THE BEE COMMUNITY AND ITS RELATIONSHIP TO CANOLA SEED PRODUCTION IN HOMOGENOUS AGRICULTURAL AREAS

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Abstract—Canola crop productivity is benefited by bee pollination and it has been shown that bee communities can be affected by landscape composition. The aim of this study was to analyse the bee community and its relationship to canola seed production in agricultural areas. The density, abundance and richness of floral visitors of *Brassica napus* cultivar Hyola 61 in six commercial fields in southern Brazil were studied, and their relationships with seed production and the ratio of semi-natural, forested and agricultural areas surrounding the crops were examined. It was observed that canola fields of southern Brazil are surrounded by a homogeneous landscape dominated by agricultural areas. The survey of bees detected a low abundance and richness of native bees in contrast to the high abundance of *Apis mellifera*. Except for a negative correlation between the abundance of honey bees and the proportion of forested areas within a 2000 m radius from the field (R = -0.90; P = 0.012), no other correlations were found among bee abundance and richness and landscape composition. Although there was not a relationship between *A. mellifera* and seed set, there was a positive correlation between insect density and seed weight per plant (R = 0.87; P = 0.024). As honey bees were the most captured insect (79%), much of the pollination in this system was probably achieved by honey bees.

Keywords: Brassica napus, Apis mellifera, *yield, crop pollination, landscape*

INTRODUCTION

Human activity in the biosphere has altered ecosystems and often threatens their capacity to provide services that are essential to human survival (Kremen 2005). One of these services is pollination, which is fundamental to the maintenance of biodiversity, floristic composition (Biesmeijer et al. 2006; Potts et al. 2010) and food production (Gallai et al. 2009). In this context, the decline in native (Cameron et al. 2011) and managed (Potts et al. 2010) bee populations can pose a threat to pollination services globally and, consequently, to agriculture. To estimate the risk of a pollination crisis in the global market, the role of non-managed pollinators for different crops should be investigated (Jauker et al. 2012).

Native pollinators can increase the productivity of crops and thus constitute an important natural resource, even though their populations are sometimes insufficient to adequately pollinate crops in environments of intensive agriculture (Klein et al. 2007). Agricultural areas with small fragments of natural habitats are suffering losses in productivity because of the lack of native pollinators (Gallai et al. 2009; Richards 2001; Ricketts 2004). According to Morandin et al. (2007), areas of intensive agriculture that exhibit homogeneous landscape structures are detrimental to native bee populations and have lower potential for canola production, for example, than areas with diverse vegetation.

Canola production is greatly influenced by pollen vectors such as wind, gravity and insects, especially *Apis mellifera* (Sabbahi et al. 2005; Duran et al. 2010; Bommarco et al. 2012). Recent studies have shown that native bees are also efficient pollinators of canola flowers (Ali et al. 2011; Jauker et al. 2012) and that the elevated abundance of these bees increases the productivity of crops (Morandin & Winston 2005). Although it is self-fertilising, canola does not produce a large number of siliques in the absence of insect pollinators (Sabbahi et al. 2005); bees enhance seed quantity and quality, and thus market value of the crop (Duran et al. 2010; Ali et al. 2011; Bommarco et al. 2012; Jauker et al. 2012).

The cultivation of canola in Brazil is increasing, with Rio Grande do Sul having the largest planted area (Tomm 2007). Considered the third largest global commodity among oleaginous crops, canola currently accounts for 15% of vegetable oil production and is eclipsed only by soy and palm (Carvalho 2011). Canola is also used in the production of biodiesel and animal feed and represents an important alternative for crop rotation, with the potential to increase employment and revenue (Carvalho 2011; Vargas et al. 2011).

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The landscapes surrounding the fields chosen for the present study were similar in land use characteristics. Moreover, there is no traditional use of bees for pollinating, and no studies on the abundance and diversity of bees in the area have been performed. In this context, this study is diagnostic in nature, providing an approximation of services that pollinators render to the canola crop. Considering the economic importance of canola and the fact that the increase in productivity mediated by pollinators varies according to the crop and environmental conditions (Kevan & Eisikowitch 1990; Sabbahi et al. 2005), the present study sought to understand the relationships between the landscape and the assemblage of pollinators in seed production.

MATERIALS AND METHODS

Study areas

The study was performed in the municipality of Guarani das Missões (28°08'27"S and 54°33'29"W) located in the northeast of Rio Grande do Sul, southern Brazil, from July to October of 2011. According to the classification of Köppen, this region possesses a humid temperate climate with hot summers (Cfa).

