Stingless Bees as Alternative Pollinators of Canola

SIDIA WITTER,^{1,2} PATRÍCIA NUNES-SILVA,³ BRUNO B. LISBOA,⁴ FLAVIA P. TIRELLI,³ ARONI SATTLER,⁵ SUZANE BOTH HILGERT-MOREIRA,⁶ and BETINA BLOCHTEIN³

J. Econ. Entomol. 108(3): 880-886 (2015); DOI: 10.1093/jee/tov096

ABSTRACT Alternative pollinators can ensure pollination services if the availability of the managed or most common pollinator is compromised. In this study, the behavior and pollination efficiency of *Apis mellifera* L. and two species of stingless bees, *Plebeia emerina* Friese and *Tetragonisca fiebrigi* Schwarz, were evaluated and compared in flowers of *Brassica napus* L. 'Hyola 61'. A. *mellifera* was an efficient pollinator when collecting nectar because it effectively touched the reproductive organs of the flower. In contrast, stingless bees were efficient pollinators only when collecting pollen. The number of pollen grains deposited on the stigma after a single visit by worker bees of the three species was greater than the number of grains resulting from pollination without the bee visits. On average, the three species deposited enough pollen grains to fertilize all of the flower ovules. *A. mellifera* and *P. emerina* had similar pollination efficiency because no significant differences were observed in the characteristics of the siliques produced. Although *T. fiebrigi* is also an effective pollinator, the seed mass produced by their pollination was lower. Native bees promoted similar rates of fruit set compared with *A. mellifera*. Thus, *P. emerina* has potential to be used for pollination in canola crops.

KEY WORDS Apis mellifera, Plebeia emerina, Tetragonisca fiebrigi, pollination, Brassica napus

Pollination ecosystem services are important for both ecosystems (Constanza et al. 1997) and agriculture (Ricketts et al. 2008). While the nature of this service ensures the reproduction and maintenance of angiosperm populations (Ollerton et al. 2011). In agroecosystems, pollination is responsible for the production and quality of 35% of the volume of the global production of fruits and seeds (Klein et al. 2007). The level of pollination dependence varies between crops, and 33% of crops rely to some degree on that service (Klein et al. 2007). In agriculture, bees are the most important pollinators, and among them, A. *mellifera* L. is the primary species managed for pollination (Delaplane and Mayer 2000, Ricketts et al. 2008). The decline of wild and managed populations of this species (Moritz et al. 2007, Van Engelsdorp et al. 2008, Potts et al. 2010) has generated concern for the stability of pollination services and its consequences on global food production (Natural Research Council 2006).

Despite the recognized efficiency of *A. mellifera* as a pollinator of various crops, a recent worldwide analysis

determined that wild bees are generally more effective than *A. mellifera* and are an important source of pollination (Garibaldi et al. 2013). The identification and management of alternative pollinators aims to ensure continuity of pollination services if the main pollinator becomes unavailable (Slaa et al. 2006). In addition, the use of native pollinators encourages the conservation of these species (Freitas et al. 2009).

Non-Apis pollinators have been identified for some crops, such as strawberry (Malagodi-Braga and Kleinert 2004, Witter et al. 2012), tomato (Cauich et al. 2004; Del Sarto et al. 2005; Hogendoorn et al. 2000, 2006; Palma et al. 2008; dos Santos et al. 2009), and eggplant (Nunes-Silva et al. 2013; but also see Klein et al. 2007 for more information). These studies used different approaches to evaluate the pollination efficacy of bee species. Generally, researchers tend to evaluate the pollination efficiency of a particular pollinator species based on the quantity and quality of fruits and seeds produced by comparing the bee-pollinated flowers with a control group that was not visited by bees. Another possible approach is to evaluate the behavior of the pollinator and assess its ability to transfer enough pollen to fertilize the flower ovules (Kremen et al. 2009, Rader et al. 2009, Ne'eman et al. 2010, Nunes-Silva et al. 2013).

Canola (*Brassica napus* L.) is an oilseed crop produced in several countries around the world, and *Apis mellifera* L. is considered to be an important pollinator of this crop (Sabbahi et al. 2005, Duran et al. 2010, Bommarco et al. 2012, Stanley et al. 2013, Woodcock et al. 2013). In addition to the honey bees, wild bees from the crop growing regions have also been identified as pollinators (Ali et al. 2011, Jauker et al. 2012).

© The Authors 2015. Published by Oxford University Press on behalf of Entomological Society of America. All rights reserved. For Permissions, please email: journals.permissions@oup.com

¹ Fundação Estadual de Pesquisa Agropecuária - Vale do Taquari, Centro de Pesquisa Emílio Schenk, Caixa Postal 12. Taquari, RS 95860-000, Brazil.

²Corresponding author, e-mail: siwitter@gmail.com.

³ Pontificia Universidade Católica do Rio Grande do Sul (PUCRS), Faculdade de Biociências, Laboratório de Entomologia, Porto Alegre, Brazil.

⁴ Fundação Estadual de Pesquisa Agropecuária, Laboratório de Microbiologia Agrícola, Porto Alegre, Brazil.

