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RESEARCH ARTICLE - BEES

Pollination Services Provided by *Melipona quadrifasciata* Lepeletier (Hymenoptera: Meliponini) in Greenhouses with *Solanum lycopersicum* L. (Solanaceae)

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Abstract

Stingless bees are considered particularly promising for use as commercial pollinators. However, studies testing the effectiveness of these bees in pollinating grape tomatoes in greenhouses are not yet available. This work aimed to analyze the floral biology of the grape tomato and evaluate whether the additional pollination performed by *Melipona quadrifasciata* Lepeletier generates an effective increase in production and improves the quality of this variety of tomato when grown in greenhouses. The research took place in two greenhouses located in Araguari, Minas Gerais state, Brazil. In one of them, we only used a mechanical method of pollination and, in the other, pollination by the mechanical method was associated with pollination performed by bees. The productivity was compared by recording the number of flowers and fruits formed on different branches, and tomato quality was assessed by analyzing their size, weight, number of seeds and total sugar content. Tomatoes originating from flowers visited by *M. quadrifasciata* workers produced about 47% more seeds and their concentration of sugar was approximately 14% higher. These results suggest that using *M. quadrifasciata* for tomato pollination shows to be promising, since their use as pollinators entails positive effects on fruit quality.

Introduction

Ecosystem services are benefits provided by organisms that interact with each other for human welfare (Daily, 1997). Among them, pollination has proven to be an essential service, especially that performed by insects, accounting for approximately 35% of global agricultural production (Klein et al., 2007). In this context, bees play a key role in various ecosystems (Bawa, 1990). They have increasingly been spotlighted as the main agent responsible for pollinating many species of native and cultivated plants, since they ensure the maintenance of genetic variability in the former and the productivity and fruits quality in the latter (Kremen, 2005).

It is estimated that of a total of 40,000 pollinators, 20,000 are bees (Michener, 2007) and 73% of cultivated plant species worldwide are pollinated by these insects (FAO, 2004). In Brazil, the efficiency of pollination performed by bees has been confirmed for various crops, either in greenhouses or open areas. Among the crops grown in greenhouses, especially noteworthy are strawberry pollination, tested with *Tetragonisca angustula* (Latreille) (Cruz & Campos, 2009), *Scaptotrigona* aff. *depilis* (Moure) (Roselino et al., 2009) and *Nannotrigona testaceicornis* (Lepeletier) (Roselino et al., 2009); sweet pepper, with *Melipona subnitida* Ducke (Cruz et al., 2005), *Melipona quadrifasciata anthidioides* Lepeletier (Roselino et al., 2010) and *Melipona scutellaris* Latreille (Roselino et al., 2010); and tomato, tested with *Nannotrigona perilampoides* (Cresson) (Cauich et al., 2004; Palma et al., 2008), *M. quadrifasciata* (Del Sarto et al., 2005; Bispo dos Santos et al., 2009) and *Apis mellifera* L. (Bispo dos Santos et al., 2009).

Tomato, *Solanum lycopersicum* L. (Solanaceae), is one of the most widespread vegetable crops in the world. The fruit is widely consumed and is of great importance to the human body, mainly due to the presence of lycopene, an antioxidant that fights free radicals and slows aging. Tomatoes are grown both in open areas and in greenhouses. Their flowers are self-fertile, bisexual, do not produce nectar

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and present poricidal anthers. Therefore, in order to release the pollen and, consequently, enable pollination to occur, vibration of the anthers with consequent opening thereof is necessary (Buchmann, 1983).

When grown in open areas, pollen release is performed by the wind (McGregor, 1976; Free, 1993) and natural pollinators, especially bee species, which are capable of a vibratory behavior or buzz-pollination. In greenhouse cultivation, pollination is normally accomplished by mechanical vibration of the flowers, as an alternative to the absence of wind and natural pollinators. However, the mechanical method increases the production costs as they require additional labor and can cause damage to flowers and thus to fruits (Cribb et al., 1993; Ilbi et al., 1994; Dogterom et al., 1998).