The region has highly fragmented habitats derived from intensive agricultural use. The area is covered by pasture land, fragments of forest and fields of annual crops composed primarily of soya, wheat, maize, canola and sunflower. The soil of the area is classified as oxisol.

Six fields planted with cultivar Hyola 61 were selected. The six fields differed in area from seven to 23 ha, (field 1= 7 ha; field 2 = 9 ha; field 3 = 10 ha; field 4 = 9 ha; field 5 = 8 ha; field 6 = 23 ha). The fields were separated by a distance of more than 1 km and were cultivated by direct planting. The experiments were conducted in areas of 25 m x 50 m located 20 m from the edge of each field and began when 20% of the plants were flowering.

We examined the density, abundance and richness of pollinators in relationship to seed production, and the ratio of semi-natural, forested and agricultural areas surrounding the fields (Vaissière et al. 2011).

Landscape Analysis

To identify landscape structures, Landsat TM 5 satellite images made available by the National Institute for Space Research (INPE) were geo-referenced and processed in the computational program ENVI® (Environment for Visualising Images). Thematic maps of land use and cover were created based on the classifications in the computational program ArcGIS® over the digital mapping layer available in Hasenack & Weber (2007). The nomenclature of the program CORINE (Coordination of Information on the Environment) used for mapping is related to the inventory of human activities (Heymann et al. 1994). Level 1 of the CORINE nomenclature was used, according to the recommendation of Vaissière et al. (2011), to detect and evaluate pollination deficits in agricultural crops using three categories (agricultural areas, semi-natural areas including eucalyptus and tree/shrub savannah and forested areas). Features such as exposed soil and different crop fields were grouped in the category 'agricultural areas' because they occupy the same area during different periods of the year. Land use analysis was conducted within a radius of 500 m, 1000 m and 2000 m of the study fields.

Insect sampling and analysis of crop productivity

The abundance and richness of floral visitors was analysed in the experimental areas by capturing insects with a sweep net in six 25 m transects for five minutes each, for a total of 30 minutes of sampling. The insects collected were killed in jars containing ethyl acetate. Bees were subsequently tagged, identified by specialists and deposited in the Museum of Entomology of Pontifical Catholic University of the Rio Grande do Sul. The remaining captured insects were identified to the level of order and preserved in 70% alcohol.

The density of pollinating insects per field was obtained by counting the number of insect visitors in 500 canola inflorescences (Density per inflorescence = number of visitors/500) counting all insects visitors, not just honey bees. To accomplish this, an evaluator walked slowly for the length of four previously determined 25 m transects in each experimental area for a maximum period of 15 minutes/transect.

In each experimental area, three sampling rounds were carried out (collection by net and determination of insect density) at fixed times (10:00 h, 13:00 h and 16:00 h) in temperature conditions above 15°C, with low wind, without rain and with dry vegetation. Each sampling round was done on a different day.

Seed production was calculated by gathering five plants in four previously established lines in different positions within the experimental area of each field. Productivity parameters were evaluated by plant, not by area (e.g. per hectare). After the collection, the number of siliques per plant (n=20), the number of seeds per silique (n=10) and the net weight of seeds per plant (n=20) were calculated. The rate of fruit set was calculated by dividing the number of siliques formed by the sum of the abortions with the number of siliques formed. The aborted flowers were identified by the floral pedicel that had lost their floral elements.

Statistical analysis

The relationships between insect density, abundance of *A. mellifera*, native bees and other insects, richness of native bees, crop parameters (rate of fruit set, number of siliques per plant, number of seeds per silique, total seed weight per plant) and the different landscape variables (semi-natural, forested and agricultural areas within a radius of 500 m, 1000 m and 2000 m of the study fields) were estimated by calculating Pearson's correlation coefficients ($\alpha = 0.05$). The semi-natural area within radius of 500m could not be transformed to a normal distribution, therefore, the

TABLE 1. Data for cover (%) of semi-natural areas (SN) forested areas (F) and agricultural areas (A) within a radius of 500 m, 1000 m and 2000 m of canola fields in Guarani das Missões, RS, Brazil, 2011. SD: standard deviation.