⁵Universidade Federal do Rio Grande do Sul (UFRGS), Laboratório de Fitossanidade, Porto Alegre, Brazil.

⁶ Universidade do Vale do Rio do Sinos (Unisinos), Laboratório de Taxonomia Vegetal, São Leopoldo, Brazil.

In Brazil, there is no management of pollinators associated with canola crops; however, studies have shown that insect pollination has a positive impact on canola crop production (Rosa et al. 2010, 2011; Blochtein et al. 2013; Witter et al. 2014). Additionally, given the environmental impact generated by agriculture, these areas have a low abundance of native bees (Féon et al. 2010).

Thus, the identification of pollinators that can be managed in canola plantations is important both to create an alternative to *A. mellifera* and to raise awareness of the importance of native bees in the region for the pollination of crops and native vegetation. One group of insects with great potential as pollinators is the stingless bees (Meliponini) because beekeeping ("meliponiculture") has already been established for these species (Cortopassi-Laurino et al. 2006).

In the present study, the efficiency of pollination by *A. mellifera, Plebeia emerina* Friese, and *Tetragonisca fiebrigi* Schwarz in the canola crop was compared to assess the potential of these native bees for use in crop pollination services. To this end, the following pollination efficiency parameters were analyzed: 1) the behavior (residence time of the bee on the flower, floral resource collected, presence of pollen in the pollen basket, contact with anthers and stigmas, and specific location of resource collection) of the three bee species during visits to flower; 2) the number of pollen grains deposited on the stigma of flowers during a visit; and 3) the rate of fruit set and the production of seeds and siliques after a single visit.

Materials and Methods

Study area. The study was conducted in October and November 2012 at the Research Center of the State Foundation for Agricultural Research ("Centro de Pesquisa da Fundação Estadual de Pesquisa Agropecuária") in the municipality of Viamão, state of Rio Grande do Sul (30° 02'11" S, 51° 01'23" W), Brazil. According to the Brazilian classification system, the soil is Red-Yellow Argisol (Ultisol; Embrapa 2006), and the climate is subtropical with a humid hot summer (i.e., Cfa type according to the Köeppen classification; Brasil 1969).

Shade House and Plant Management. To avoid external insects interfering with pollination, as well as the escape of the bees being tested, the experiments were conducted in a shade house covered with white polyethylene fabric (12% shade) with a 2 by 2 mm^2 mesh. The shade house was 5 by 18 m^2 with an internal height of 2.5 m. This was divided into six compartments of 15 m² using polyethylene fabric. The soil fertility was corrected after soil chemical analysis, according to the recommendation for the canola crop (Comissão de Química e Fertilidade do Solo [CQFSRS/SC] 2004). Before sowing, supplementation of phosphorus, nitrogen, and sulfur was made using 300 kg of triple superphosphate and 75 kg of ammonium sulphate per hectare. Forty days after germination, 225 kg of ammonium sulfate per hectare was applied.

In each subunit of the shade house, 2 by 4 m^2 plots were established in July 2012, with a lateral distance of 0.5 m between the plot of one subunit and the next, in which canola 'Hyola 61' was sown in rows spaced at 0.4 m. This resulted in a final density of ~35 plants per square meter, which is similar to the 40 plants per square meter found in commercial fields.

Introduction of Bee Colonies. Three species of social bees were used: A. mellifera, P. emerina, and T. fiebrigi. P. emerina ("mirim") and T. fiebrigi ("jataf") colonies were established in standard hives (31 by 15 by 7 cm³), and the honey bees in standard Langstroth hives with five frames each. When the crop displayed $\sim 10\%$ flowering, only one bee colony was placed per compartment of the shade house, resulting in the use of two subunits per species. Hives were on 1-m-tall wooden stands on the side of each plot.

A. mellifera was chosen because this species has been widely referred to in the literature as an effective pollinator of canola. The choice of *P. emerina* and *T. fiebrigi* was based on the presence of wild nests located in canola growing areas in southern Brazil (Coleção de Abelhas, MCP 2013). Additionally, these species are hardy and easy to manage.

P. emerina is distributed in Brazil, in the States of Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo, and in Paraguai, in the Cordillera and Misiones regions (Moure et al. 2007). These bees are gentle and they build their nests in tree hollows and spaces in walls (Nogueira-Neto 1997).

T. fiebrigi is distributed in Argentina (Misiones and Tucumam regions), Bolivia (Santa Cruz region), and Brazil, in the States of Mato Grosso, Mato Grosso do Sul, Paraná, Rio Grande do Sul, and São Paulo (Moure et al. 2007). This is one of the most common species of Meliponini, which nests in tree hollows, spaces in walls, and other types of human construction (Nogueira-Neto 1997). Colonies have from 2,000 to 5,000 individuals (Nogueira-Neto 1997).

Bee Foraging Behavior. The behavior of the workers was analyzed using direct observations of the bees during their visits to flowers throughout the day beginning at 0830 hours. We observed 65 visits of *A. mellifera*, 69 of *P. emerina*, and 60 of *T. fiebrigi.* For each visit (observation unit), the following aspects of bee behavior were recorded: residence time of the bee on the flower; floral resource collected (nectar or pollen); the presence of pollen in the pollen basket; whether there was contact with anthers and stigmas; and specific location of resource collection (anther position, lateral, or median nectaries).

