits in three hours of observation during the flowering peak.

| Pollinator | Number of flower visits |
|-----------------------------------|-------------------------|
| <i>Exomalopsis analis</i> Spinola | 47 |
| <i>Centris tarsata</i> Smith | 16 |
| <i>Epicharis</i> sp. | 2 |
| <i>Bombus morio</i> Swederus | 2 |
| <i>Eulaema nigrita</i> Lepeletier | 1 |

TABLE 2. Gains in tomato plant crops obtained from bagged and not bagged flowers treatments. Compared with paired Student's *t*-test with 95% significance (N: sample number; *t*: Student's *t* test; *df*: degree of freedom; *P*: statistical significance; %: Percentage gain of the "not bagged" treatment in relation to "bagged")

| | Flowers | Average | N | <i>t</i> | <i>df</i> | <i>P</i> | % |
|---------------------------------------|------------|--------------|----|----------|-----------|----------|--------|
| Pollen load (No. of pollen grains) | Not bagged | 182.51±86.68 | 37 | 5.67 | 36 | 0.00 | 168.31 |
| | Bagged | 67.83±68.33 | | | | | |
| Fruit set | Not bagged | 0.82±0.22 | 34 | 9.55 | 33 | 0.00 | 64.48 |
| | Bagged | 0.50±0.22 | | | | | |
| Tomato paste (g) | Not bagged | 70.69±20.40 | 31 | 4.91 | 30 | 0.00 | 50.21 |
| | Bagged | 47.06±26.88 | | | | | |
| Tomato size (mm) | Not bagged | 51.12±5.43 | 27 | 2.66 | 26 | 0.01 | 9.72 |
| | Bagged | 46.59±8.14 | | | | | |
| Seeds (No.) | Not bagged | 183.94±46.34 | 36 | 12.37 | 35 | 0.00 | 208.46 |
| | Bagged | 59.63±38.54 | | | | | |

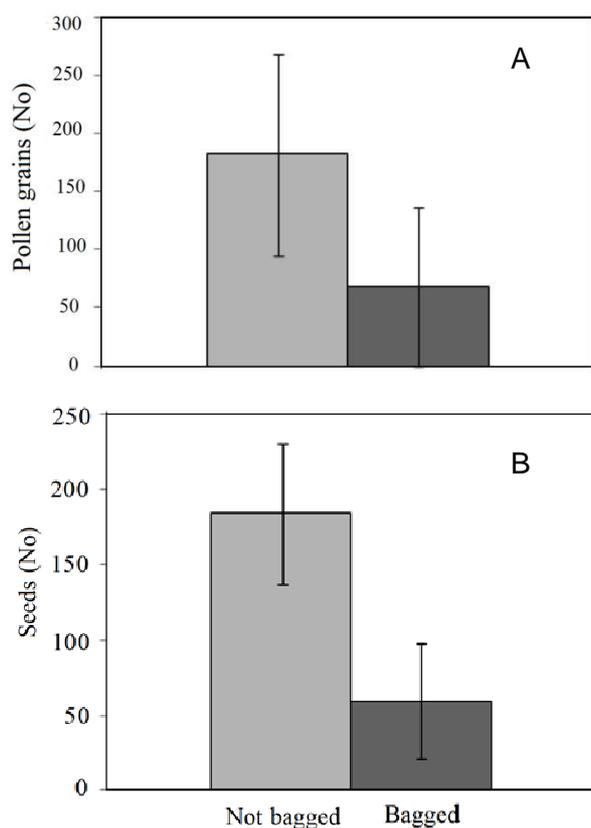


FIGURE 1. (A) Mean number (\pm SE) of pollen grains on the stigma of bagged and non bagged flowers. Compared with paired Student's *t*-test at 95% significance ($t = 5.678$; $P = 0.0000$). (B) Mean number (\pm SE) of seeds in the fruit of bagged and non bagged flowers. Compared with paired Student's *t*-test at 95% significance ($t = 12.37$; $P = 0.000$).

The fruit set was on average 64.48% ($t = 9.55$; $P = 0.000$) larger from not-bagged inflorescences than from bagged inflorescences (Table 2). In addition, not-bagged flowers produced 50.21% ($t = 4.91$; $P = 0.000$) heavier fruits than bagged flowers (Figure 2). The size of the fruits also was

significantly difference between bagged and not-bagged treatments: Fruit from not-bagged flowers were 9.72% larger than fruit from bagged flowers ($t = 2.66$; $P = 0.01$).

Seed number increased 208.5% between treatments ($t = 12.37$; $P = 0.000$) (Figure 1B). The correlation between fruit mass and number of seeds was high ($r^2 = 0.7047$; $r = 0.8395$; $P = 0.00003$; $y = 28.2011 + 0.3608 \cdot x$) (Figure 3).