	Area (%)								
Field	SN 500	SN 1000	SN 2000	F 500	F 1000	F 2000	A 500	A 1000	A 2000
1	0.2	0.3	0.4	10.0	6.0	12.0	90.0	94.0	87.0
2	0	0	0.2	2.5	9.0	16.0	97.0	91.0	81.0
3	0	0	0	21.0	13.0	13.0	74.0	79.0	82.0
4	0.3	1.0	1.6	5.0	8.0	9.8	95.0	90.0	88.0
5	0	2.0	2.7	1.2	9.0	11.0	99.0	90.0	85.0
6	0	0	8.4	2.0	7.0	10.0	98.0	93.0	81.0
Mean ± SD	0.1 ± 0.2	0.6 ± 0.8	2.2 ± 3.2	7.0 ± 7.6	8.7 ± 2.4	12.0 ± 2.3	92.2 ± 9.5	89.5 ± 5.4	84.0 ± 3.1

Spearman correlation was performed to test associations with this variable. To determine the relationship between insect density and total seed weight per plant, a linear regression equation ($y_{ij} = a + bx + \varepsilon_{ij}$) was fit ($P < 0.05$). The assumptions of normality of data (Kolmogorov–Smirnov test; $P > 0.05$) and homogeneity of variances (Levene´s test, $P > 0.05$) were checked for all variables studied and, transformations were performed when necessary. We also determined that there was no collinearity among variables, except, as expected, within the different descriptors of landscape composition (i.e. A500 and F500, R = -0.99, $P <$ 0.001; A2000 and SN500, R = 0.86, $P = 0.029$; see Tab. 1).

All analyses were carried out with JMP statistical software (version 8.2, SAS Institute, Inc., Cary, NC).

RESULTS

Landscape

Agricultural areas predominated in the study region and occupied an average of 84% of the landscape within a 2000 m radius of the fields evaluated, while forested and semi-natural areas covered only 2.2% and 12% of the area, respectively (Tab. 1). The percentage of agricultural areas differed little among fields and corresponded to 74% to 99% of the surrounding area within a radius of 500 m, 79% to 94% within a radius of 1000 m and 81% to 88% within a radius of 2000 m (Tab. 1).

Abundance, richness and density of insects

We captured 2,298 insects visiting canola flowers. These insect belonged mainly to the orders Hymenoptera (83%), Coleoptera (9%) and Diptera (7%), while representatives of Lepidoptera, Hemiptera and Neuroptera represented less than 1% (Tab. 2). *Apis mellifera* was the most abundant species, representing 79% of the insects captured (mean number of specimens sampled per field: 304.5 ± 73); 3% of the recorded specimens were native bees (mean number of specimens sampled per field: 9.8 ± 8.7).

A. mellifera was the most abundant (97%) species of bee, followed by native bees (3%) of the families Andrenidae, Halictidae and Apidae (Tab. 2 and 3). The abundance of *A. mellifera* and native bees and the richness of native bees varied among fields (Tab. 3).

Relationship between insect density and crop productivity

There was a positive correlation between insect density and the total seed weight per plant (R = 0.87; $P = 0.024$; Fig. 1). However, the rate of fruit set (R = 0.36; $P =$ 0.484), the number of siliques per plant (R = 0.70; $P =$ 0.121) and the number of seeds per silique (R = 0.64; $P =$ 0.173) were not correlated with insect density.

Abundance and diversity of bees, landscape and canola productivity

The abundance of *A. mellifera* was negatively affected by the proportion of forested areas within a 2000 m radius of fields (R = -0.90; $P = 0.012$). However, no significant correlation was observed between the abundance and richness of native bees and any landscape variables evaluated (Tab. 4).

In relation to crop productivity, no significant correlations were observed between the abundance of *A. mellifera* and the parameters of crop productivity analysed (rate of fruit set: R = 0.65, $P = 0.158$; number of siliques/plant: R = 0.43, $P = 0.396$; number of seeds/silique: R = -0.01, $P = 0.981$; weight of seeds/plant: R = 0.22, $P = 0.668$). In the case of native bees, there was a negative correlation between their abundance and the number of seeds per silique (R = -0.92; $P = 0.008$), but not between their abundance and the rate of fruit set (R = 0.14, $P = 0.784$), the number of siliques/plant (R = -0.24, $P =$ 0.640) and the weight of seeds/plant (R = -0.72, $P =$ 0.103). There was also a negative correlation between the richness of native bees and the number of seeds per silique (R = -0.86; $P = 0.025$), but no significant correlations were observed between their richness and the rate of fruit set (R = 0.36, $P = 0.476$), the number of siliques/plant (R = -0.15, $P = 0.778$) and the weight of seeds/plant (R = -0.67, $P =$ 0.147). The abundance of other insects was not correlated