Pollination Efficiency of Bee Species. The efficiency of pollination by *A. mellifera, T. fiebrigi,* and *P. emerina* was compared by the number of pollen grains deposited on stigmas, the rate of fruit set, the number of seeds or silique, and the silique and seed mass after one visit. To assess the deposition of pollen grains on the stigma for each species of bee, flower buds in pre-anthesis (n = 30) were marked and covered with bags (5×6 cm) made of fine mesh white fabric, which did not allow insects to contact the flowers. Immediately after flower bud opening, a bee was

allowed to visit and the flower was covered again after the bee left. Other flower buds (n = 30) were covered to assess the number of pollen grains deposited on the stigma that had no visiting insects (control).

The pistils of the flowers were collected 24 h after the treatments and fixed for 1 h in a solution of 45% glacial acetic acid and 70% ethanol (3:1), after which they were transferred to 70% ethanol (Dafni et al. 2005). In the laboratory, each pistil was placed on a histological slide with glycerinated gelatin stained with fuchsin. The slide was heated on a hotplate to melt the gelatin. After melting, a cover slip was placed on the material by applying light pressure to crush the pistil. The number of pollen grains deposited on the stigma and the number of ovules present in the ovaries were counted manually using a light microscope.

To analyze parameters related to fruiting, flower buds in pre-anthesis were covered with fine mesh fabric (A. mellifera: n = 56; P. emerina: n = 45; T. fiebrigi: n = 45) and a forager's visit was allowed at 3 or 24 h after the flower opening. The flower was then covered until senescence. Buds in pre-anthesis were also covered to assess production without insect pollination (control; n = 24). The resulting siliques were collected when mature, and the number of siliques formed in each treatment group was recorded. The collected siliques were evaluated for weight, length, seed number, and seed mass.

Data Analysis. The behavior of the bees during flower visits was compared by evaluating the number of anthers touched and the duration of visits by foragers. Because the data were not normally distributed (Shapiro–Wilk, P < 0.001), differences between species were compared using the Kruskal–Wallis analysis of variance (ANOVA; $\alpha = 0.05$) with comparison by Dunn's post hoc test ($\alpha = 0.05$). The number of foragers that contacted the stigma was compared among species using the chi-square test ($\alpha = 0.05$).

The number of pollen grains deposited on the stigma of canola flowers by the three bee species and in controls was compared using the Kruskal–Wallis ANOVA ($\alpha = 0.05$) with comparison by Dunn's post hoc test ($\alpha = 0.05$) because these data were also not normally distributed (Shapiro–Wilk, P < 0.001). Two extreme outliers were removed from the A. mellifera data set because the number of pollen grains on these stigmas was 2.8 (500) and 4.8 (900) times greater than the maximum amount of pollen grains found in the stigma (186). We suspected that there was deposition of pollen grains on stigmas by another source during handling.

To examine the correlation between the duration of the visits by bees and 1) the number of pollen grains deposited on the stigma of flowers and 2) the number and 3) mass of the seeds, the Spearman's correlation test ($\alpha = 0.05$) was used because, except for the number of seeds, the variables were not normally distributed (Shapiro–Wilk, P < 0.001). The data for the three species were pooled for this analysis.

The fruit rate set in the treatments with visits of A. mellifera, P. emerina, and T. fiebrigi, and also without interference from insects was compared using the chisquare test ($\alpha = 0.05$). To compare the characteristics of the siliques formed after visits by A. mellifera, P. emerina, and T. fiebrigi, and also without interference from insects, the silique mass and length, the number of seeds per silique, and the total seed mass were analyzed using an ANOVA ($\alpha = 0.05$) because the variables were normally distributed (Shapiro–Wilk, P > 0.05). The differences between groups were evaluated using Tukey's test ($\alpha = 0.05$). The data presented refer to the combination of the three and 24-h-old flowers. Differences were not detected for the silique mass and length, number of seeds per silique, or total seed mass between the flowers of different ages (Mann–Whitney, P > 0.05).

Statistical analyses were performed using the software BioEstat and Statistica. For the chi-square tests the chisq test function in "stats" package for R program (R Core Team, 2014, Vienna, Austria) was used.

Results

Bee Foraging Behavior. A. mellifera collected nectar from the nectaries located at the base of the short stamens by accessing them through the corolla opening. The foragers were never observed collecting pollen, even though they do it (Nedić et al. 2013). Both the P. emerina and T. fiebrigi workers used the opening between the sepals to reach the nectaries located at the base of the long stamens without contacting the reproductive organs of the flowers (Fig. 1). However, both P. emerina and T. fiebrigi were frequently observed actively collecting pollen. During pollen collection, the bees landed on the corolla, walked on the flower, and touched the reproductive organs. The data to evaluate pollination efficiency were recorded by analyzing the behavior during nectar-gathering visits for A. mellifera and pollen-gathering visits for both P. emerina and T. fiebrigi.