For this reason, the number of studies involving the management of pollinators in greenhouses has grown steadily in recent years. Among tomato pollinators in greenhouses are *Amegilla chlorocyanea* (Cockerell) (Hogendoorn et al., 2006), bees of the genus *Xylocopa* (*Lestis*) Lepeletier & Serville (Hogendoorn et al., 2000) and *Bombus* Latreille (Morandin et al., 2001), and the stingless bees *N. perilampoides* (Cauich et al., 2004; Palma et al., 2008) and *M. quadrifasciata* (Del Sarto et al., 2005; Bispo dos Santos et al., 2009; Hikawa & Miyanaga, 2009).

Stingless bees are particularly promising for use as commercial pollinators (Cruz & Campos, 2009), given that they do not present a functional sting, are easy to handle (usually low aggressive-nonstinging), have populous and perennial nests, present a marked worker recruitment behavior and stock a large amount of food (Heard, 1999). *M. quadrifasciata* is a stingless bee that has a wide distribution throughout Brazil and is present in Argentina and Paraguay (Camargo & Pedro, 2012). This species proved to be efficient in pollinating different tomato varieties. However, studies testing its pollination efficiency on grape tomato, grown mostly in greenhouses, are yet to be performed. Accordingly, this study aimed to: (1) analyze the floral biology of grape tomato to allow a richer discussion about the effectiveness of pollinators; and (2) evaluate the pollination efficiency of *M. quadrifasciata* on grape tomato in greenhouses, to verify whether introducing nests of this species generates an effective increase in production and improves fruit quality.

Material and Methods

Study area

The study was conducted in two identical greenhouses located in "Chácara Paraíso", in the municipality of Araguari (18º39'3.55"S/48º11'7.51"W), Minas Gerais state, Brazil. This municipality is configured as a hub of food production, with approximately 98,487 ha of arable area and an estimated production of 70,631 tons of tomatoes per year (IBGE, 2012).

The regional climate is marked by two distinct seasons, the rainy season that extends from October to March and the dry season, from April to September. Annual rainfall varies between 1,160 and 1,460 mm/year and the average annual temperature is between 23 and 25ºC, being uniform throughout the year (Alves & Rosa, 2008).

The greenhouses comprised approximately 1,344 m^2 (48 m x 28 m), covered at the top with an Extra Long Life (ELL) Diffuser Antivirus plastic film with ultraviolet filter, and fully enclosed on the sides with anti-aphid screens. The greenhouses presented 24 planting rows, and each of these had an average of 112 tomato plants, totaling 2,688 plants.

Floral biology

The floral biology analyzes were performed in one of the two greenhouses. The parameters evaluated included aspects of floral morphology, number of open flowers per plant, duration of the flower, pollen viability and stigma receptivity.

The structures of a total of 20 flowers of different plants were analyzed. The floral diameter and length of anther, pistil and style were measured using a digital pachymeter. Counting the number of open flowers per plant was performed on 40 different randomly selected individuals two months after the onset of flowering.

Fifty floral buds were marked and monitored to determine the time of flowers opening, duration of anthesis and flower senescence. Pollen viability was tested indirectly by staining 20 pre-anthesis flower buds from different plants with 2% acetic carmine (Kearns & Inouye, 1993), and estimated through observation of the first 300 pollen grains from each bud using an optical microscope.

Stigma receptivity was tested in 10 buds three days before anthesis, 10 buds in pre-anthesis and a total of 55 newly opened flowers from 7 am to 6 pm (five flowers every hour) using 3% hydrogen peroxide (H_2O_2). Receptivity was detected by the formation of air bubbles (Kearns & Inouye, 1993).

*Pollination experiments with **M. quadrifasciata***

For pollination experiments, the plants presented the same development time in both greenhouses. One greenhouse was used as control, where only mechanical method of pollination was performed (flowers were vibrated using a leaf blower). In the other greenhouse, six nests of *M. quadrifasciata* with similar population sizes were introduced in March 2012 at the onset of flowering. In this greenhouse, pollination occurred by the mechanical method associated with pollination by bees.

Given the difficulty in bee orientation inside the greenhouse (Bartelli et al., 2014), before the introduction of bees, the old foragers were removed to avoid their loss in the enclosed space, following the methodology used by Cauich et al. (2004). Moreover, for the same reason, the nests were placed in the greenhouse after dark (Cuypers, 1968).