Family	Tribe	Species	Abundance
Apidae	Apini	*A. mellifera*	1827
	Meliponini	*Plebeia nigriceps*	1
		Plebeia emerina	6
		Schwarziana quadripunctata	6
		Tetragonisca fiebrigi	23
		Trigona spinipes	10
	Bombini	*Bombus pauloensis*	1
	Xylocopini	*Xylocopa* sp.	1
	Exomalopsini	*Exomalopsis* sp.	6
Andrenidae	Protandrenini	*Psaenythia* sp.	1
Halictidae	Augochlorini	*Augochlorella* sp.	1
		Augochlora sp.	2
		Augochloropsis sp.	1
		Neocorynura sp.	1
Total			1887

TABLE 2. Bees captured in six canola fields during the flowering period (July to October 2011) in Guarani das Missões, northern part of Rio Grande do Sul, Brazil.

with the number of seeds per silique (R = 0.31; $P = 0.550$), the rate of fruit set (R = -0.28, $P = 0.585$), the number of siliques/plant (R = -0.33, $P = 0.524$) and the weight of seeds/plant (R = -0.07, $P = 0.891$).

DISCUSSION

This study verified that the canola fields of southern Brazil are surrounded by a homogeneous landscape dominated by agricultural areas (Tab. 1). The survey of bees detected a low abundance and richness of native bees in contrast to the high abundance of *A. mellifera* (Tab. 2 and 3). Although a relationship between *A. mellifera* and seed production was not found, probably much of the pollination in this system is achieved by this species, because it was the most captured insect (79%). Bommarco et al. (2012) also suggested that because of their high abundance, honey bees were probably the major contribution to canola pollination in their study.

We did find that total density of flower visiting insects influences seed weight per plant, showing that the role of insect pollinators was most apparent when pooling the honey bees with the remaining 21% of insects. However, no significant correlation was found between the abundance of non-bee insects (on their own) and seed production. It is possible that the other insects complement the pollination provided by honey bees. Some Diptera (e.g. Syrphidae), which were found in the fields studied, can also contribute to the pollination of this crop (Jauker and Wolters 2008; Rader et al. 2011). It can be concluded that biotic pollination as a whole is important for canola seed production.

TABLE 3. Seed production, abundance of Apis mellifera, native bees and other insects, and richness of bees in six canola fields from July to October 2011, (flowering period) in Guarani das Missões, northern part of Rio Grande do Sul, Brazil.

Field	Rate of fruit set per plant (%)	Number of siliques per plant	Seed weight per plant (g)	*A. mellifera* abundance	Native bees abundance	Native bees richness	Other insects abundance
1	76.7 ± 8.4	264.4 ± 149.2	12.5 ± 7.6	296	4	3	116
2	75.3 ± 14.4	180.8 ± 81.3	7.8 ± 4.0	167	10	4	49
3	75.7 ± 9.0	258.4 ± 153.4	11.8 ± 6.4	332	12	5	5
4	81.4 ± 4.1	331.9 ± 103.1	14.0 ± 12.1	353	25	8	16
5	81.2 ± 9.5	548.7 ± 235.3	6.6 ± 3.1	307	3	3	19
6	81.4 ± 5.0	328.6 ± 126.4	17.7 ± 5.6	372	6	5	48
Mean ± SD	-	-	-	304 ± 73	10 ± 9	5 ± 2	42 ± 40

TABLE 4. Correlation coefficients (R) among bee abundance and richness of canola fields and landscape variables. SN: seminatural areas. F: forested areas. A: agricultural areas. The numbers after SN, F and A correspond to the radius (m) within the fields. *significant at α=0.05. # Spearman correlation coefficients; +Pearson correlation coefficients.

Landscape categories	*Apis mellifera* abundance		Native bees abundance		Native bees richness		Other insects abundance	
	R	*P*	R	*P*	R	*P*	R	*P*
SN 500#	0.10	0.848	0.34	0.512	0.26	0.617	0.07	0.899
SN 1000+	0.17	0.744	-0.02	0.963	-0.06	0.900	-0.31	0.552
SN 2000+	0.53	0.274	-0.23	0.654	0.09	0.853	-0.04	0.934
F 500+	0.19	0.717	0.13	0.803	0.05	0.915	-0.11	0.832
F 1000+	-0.05	0.918	0.19	0.716	0.10	0.849	-0.72	0.103
F 2000+	-0.90*	0.012	-0.17	0.747	-0.40	0.429	0.13	0.811
A 500+	-0.17	0.743	-0.13	0.798	-0.06	0.903	0.18	0.728
A 1000+	0.37	0.469	-0.41	0.425	-0.27	0.606	0.67	0.146
A 2000+	0.29	0.579	0.34	0.507	0.24	0.643	0.208	0.692

provided by honey bees. Some Diptera (e.g. Syrphidae), which were found in the fields studied, can also contribute to the pollination of this crop (Jauker and Wolters 2008; Rader et al. 2011). It can be concluded that biotic pollination as a whole is important for canola seed production.