During the visits, most A. mellifera (63.1%) and P. emerina (55.1%) foragers made contact with the stigma, but fewer than half of T. fiebrigi contacted the stigma (46.7%); however, it was not significant different ($\chi^2 = 3.4$, df = 2, P > 0.05; Table 1). There was a difference in the number of anthers contacted by the foragers of different species during a visit (H = 37.2, df = 2, P < 0.001, Table 1). A. mellifera made contact with a greater number of anthers than P. emerina and T. fiebrigi (P < 0.05; Table 1), while the number of anthers contacted by the latter species were similar (P > 0.05; Table 1).

The visit duration of the three bee species studied also differed (H = 110.8, df = 2, P < 0.0010; Table 1). The visits from *A. mellifera* were shorter than those from *P. emerina* and *T. fiebrigi* (P < 0.05; Table 1), with the latter two not differing in duration (P > 0.05; Table 1).

Pollination Efficiency.

Deposition of Pollen on Stigmas. There was a significant difference in the number of grains deposited on the stigma of canola flowers by the three bee species and by self-pollination (H = 23.3, df = 3, P < 0.0001; Fig. 2). The deposition of pollen grains in the absence



Fig. 1. Flower of *B. napus* and the behavior of bees during nectar collection. (A) The location on the flower of the lateral and median nectaries. (B) *A. mellifera* visit through the corolla opening for nectar collection in the lateral nectaries located at the base of the short stamens. (C) Visit through the outside of the flower by *P. emerina* to collect nectar in median nectaries located at the base of the long stamens, exposed between the sepals. Illustration by Flávia Tirelli.

Table 1. Number of anthers contacted during canola flower visits by *A. mellifera*, *P. emerina*, and *T. fiebrigi* (*B. napus* Hyola 61), the duration of the visits and the percentage (%) of foragers that made contact with the stigma

Treatment	No. of touched anthers	Visit duration (s)	Percentage of foragers contacting the stigma	N
A. mellifera	$5.6 \pm 1.1a$	$\begin{array}{c} 14.4 \pm 9.2a \\ 65.7 \pm 41.7b \\ 88.0 \pm 53.0b \end{array}$	63.1%ns	65
P. emerina	$4.6 \pm 1.3b$		55.1%ns	69
T. fiebrigi	$4.4 \pm 1.5b$		46.7%ns	60

Number of touched anthers and visit duration: different letters (a and b) indicate significant differences by Kruskal–Wallis test with post hoc comparison by Dunn's method (P < 0.05). Percentage of foragers contacting the stigma: not significant (ns) at $\alpha = 0.05$ by the chi-square test.



Fig. 2. Number of pollen grains deposited on the stigma of canola flowers (*B. napus* Hyola 61) by *A. mellifera* (AM), *P. emerina* (PE), and *T. fiebrigi* (TF) during a visit, and by self-pollination (SP). Boxplot: boxes indicate the distribution of 50% of values, solid horizontal line indicates the median, dashed horizontal line indicates the mean, whisker indicates the SE (>90% and <10%), and filled circles indicate outliers. Different letters (a and b) indicate significant differences by Kruskal–Wallis test with post hoc comparison by Dunn's method (P < 0.05).

of insect pollination (self-pollination) was lower than that by visits from bees of the three species (P < 0.05; Fig. 2). However, there was no significant difference in the number of pollen grains deposited by A. *mellifera*, P. emerina, or T. fiebrigi (P > 0.05; Fig. 2).

The average number of ovules present in the ovaries of flowers of canola was 28 ± 4 (n = 118). All three bee species deposited a sufficient number of grains to fertilize all ovules (A. mellifera: 38 ± 45 , n = 40; P. emerina: 30 ± 24 , n = 29; T. fiebrigi: 38 ± 39 , n = 30); however, self-pollination was insufficient (5 ± 6 , n = 17, Fig. 2). There was no correlation between the duration of the visits by the three bee species and the number of pollen grains deposited on the stigma of flowers (r = -0.09; P > 0.05).

Production of Siliques and Seeds. Pollination by *P. emerina*, *A. mellifera* or *T. fiebrigi* resulted in a higher rate of fruit set compared with by self-pollination ($\chi^2 = 8.2$, df = 3, P < 0.05; Table 2). There was an increase of 20.8, 32.1, and 29.8% in the rate of fruit set from pollination by *A. mellifera*, *P. emerina*, and *T. fiebrigi*, respectively, compared with the rate of fruit set resulting from self-pollination (Table 2).

There was a significant difference in silique mass (F=6.05; df=3; P<0.001) and length (F=3.66; df=3; P<0.05), number of seeds per silique (F=3.93; df=3; P<0.05), and total seed mass (F=6.85; df=3; P<0.05) produced by the different pollination treatments (Table 2). Flowers without bee pollination generated siliques with lower mass, size, number of seeds, and seed mass than those pollinated by *A. mellifera* and *P. emerina* (P<0.05, Table 2). Pollination by *T. fiebrigi* increased silique length compared with silique length in the control treatment (P<0.05; Table 2).