In order to allow a homogeneous distribution of the bees on flowers, nests were arranged uniformly in the central region of the greenhouse (Free, 1993) and supported by plastic boxes installed in eucalyptus logs located within the planting rows. Containers with water, mud and cerumen (alternative source for plant resin) of *T. angustula* were placed on the plastic boxes. Nests were fed biweekly with pollen collected by *A. mellifera* (5g) macerated with sugar and water until the onset of flower visitation, and fed weekly with syrup (a mixture of *A. mellifera* honey, sugar and water in the ratio 1:1) over the entire period of confinement. For pesticide application, a common management practice, the entrances of all nests were sealed with paper, the nests themselves were protected with plastic bags and the containers covered with cardboard boxes. These protections were removed only after the pesticide had dried. Then, the entrances of the nests were unobstructed after dark.

To evaluate the pollination efficiency of *M. quadrifasciata* after initiation of foraging activities by the bees on flowers, the productivity of the two greenhouses was compared by marking 20 branches of different plants (randomly selected) in each. The total number of flowers and fruits produced over time on each of the branches was recorded.

Fruits decrease in size from the base to the apex of the branches. Thus, to determine whether the additional pollination performed by *M. quadrifasciata* improved the quality of the tomatoes, the position that each fruit occupied on the branch was taken into consideration. We collected 25 fruits originating from flowers effectively visited by bees (after observation of the visit and marking of the flower) in the experimental greenhouse and 25 corresponding fruits in the control greenhouse. All of them were collected at an intermediate stage of maturation (early in the reddish stage) and analyzed on the day of harvest.

As tomatoes decrease in size and weight from the first crop through the subsequent flowerings, depending on the age of the culture (Hill, 2001), the fruits of both greenhouses were collected during the same period. Fruit quality was evaluated by the following parameters: size (longitudinal and equatorial diameters), using a digital pachymeter; weight, using a digital balance; number of seeds, counted directly; and concentration of total sugars (°Brix), by means of an analogical portable refractometer, after removal of the seeds and maceration of the rest of the fruit.

Statistical analyzes

To verify the extent to which tomato production was dependent on the introduction of nests, we conducted an analysis of covariance (ANCOVA) for the number of fruits produced, with the number of flowers on the branches as a covariate (Zar, 2010). To determine if the quality of the fruit (size, weight, number of seeds and sugar concentration) was affected by the additional pollination effected by bees, we performed a paired t-test (Zar, 2010) for each of the analyzed parameters.

Results

Floral biology

Regarding floral morphology, the average floral diameter was 31.31 mm and the average length of anther, pistil and style were, respectively, 8.79, 10.56 and 8.01 mm (Table 1). The average number of open flowers per plant was 16.07 (Table 1) and the flowers lasted an average of five days, closing at night and opening again between 6:30 and 7 am, being totally open by 8 am.

An average of 98% of the pollen grains present in anthers was viable. The stigma of newly opened flowers was receptive during the entire period of evaluation, from 7 am to 6 pm. Even before opening, the grape tomato flowers were able to be fertilized, considering that the stigma of all buds in pre-anthesis as well as buds three days before anthesis were receptive.

Pollination experiments with ***M. quadrifasciata***

Of the six nests introduced, only two remained over the entire 7-months cycle of flowering of the tomato plants in the greenhouse. Two nests did not survive the conditions of confinement and two were removed from the greenhouse after being held in the greenhouse for three months to prevent their loss. The worker bees began the pollen collection from tomato plants after about six months of confinement.

Table 1. Minimum, maximum and mean (± standard deviation) values of the floral diameter, length of anther, pistil and style and number of open flowers per tomato plant in a greenhouse of grape tomato, in Araguari, Minas Gerais state, Brazil.

Statistical data	Floral diameter (mm)	Anther length (mm)	Pistil length (mm)	Style length (mm)	Number of flowers/plant
Minimum	28.76	8.36	9.96	7.53	5
Maximum	33.85	9.15	11.3	8.87	31
Mean	31.31	8.79	10.56	8.01	16.07
(± SD)	(± 1.33)	(± 0.22)	(± 0.36)	(± 0.39)	(± 6.39)

The introduction of nests of *M. quadrifasciata* did not generate an effective increase in production of grape tomatoes, since the average number of fruits did not differ between treatments ($F = 0.70$; df = 1; $P = 0.41$; Fig 1). However, fruits originating from flowers visited by bees presented about 47% more seeds ($/t/ = 4.231$; df = 24; $P < 0.001$) and had a sugar concentration approximately 14% higher ($/t/ = 5.91$; df = 24; $P < 0.001$) compared to those derived from flowers pollinated only by the mechanical method. Regarding the other parameters associated with fruit quality, additional pollination by bees did not generate an increase in size, i.e., longitudinal diameter ($/t/ = 0.811$; df = 24; $P = 0.425$), equatorial diameter ($/t/ = 0.053$; df = 24; $P = 0.958$) or weight ($/t/ = 0.169$; df = 24; $P = 0.867$) of the tomatoes produced (Table 2).