DISCUSSION

Our results show that the visits of native pollinators probably increase pollen doses transferred to the stigma of flowers. The buzz pollination behaviour probably contributed mainly to the deposition of self-pollen because the stigma surface is inside the anther cones of cultivated tomato varieties. On average, non-bagged flowers had 114 ± 68 more pollen grains than bagged flowers on the stigma. This difference is smaller than that found on greenhouses tomato crops with colonies of *Bombus impatiens* Cresson (Morandin et al. 2001a). Nevertheless, our results showed that the tomatoes from not-bagged flowers and probably visited by pollinators are larger, heavier and with more seeds compared to those of bagged flowers. The pollen dose added to the stigmas of tomato flowers should lead to an increase in the number of fertilized eggs and thus, an increase in the production of seeds in the fruits. Studies have shown that the number of seeds in development in tomato fruits influences the activity of the fw 2.2 gene, which is responsible for the production of stimuli for the ovary walls growth and fruit formation (Tanksley 2004; Paran & van der Knaap 2007).

It has been shown that in greenhouses with managed *Melipona quadrifasciata* bees, gains in fruit production reached 15% (Bispo dos Santos et al. 2009). With *B. impatiens*, gains reached 50% in fruit mass and up to the double in the number of seeds (Morandin et al. 2001b). Other studies showed similar results in greenhouses (Hogendoorn et al. 2006; Palma et al. 2008; Bispo dos Santos et al. 2009; Vergara & Fonseca-Buendía 2012). In open air (filed) cultivation in Mexico, Macia-Macia et



FIGURE 2. Tomatoes in cross (above) and longitudinal sections (below). The tomatoes on the left are from not bagged flowers and those on the right are from bagged flowers.

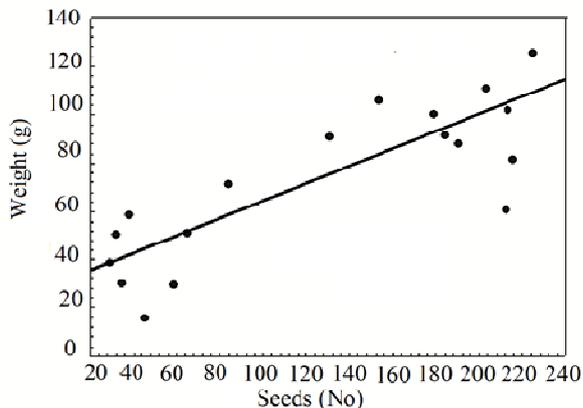


FIGURE 3. Relationship between seeds and tomato fruits mass carried out with simple linear regression ($r^2 = 0.7047$; $r = 0.8395$; $P = 0.00003$; $y = 28.2011 + 0.3608 \cdot x$).

production gains of 67.91% in mass and up to 208% in the number of seeds with native bee visits. Thus, the increase in the production of fruits seems to be greater in field-grown tomato crops than in greenhouses.

It has been suggested that a practical way to increase agricultural production of open grown crops (whose pollinators are native bees) is the conservation of wild or semi-managed vegetation areas around the perimeter of the areas of cultivation (Greenleaf & Kremen 2006a; Holzschuh et al. 2008; Winfree et al. 2007). Those areas of native vegetation are important sources for feeding and nesting to native bees (Kevan et al. 1990, Kevan, 1999). The influence of native areas to bee species may reduce the deficit of pollination in tomato crops is being analyzed by our research

team in the State of Goiás where we have already identified 29 different native species visiting tomato flowers in 14 different crops in the same of our study reported herein. A further 17 species of bees were collected there in pan-traps.

Considering the relevance of native bees in nature and for food production, it is essential to understand their attributes, such as nesting, social behaviour or not, foraging behaviour (flight distance, type of food resource) and pollination (buzz pollination and other behaviours). Such studies should cover not only social bees, but also solitary and para-social bees, which have been shown to be important for pollination of many crops (for example, *Exomalopsis* in the case of tomato and pepper) (Raw 2000; Macias-Macias et al. 2009; Santos and Nascimento 2011; Burkart et al. 2011; Kremen et al. 2011; Giannini et al. 2012; van der Valk et al. 2013). Knowledge on the biology of those bees is of utmost importance in order to propose management and conservation strategies to the government and also to implement friendly practices by tomato producers and other pollinator-dependent crops in the State of Goiás and other areas where these species may occur. The use of pesticides for conventional tomato production negatively impacts native pollinators but the extent of that impact is not known for tomato production in Brazil and is part of our continuing research program.

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