The siliques resulting from visits by A. *mellifera* and *P. emerina* were similar in mass, size, number of seeds, and total seed mass (P > 0.05; Table 2). The siliques and seeds produced from flowers pollinated by A. *mellifera* were heavier than those produced by T. *fiebrigi* (P < 0.05; Table 2); however, the size and number of

Treatment	No. of siliques	Mean silique mass (mg)	Mean silique length (cm)	Mean number of seeds per silique	Mean total seed mass per silique (mg)	Fruit set rate
A. mellifera	36	$71.4 \pm 32.6a$	4.7 ± 1.1 a	$12 \pm 5a$	$38.3 \pm 19.1a$	64.3%a
P. emerina	34	$68.9 \pm 34.4 ab$	$4.8 \pm 1.3a$	$12 \pm 6a$	$33.6 \pm 16.9 ab$	75.6%a
T. fiebrigi	33	$51.1 \pm 25.9 bc$	$4.4 \pm 1.1a$	$10 \pm 5ab$	$23.9 \pm 15.1 bc$	73.3%a
Self-pollination (control)	10	$37.8 \pm 21.7 \mathrm{c}$	$3.5 \pm 1.0 \mathrm{b}$	$6 \pm 4b$	$16.9 \pm 1.2c$	43.5%b

Table 2. Characteristics of siliques and the rate of fruit set resulting from self-pollination (control: without visits) and visits by A. mellifera, P. emerina, and T. fiebrigi workers

All variables, except fruit set rate: different letters (a, b, and c) indicate significant differences by ANOVA with post hoc comparison using Tukey's test (P < 0.05). Fruit set rate: different letters (a, b, and c) indicate significant differences by the chi-square test (P < 0.05).

seeds from the siliques were similar (P > 0.05; Table 2). All quality parameters of the siliques produced by *P. emerina* were similar to those resulting from the visits by *T. fiebrigi* (P > 0.05; Table 2). There was no correlation between the visit durations of the three bee species (r = 0.004; P > 0.05) and the number and the mass of the seeds produced by the siliques (r = -0.18; P > 0.05).

Discussion

B. napus flowers have two pairs of nectaries. Lateral nectaries are located on the inner base of each filament of the short stamens and the median nectaries arise from the external junction of the bases of the long stamen filaments (Davis et al. 1986, 1994, 1996; Nedić et al. 2013). The nectaries are visible and accessible both from the flower top and side (Ali et al. 2011). In the present study, the three bee species selected different nectaries to obtain nectar, and this behavior influences the efficiency of flower pollination. A. mellifera visited the canola flower from the top (through the corolla opening) to collect nectar only from the lateral nectaries, thereby touching the reproductive organs. The pollen grains stick to the insect during the flower visits and are therefore obtained indirectly. The preference of this species for using the lateral nectaries corroborates the finding from previous studies on other canola cultivars (Adegas and Nogueira-Couto 1992, Rosa et al. 2010). P. emerina and T. fiebrigi workers collect nectar in the median nectaries that are exposed between the sepals. These nectar gatherers do not pollinate the crop because they do not touch the reproductive organs during their visits to the flowers. This side feeding on canola flowers (i.e., this "illegitimate" nectar collection) has also been observed in *Apis florea* F. and some Diptera species (Eristalinus sp., Euphumosia sp., and Musca sp.; Ali et al. 2011).

The lateral pair of canola nectaries yields more nectar than the median pair, and the lateral pair secretes 95% of the carbohydrates produced by the flower (Davis et al. 1994, Nedić et al. 2013). Additionally, nectar from the lateral glands has higher glucose or fructose levels (Davis et al. 1986). The preference of *A. mellifera* for *B. napus* varieties with a higher volume of nectar and high sugar concentrations (Abrol 2007) might explain the choice of lateral nectaries for nectar collection by *A. mellifera* that was observed in the present study. The "illegitimate" collection of nectar ("robbing") by stingless bees could be attributed to the inability of *P. emerina* and *T. fiebrigi* to reach the lateral nectaries. *A. mellifera* workers also rob nectar from this crop (Delaplane and Mayer 2000). According to Ali et al. (2011), side feeding by insects on canola flowers is a way to obtain nectar with less effort. Nectar robbers may have direct and indirect effects on plant reproductive success, but their presence does always not have negative effects on pollination (Irwin et al. 2010, Fumero-Caban and Melendes-Ackermann 2012). Thus, despite acting as nectar robbers, *P. emerina* and *T. fiebrigi* are effective pollinators when they collect pollen.

Another aspect of pollinator behavior related to plant reproductive success is the duration of the flower visits. In the present study, we determined that the duration *A. mellifera*'s visit to flowers to collect only nectar was shorter than that of native bees to collect pollen (Table 1). Singh et al. (2006) and Adegas and Nogueira-Couto (1992) observed that the duration of visits of a pollinator of *B. napus* flowers depends on the resource collected and that *A. mellifera* spends more time per visit to collect nectar and pollen than when collecting only nectar.