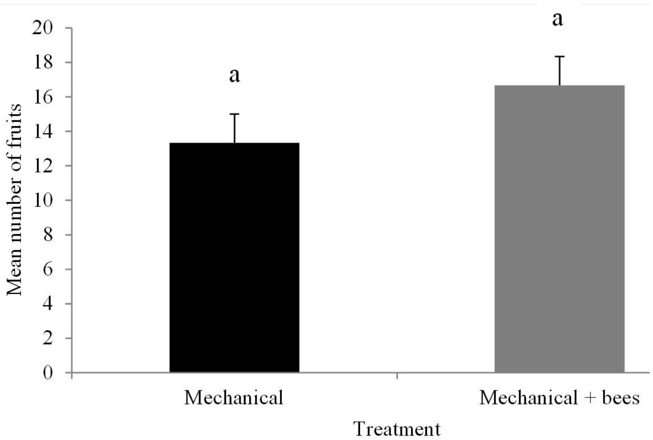

Fig 1. Mean number (± standard error) of fruit set per branch in the two greenhouses of grape tomato, one control (mechanical method of pollination alone) and the other where *Melipona quadrifasciata* bees were introduced (pollination by mechanical method + pollination by bees), in Araguari, Minas Gerais state, Brazil. The same letters indicate no significant difference between treatments.

Table 2. Mean values (± standard deviation) of each of the parameters related to fruit quality in the two different treatments (mechanical method of pollination alone and pollination by mechanical method + pollination by bees), in Araguari, Minas Gerais state, Brazil.

Fruit attribute	Pollination treatment	
	Mechanical	Mechanical + bees
Longitudinal diameter (mm)	29.14 (± 1.89)a	29.67 (± 2.81)a
Equatorial diameter (mm)	21.40 (± 1.82)a	21.38 (± 1.89)a
Weight (g)	7.261 (± 1.512)a	7.196 (± 1.733)a
Number of seeds	56.92 (± 23.18)a	83.72 (± 23.30)b
Concentration of sugars (ºBrix)	6.51 (± 0.51)a	7.40 (± 0.69)b

Discussion

Through genetic improvement, several varieties of tomatoes were and have been bred to meet the different tastes of consumers. Among those, grape tomato has become increasingly popular, since the sweetness, small size and elongated shape (similar to grape) of the fruits make them attractive for culinary use, for example, as ingredients in salads (Hill, 2001). Hence the importance of knowing its productive potential, and efficiency in fruit formation. In relation to this, the studied tomato variety shows promising prospects. Two important aspects to consider are the large percentage of viable pollen grains present in anthers and the extensive period of stigma receptivity. The stigma showed receptivity three days before anthesis and in newly opened flowers, over an extended period of the day.

Results similar to those obtained in the present study were found for the long-life tomato, variety Rodas, regarding the time of flowers opening (Del Sarto et al., 2005). When compared to variety Forty, grape tomato flowers are larger and the number of open flowers per plant is considerably higher (unpublished data). As expected, grape tomato flowers have the anthers longer than the styles, like other tomato varieties, favoring the occurrence of self-pollination.

Different results have been found in various studies that used stingless bees for tomato pollination in greenhouses (Cauich et al., 2004; Del Sarto et al., 2005; Palma et al., 2008; Bispo dos Santos et al., 2009; Hikawa & Miyanaga, 2009). In one such study, the inefficiency of *M. quadrifasciata* in increasing tomato production was explained by the overlap of only 30 minutes between the peak activity of the workers and the period of highest stigma receptivity of the flowers (Del Sarto et al., 2005). However, as previously mentioned, the stigma grape tomato flowers are receptive from 7 am to 6 pm, a time interval that covers almost the whole period of foraging by *M. quadrifasciata* workers (Bartelli et al., 2014).