Native bees

The landscape surrounding the fields studied was homogeneous and dominated by agricultural areas, presenting low abundance and richness of native bees. Although there was no observed correlation among the abundance and the richness of native bees and the different categories of landscape, other studies show that landscape homogeneity can explain low abundance and richness of bees (Steffan-Dewenter et al. 2002). Areas of intensive agriculture that exhibit homogenous landscape structures can limit the maintenance of native bee populations (Morandin et al. 2007). Moreover, the ability of the habitat to offer places for nesting and feeding, the diversity of plants in the area of the plantation and the crop management are essential for the maintenance of bee fauna in areas of cultivation (Kennedy et al. 2013). Thus, in the study region, the presence of widespread monocultures could be one of the causes of the low abundance and richness of native bees detected.

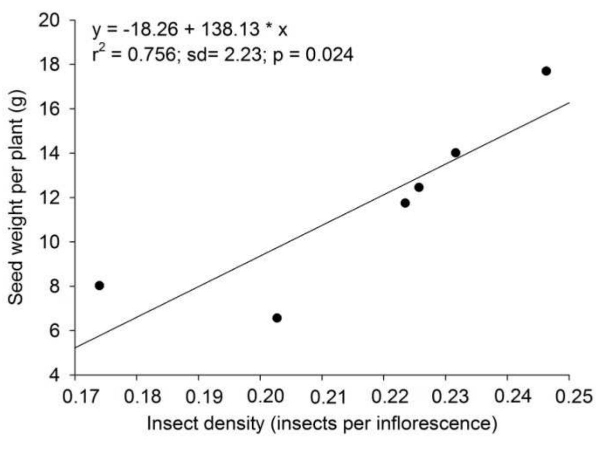

FIG. 1. Linear regression between canola (*Brassica napus* L.) seed weight per plant in relation to the density of insects, calculated as the number of visits per inflorescence over 500 inflorescences in a 15 minute survey period.

We expected to find a positive correlation between the richness and abundance of native bees and semi-natural and forested habitats because, in general, the abundance of native bees is greater near natural areas (Brosi et al. 2007; Morandin et al. 2007). However, there were no semi-natural areas within a 500 m radius of four of the fields studied, very few semi-natural areas near the other two fields, and the forested areas were relatively small when compared to agricultural areas (Tab. 1). Probably, this fact allied to the small number of native bees sampled did not permit the finding of a correlation between the richness and abundance of bees and the presence of semi-natural areas.

We found a negative correlation between native bee abundance and the number of seeds per silique, but, have no explanation for this result. This was the only significant relationship observed when correlating native bee abundance and richness and the analysed parameters of crop productivity.

Honey bees and insect density

A. mellifera was much more abundant (97% of the bees collected) than native bees. We found a negative correlation between the abundance of this species and the occurrence of forested fragments within a radius of 2000 m of the fields. Brosi et al. (2007) showed that, at forest edges only 5% of sampled bees were *A. mellifera*, but far from forests the percentage of *A. mellifera* increased to 45%.

The positive impact of insect density on seed weight of canola found in the present study is most likely associated with the high abundance of *A. mellifera* in the fields evaluated, as this bee is considered one of the main pollinators of canola (Sabbahi et al. 2005). Bommarco et al. (2012) found an increase of 18% in seed weight per plant in parcels that insects could freely visit and also associated the contribution of *A. mellifera* to the productivity of canola due to its abundance. The contribution of pollinating services performed by *A. mellifera* to numerous parameters of canola productivity has been widely documented (Sabbahi et al. 2005; Duran et al. 2010; Ali et al. 2011; Jauker et al. 2012).

Implications for agriculture

The only abundant pollinator we detected was *A. mellifera*. However, the extensive losses of honey bee colonies that have occurred in the last 20 years have increased concerns about the conservation of native bee populations in many regions of the world (Watanabe 1994; Biesmeijer et al. 2006; Potts et al. 2010; Grixti et al. 2009; Cameron et al. 2011). Although there is no evidence that managed and wild colonies of *A. mellifera* are decreasing in number in this part of Brazil, the low abundance and richness of native bees found in this study raises awareness for the importance of conserving native bees. If there were to be a decrease in honey bee populations, the lack of native pollinators can cause losses in productivity (Gallai et al. 2009; Richards 2001; Ricketts 2004). It is suggested that canola growers provide suitable habitats for native bees and promote pollinator-friendly measures for conserving their populations.

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