According to Ivey et al. (2003), the most effective species at transferring pollen grains is the one that spends more time visiting the flower, which also suggests that slow foraging pollinators should increase the pollination success of a plant. However, the present study identified no increase in the number of pollen grains deposited on the stigma of flowers with increasing duration of visits. Furthermore, the number of pollen grains deposited on the stigma of the flower in a visit was similar for all three bee species (Fig. 2), despite differences in the duration of the visit (Table 1). The absence of bee visits resulted in a deposition of pollen grains that was insufficient to fertilize all ovules (Fig. 2), thereby confirming the importance of insect pollination to increase the productivity of B. napus Hyola 61 crops.

The rate of fruit set in the absence of pollinators was 43.5%. This increased to 64.3% in the presence of *A. mellifera* and to values >70% with the visit of the native bees. These results are in agreement with the findings of Garibaldi et al. (2013) that compared the efficiency of *A. mellifera* and native insects on rates of fruit set in 41 crops and found that native insects are more efficient with regards to the rate of fruit set of crops than *A. mellifera*.

Managed colonies of *A. mellifera* have become the largest source of pollinators on the planet, and can increase productivity by up to 50% for the canola crop (Sabbahi et al. 2005, Duran et al. 2010). However, the losses of colonies of this species recorded in the past 20 years increases the concern for the conservation of native bee populations (Watanabe 1994). There is also evidence that the management of *A. mellifera* supplements, but does not replace, the pollination services provided by native insects (Garibaldi et al. 2013).

The present study revealed that *P. emerina*, a species of social native bee from canola-growing regions in southern Brazil, has the potential to be used and managed for canola pollination because pollination by this species improved the rate of fruit set rate comparing to the other two species and self-pollination and produced siliques with the same characteristics of the ones produced by *A. mellifera*. This finding was true despite the small body size (~4 mm; Friese 1900). *T. fiebrigi*, despite its role as a pollinator, was not as efficient as *A. mellifera*, as the former produced lighter seeds (Table 2). The use of native bee species for the pollination of canola is not only beneficial to the crop yield but can also promote the conservation of native pollinators.

Acknowledgments

We give thanks to the following. Guilherme Abreu, an Agronomy student at UFRGS, for his support during field work. Tatiana Kaehler for helping with the laboratory activities. The employees of the State Foundation for Agricultural Research ("Fundação Estadual de Pesquisa Agropecuária"), Darci Vianna Fucilini and José Átila Feijó, for their support in managing the crop. The National Council of Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) and FAO (Food and Agriculture Organization of the United Nations)/ UNEP (United Nations Environment Program)/MMA (Ministério do Meio Ambiente [Ministry of the Environment])/ FUNBIO (Fundo Brasileiro para a Biodiversidade [Brazilian Biodiversity Fund]) for financial support.

References Cited

- Abrol, D. P. 2007. Foraging behavior of Apis mellifera L. and Apis cerana F. as determined by the energetics of nectar production in different cultivars of Brassica campestris var. toria. J. Apic. Sci. 51: 19–24.
- Adegas, J.E.B., and R. H. Nogueira-Couto. 1992. Entomophilous pollination in rape (*Brassica napus* L. var oleifera) in Brazil. Apidologie 23: 203–209.
- Ali, M., S. Saeed, A. Sajjad, and A. Whittington. 2011. In search of the best pollinators (*Brassica napus* L.) production in Pakistan. Appl. Entomol. Zool. 46: 353–361.
- Blochtein, B. P. Nunes-Silva, R. Halinski, L. A. Lopes, and S. Witter. 2013. Comparative study of the floral biology and of the response of productivity to insect visitation in two rapeseed cultivars (*Brassica napus* L.) in Rio Grande do Sul. Braz. J. Biol. 74: 787–794.
- Brasil. 1969. Atlas Climatológico do Brasil [Climate Atlas of Brazil]. 1969. Ministério da Agricultura, Escritório de meteorologia [Ministry of Agriculture, Weather office], Rio de Janeiro.
- Cauich, O., J.J.G. Quezada-Euán, J. O. Macias-Macias, V. Reyes-Oregel, S. Medina-Peralta, and V. Parra-Tabla. 2004. Behavior and pollination efficiency of *Nannotrigona*

perilampoides (Hymenoptera: Meliponini) on greenhouse tomatoes (*Lycopersicon esculentum*) in subtropical México. J. Econ. Entomol. 97: 172–179.