Biology and foraging behavior differ between species of stingless bees and, in addition, the pollination requirements of different cultivated plant species and different varieties of the same species are not identical. Such factors must be taken into consideration when making comparisons. Despite different tomato varieties being used, a more direct comparison can be made with a study developed by Del Sarto et al. (2005). According to these authors, two colonies of *M. quadrifasciata* would be sufficient to pollinate a greenhouse with 858-1,534 tomato plants (a proportion of one nest per 429–767 plants). These estimates were made considering that, in a strong nest of *M. quadrifasciata*, about 6% (Jarau et al., 2000) of an approximate total of 890 bees (Michener, 1974) are forager workers. Moreover, for pollen collection, each worker visits 28–50 tomato flowers per trip (Del Sarto et al., 2005). In the present study, however, beyond the small number of active forager workers, the density was 0.001 nest/m^2 and the proportion was one colony to 1,344 tomato plants, insufficient for an increase in production. Thus, considering the size of the studied greenhouse, the inability of *M. quadrifasciata* to increase the production of tomatoes in this study is probably related to the low density of nests inside the greenhouse, inasmuch as only two of the six that were introduced remained at the end of the experiments. Additionally, the number of active forager workers in each of the nests was very low compared to the number of flowers available for pollen collection.

Nevertheless, in general, an improvement could be observed in fruit quality. The highest number of seeds found in the tomatoes originated from flowers visited by *M. quadrifasciata* workers, denoting the efficiency of this species on tomato pollination. Compared to the mechanical method, the buzz pollination behavior carried out by several species of bees, such as *M. quadrifasciata*, results in an increase in the number of pollen grains deposited on the stigma of the flowers, thereby increasing the quantity of seeds in the fruits (Hogendoorn et al., 2000).

Although several studies had shown the existence of a positive correlation between the number of seeds and the size and weight of tomatoes (Dempsey & Boynton, 1965; Imanishi & Hiura, 1975; Hogendoorn et al., 2010), there was no difference in these last two parameters between the treatments performed. Among other factors, the size and weight of the fruit are related to the amount of water used in irrigation, determining a higher or lower concentration of soluble components. Furthermore, high pluviometric indices also trigger the production of larger tomatoes, but with lower nutrient content and less accentuated flavour (Casquet, 1998). However, the greenhouses used were positioned one beside the other and were managed the same way, eliminating such possibilities.

Our knowledge about the variety of tomato studied is still fairly rudimentary. However, the fruits apparently develop to an equal extent regardless of the degree of pollination. In other words, they grow independent of the number of pollen grains deposited on the stigma of the flowers and, consequently, of the number of seeds. This might explain the similarity in relation to the size and weight attributes found between fruits pollinated only by the mechanical method and those additionally pollinated by bees.

Nevertheless, tomatoes originating from flowers visited by bees had a considerably higher concentration of sugars. Little is known about the mechanisms involved in accumulating sugars in the process of tomato development (Damon et al., 1988). However, the higher concentration of carbohydrates in fruits resulting from the additional pollination carried by the bees may be related to the greater number of seeds therein. Through the production of hormones, the number of seeds may modify and stimulate the processes of division and cell expansion, increasing the storage capacity for soluble solids in the cells (Gillaspy et al., 1993; Prudent et al., 2009).

From the food science standpoint, quality comprises the features that distinguish the individual units of a product, being significant in determining the degree of acceptability by the purchaser. Regarding tomatoes, quality is strongly associated with the soluble solids content, and sugars are the main components of this fraction (Damon et al., 1988). Thus, based on the results obtained in this study, one can conclude that the use of *M. quadrifasciata* for tomato pollination is promising, since the effects on the quality of the tomatoes originating from flowers visited by workers were inequivocally positive.

In the last few years, the use of stingless bees in the Brazilian agricultural systems under greenhouse conditions has become increasingly widespread and presented exceptional results (Cruz & Campos, 2009), contrasting with the alternative use of exotic pollinators and providing greater awareness of the importance of conservation of stingless bees. However, it is still necessary to improve techniques of nest multiplication and species management specific to different species, especially in protected environments. Such actions would provide the availability and utilization of these insects in agriculture on a large scale. With improvements in these management techniques, the appropriate density of bees for pollination of different crops could be determined by maintaining greater numbers of nests inside greenhouses. Even so, the use of *M. quadrifasciata* is already a good strategy for producers, since the additional pollination performed by bees improves tomato quality.

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