- (CQFSRS/SC) Comissão de Química e Fertilidade do Solo [Soil Chemistry and Fertility Comisison]. 2004. Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina [Fertilization and liming manual for the States of Rio Grande do Sul and Santa Catarina). Sociedade Brasileira de Ciência do Solo [Brazilian Society of Soil Science], Porto Alegre.
- (MCP) Coleção de Abelhas [Bee Collection]. 2013. (http:// www.splink.org.br). Last accessed 10 January 2014.
- Constanza, R., R. D'arger, R. Grootr, S. Farberk, M. Grasso, B. Hannon, K. Limburg, S. Naeem, J. Paruelo, R. G. Raskin, et al. 1997. The value of the world's ecosystem services and natural capital. Nature 387: 253–260.
- Cortopassi-Laurino, M., V. L. Imperatriz-Fonseca, D. W. Roubik, A. Dollin, T. Heard, I. Aguilar, G. C. Venturieri, C. Eardley, and P. Nogueira-Neto. 2006. Global meliponiculture: Challenges and opportunities. Apidologie 37: 275–292.
- Dafni, A., P. G. Kevan, and B. C. Husband. 2005. Practical pollination biology. Enviroquest, Canada.
- Davis, A. R., R. L. Peterson, and R. W. Shuel. 1986. Anatomy and vasculature of the floral nectaries of *Brassica napus* (Brassicaceae). Can. J. Bot. 64: 2508–2516.
- Davis, A. R., V. K. Sawhney, L. C. Fowke, and N. H. Low. 1994. Floral nectar secretion ploidy in *Brassica rapa* and *Brassica napus* (Brassicaceae). I. Nectary size and nectar carbohydrate production and composition. Apidologie 25: 602–614.
- Davis, A. R., L. C. Fowke, V. K. Sawhney, and N. H. Low. 1996. Floral nectar secretion ploidy in *Brassica rapa* and *Brassica napus* (Brassicaceae). II. Quantified variability of nectary structure and function in rapid-cycling lines. Ann. Bot. 77: 223–234.
- Delaplane, K. S., and D. F. Mayer. 2000. Crop pollination by bees. CABI Publishing, Wallingford, United Kingdom.
- Del Sarto, M.C.L., R. C. Peruquetti, and L.A.O. Campos. 2005. Evaluation of the neotropical bee *Melipona quadrifasciata* (Hymenoptera: Apidae) as pollinator of greenhouse tomatoes. J. Econ. Entomol. 98: 260–266.
- dos Santos, S. A., A. C. Roselino, M. Hrncir, and L. R. Bego. 2009. Pollination of tomatoes by the stingless bee *Melipona* quadrifasciata and the honey bee *Apis mellifera* (Hymenoptera, Apidae). Genet. Mol. Res. 8: 751–757.
- (Embrapa) Empresa Brasileira de Pesquisa Agropecuária [Brazilian Agricultural Research Corporation]. 2006. Sistema brasileiro de classificação de solos [Brazilian soil classification system]. EMBRAPA - SPI, Rio de Janeiro.
- Friese, H. 1900. Neue Arten Der Bienengattungen Melipona. III. Und Trigona. Jur. Termeszetr. Füs. 23: 381–394.
- Freitas, B. M., V. L. Imperatriz-Fonseca, L. Medina, A. M. Kleinert, L. Galetto, G. Nates-Parra, and J. G. Quezada-Euán. 2009. Diversity, threats and conservation of native bees in the Neotropics. Apidologie 40: 332–346.
- Fumero-Caban, J. J., and E. J. Melendez-Ackerman. 2012. Effects of nectar robbing on pollinator behavior and plant reproductive success of *Pitcornia angustifolia* (Bromeliaceae). Plant Spec. Biol. 28: 224–234.
- Féon, V. L., A. Schermann-Legionnet, Y. Delettre, S. Aviron, R. Billeter, R. Bugter, F. Hendrickx, and F. Burel. 2010. Intensification of agriculture, landscape composition and wild bee communities: A large scale study in four European countries. Agric. Ecosyst. Environ. 137: 143–150.
- Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aisen, R. Bommarco, S. A. Cunningham, C. Kremen, L.

G. Cavalheiro, L. D. Harder, O. Afik, et al. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339: 923–924.

- Hogendoorn, K., Z. Steen, and M. P. Schwarz. 2000. Native Australian carpenter bees as a potential alternative to introducing bumble bees for tomato pollination in greenhouses. J. Apic. Res. 39: 67–74.
- Hogendoorn, K., C. L. Gross, M. Sedgley, and M. A. Keller. 2006. Increased tomato yield through pollination by native Australian Amegilla chlorocyanea (Hymenoptera: Anthophoridae). J. Econ. Entomol. 99: 829–833.
- Irwin, R. E., J. L. Bronstein, J. S. Manson, and L. Richardson. 2010. Nectar robbing: Ecological and evolutionary perspectives. Annu. Rev. Ecol. Evol. Syst., 41, 271–292.
- Ivey, C. T., P. Martinez, and R. Wyatt. 2003. Variation in pollinator effectiveness in swamp milkweed, Asclepias incarnate (Apocynaceae). Am. J. Bot. 90: 214–225.
- Jauker, F., B. Bondarenko, H. C. Becker, and I. Steffan-Dewenter. 2012. Pollination efficiency of wild bees and hoverflies provided to oilseed rape. Agric. For. Entomol. 14: 81–87.
- Klein, A. M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremer, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B 274: 303–313.
- Malagodi-Braga, K. S. and A.M.P. Kleinert. 2004. Could Tetragonisca angustula Latreille (Apinae, Meliponini) be effective as strawberry pollinator in greenhouses? Aust. J. Agric. Res. 55: 771–773.
- Moritz, R.F.A., F. B. Kraus, P. Kryger, and R. M. Crewe. 2007. The size of wild honeybee populations (*Apis mellifera*) and its implications for the conservation of honeybees. J. Insect Conserv. 11: 391–397.
- Natural Research Council. 2006. Status of pollinators in North America, National Academic Press, Washington, DC.
- Nedić, N., M. Mačukanović-Jocić, D. Rančić, B. Rørslett, I. Šoštarić, Z. D. Stevanović, M. Mladenović. 2013. Melliferous potential of *Brassica napus* L. subsp. *napus* (Cruciferae). Arthropod Plant Interact. 7: 323–333.
- Ne'eman, G., A. Jürgens, L. Newstrom-Lloyd, S. G. Potts, and A. Dafni. 2010. A framework for comparing pollinator performance: Effectiveness and efficiency. Biol. Rev. 85: 435–451.
- Nogueira-Neto, P. 1997. Vida e criação de abelhas indígenas sem ferrão, Nogueirapis, São Paulo.
- Nunes-Silva, P., M. Hrncir, C. I. Silva, Y. Roldao, and V. L. Imperatriz-Fonseca. 2013. Stingless bees, *Melipona fasciculata*, as efficient pollinators of eggplant (*Solanum melongena*) in greenhouses. Apidologie 44: 537–546.
- **Ollerton, J., R. Winfree, and S. Tarrant. 2011.** How many flowering plants are pollinated by animals? Oikos 120: 321–326.
- Palma, G., J.J.G. quezada-Euán, V. Reyes-Oregel, V. Meléndez, and H. Moo-Valle. 2008. Production of greenhouse tomatoes (*Lycopersicon esculentum*) using *Nannotrigona perilampoides*, *Bombus impatiens* and mechanical vibration (Hym.: Apoidea). J. Appl. Entomol. 132: 79–85.

- Potts, S. G., S.P.M. Roberts, R. Dean, G. Marris, M. A. Brown, R. Jones, P. Neumann, and J. Settele. 2010. Declines of managed honeybees and beekeepers in Europe? J. Apic. Res. 49: 15–22.
- Rader, R., B. G. Howlett, S. A. Cunningham, D. A. Westcott, L. E. Newstrom-Lloyd, M. K. Walker, D.A.J. Teulon, and W. Edwards. 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. J. App. Ecol. 46: 1080–1087.
- R Core Team. 2014. R: A language and environment for statistical computing. The R Foundation for Statistical Computing, Vienna, Austria.
- Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, et al. 2010. Apis mellifera (Hymenoptera: Apidae) as a potential Brassica napus pollinator (cv. Hyola 432) (Brassicaceae) in Southern Brazil. Braz. J. Biol. 70: 1075–1081.
- Rosa, A. S., B. Blochtein, and D. K. Lima. 2011. Honey bee contribution to canola pollination in Southern Brazil. Sci. Agric. 68: 255–259.
- Sabbahi, R., D. Oliveira, and J. Marceu. 2005. Influence of honey bee (Hymenoptera: Apidae) density on production of canola. J. Econ. Entom. 98: 367–372.
- Sing, J., O. P. Agrawal, and R. C. Mishra. 2006. Foraging rates of different *Apis* species visiting parental lines of *Bras*sica napus L. Zoos' Print J. 21: 2226–2227.
- Slaa, E. J., C.L.A. Sánchez, K. S. Malagodi-Braga, and F. E. Hofstede. 2006. Stingless bees in applied pollination. Practice and perspectives. Apidologie 37: 293–315.
- Stanley, D. A., D. Gunning, and J. C. Stout. 2013. Pollinators and pollination of oilseed rape crops (Brassica napus L.) in Ireland: Ecological and economic incentives for pollinator conservation. J. Insect Conserv. 17: 1181–1189.
- Van Engelsdorp, D., J. Hayes, Jr., R. M. Underwood, and J. Pettis. 2008. A survey of honey bee colony losses in the U.S., Fall 2007 to Spring 2008. PLoS ONE 3: e4071.
- Witter, S., B. Blochtein, P. Nunes-Silva, F. P. Tirelli, B. B. Lisboa, C. Bremm, and R. Lanzer. 2014. The bee community and its relationship to canola seed production in homogenous agricultural areas. J. Poll. Ecol. 12: 15–21.
- Witter, S. B. Radin, B. B. Lisboa, J. G. Teixeira, and V. L. Imperatriz-Fonseca. 2012. Desempenho de cultivares de morango submetidas a diferentes tipos de polinização em cultivo protegido [Performance of strawberry cultivars subjected to different types of pollination in a greenhouse]. Pesq. Agropec. Bras. 47: 58–65.
- Woodcock, B. A., M. Edwards, J. Redhead, W. R. Meek, P. Nuttall, S. Falk, M. Nowakowski, and R. F. Pywell. 2013. Crop flower visitation by honeybees, bumblebees and solitary bees: Behavioural differences and diversity responses to landscape. Agric. Ecosyst. Environ. 171, 1–8.
- Watanabe, M. E. 1994. Pollination worries rise as honeybees decline. Science 265: 1170.

Received 13 January 2014; accepted 2 April 2015.