SCIENTIFIC NOTE

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EVALUATION OF YIELD COMPONENT TRAITS OF HONEYBEE-POLLINATED (*Apis mellifera* L.) RAPESEED CANOLA (*Brassica napus* L.)

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ABSTRACT

Recent introduction of hybrid varieties raises the question if bees (*Apis mellifera* L.) contribute as pollinator agents in developing the full yield potential of rapeseed (*Brassica napus* L.). In order to evaluate the yield achieved by *B. napus* cv. Artus pollinated by *A. mellifera* testing was carried out in the district of Freire, La Araucanía Region, Chile. This consisted in isolating or excluding rapeseed plants from pollinators with exclusion cages. Treatments applied were total exclusion (T1), partial exclusion (T2) and free pollination (T0) with a density of 6.5 hives ha^{-1}, in order to determine the following yield components traits: grains per silique, siliques per plant, 1000 grain weight and yield. The experimental design used was randomized complete blocks with three treatments and three replicates. Results obtained show that the parameter least affected by bee intervention was the grains per silique variable. In contrast, siliques per plant and 1000 grain weight parameters presented significant differences, contributing to a yield greater than 5 t ha^{-1}; which represented a figure 50.34% higher than in the treatment without bees. It may be concluded that the inclusion of bees in crops is fully justified as a production tool.

Key words: bee hives, pollination.

INTRODUCTION

Rapeseed canola is an oleaginous crop, the oil of which is the most valuable component of its seeds (Amjad, 2010). The oil has a low content of erucic acid (Mandal *et al*., 2002; Aytaç *et al*., 2006) and is considered to be among the best quality oils for human consumption (Porter and Crompton, 2008).

The area planted with rapeseed canola in Chile during the 2007-2008 season was approximately 17 250 ha, established mainly in La Araucanía Region and in the South of the Bio-Bio Region (Iglesias, 2008). The mean national yield of rapeseed canola (INE, 2008), is 3.6 t ha^{-1}; the highest national yields are produced in La Araucanía and Maule Regions, with 3.7 and 3.5 t ha^{-1}, respectively.

Pollination is a basic ecological process, essential for the maintenance of viability and diversity (Potts *et al*., 2006; Klein *et al*., 2007), and agriculture today has become even more dependent on pollinators (Aizen *et al*., 2009), with about 35% of global plant food production depending on plants which require these agents. One third of the human diet is constituted by vegetables, legumes and fruits pollinated by insects, of which more than 80% are honeybees (*Apis mellifera* L.) (Klein *et al*., 2007; Hu *et al*., 2008), the most prominent and efficient pollinators (Danforth *et al*., 2006; Evans and Spivak, 2006; Thapa, 2006). For rapeseed, although the wind is the principal vector in terms of the distances over which pollen is transported (Hoyle *et al*., 2007), bees are the principal pollinators, being the most abundant insects in the cultivars and varieties of rapeseed used for seed production (Westcott and Nelson, 2001; Pordel *et al*., 2007). Thus the introduction of hives of *A. mellifera* in controlled pollination helps to increase the production of crops such as rapeseed (*Brassica napus*) (Sabbahi *et al*., 2006), since this plant is characterized by the production of abundant pollen and good quality nectar at relatively high concentrations of sugar, in flowers with a color and structure which are attractive to insects, particularly bees (Office of the Gene Technology Regulator, 2002; Smith, 2002; Sabbahi *et al*., 2006).

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Considering these data, the objective of this experiment was determining the yield of *B. napus* cv. Artus pollinated by *A. mellifera*, under field conditions, measuring yield components such as number of seeds per silique, number of siliques per plant, 1000 grain weight and yield (t ha^{-1}). In addition, we determined the percentage difference in rapeseed canola yield obtained by introducing bee hives into the crop field.

MATERIALS AND METHODS

The experiment was conducted during the 2005-2006 crop season in the municipal district of Freire, Cautín Province, La Araucanía Region (39°08' S; 72°20' W). The farm had 46 ha planted with rapeseed canola, already established when the experiment was initiated. Of this area, 21 ha were cv. Spirit, 15 ha cv. Bilbao, and 10 ha of cv. Artus, a German winter type hybrid which is well suited to the area and has become positioned as one of the most productive and economically attractive alternatives (Iglesias, 2008). It was sown on 26 April 2005, with 3 kg ha^{-1} of seed, in rows spaced at 30 cm.

The experimental period extended over the crop's flowering months, from the end of the stage of flower bud production (5 October) until the end of the flowering sub-stage (19 December), according to Meier (2001).

A study area in the 'Artus' field was defined after visual examination to ensure treatment homogeneity represented by morphologically similar, vigorous and healthy plants. Plants were isolated from pollinator insects during this period by means of cages. Treatment T1, termed total exclusion, was isolated from all insects using wood-framed cages 2 m long, 1 m wide and 2 m high, covered with a white nylon cloth, 1 mm mesh. Thus, all insects larger than 1 mm in diameter were excluded. In treatment, T2, partial exclusion, plants were isolated from honeybees and other insects similar to or larger than honeybees. A similar cage structure was used, but covered with a net made of white polypropylene filaments (Raschel), 2.5 mm mesh. In free pollination treatment (T0), considered control, rapeseed canola plants were grown in a pre-defined and marked area with no intervention, subject to free insect pollination. For pollination, 300 honeybee hives were located at two sites in the farm, the first one 150 m from the experiment in a Southwesterly direction, and the second one 200 m away from the experiment also in a Southwesterly direction. This meant a density of 6.5 hives ha^{-1}.

The central 1 m^2 of each plot was harvested manually on 30 January 2006, eliminating border effects. Once the plants were harvested, yield components were measured, number of siliques per plant, number of grains per silique, and 1000 grain weight in a digital scale, with grains at 7.4% moisture. Yield was estimated in the 1 m^2 harvested.

The experimental design was randomized complete blocks, one factor with three treatments and three replicates. The data were subjected to ANOVA with 5% significance, and then analyzed using Tukey's multiple range comparison test. All analyses were conducted with the computer program SPSS version 12.0 (SPSS, 2003).

RESULTS AND DISCUSSION

Yield components

The free pollination treatment had a mean of 24.00 grains per silique; Young *et al.* (2004) reported that it is possible to find up to 17.4 seeds per silique; total and partial exclusion treatments induced averages of 20.06 and 23.26 grains per silique, respectively. Significant differences were observed between the total exclusion treatment and the other two treatments ($p \leq 0.05$) (Table 1). No significant differences were observed between the partial exclusion and free pollination treatments.

Sabbahi *et al.* (2005) obtained similar results counting a mean of 23 grains per silique, managed with a density of three hives per hectare. Steffan-Dewenter (2003), however, reported that the number of grains per silique was approximately five times higher and seed weight per plant was 10 times higher in experiments with high pollinator density as compared to the controls. Thus, pollination is essential for rapeseed canola production (Westcott and Nelson, 2001), and the introduction of bee hives into the fields during flowering compensates for

Table 1. Effect of treatments on yield components: grains per silique, siliques per plant, 1000 grain weight and yield.

	Grains per silique		Siliques per plant		1000 grain weight		Yield	
Treatment	**Nº**	**SD**	**Nº**	**SD**	**g**	**SD**	**t ha^{-1}**	**SD**
Total exclusion	20.06a	6.72	152.94a	61.76	5.97a	0.53	3.47a	2.12
Partial exclusion	23.26b	6.53	224.83b	91.78	5.99a	0.38	4.70b	1.28
Free pollination	24.00b	5.85	291.17c	57.94	5.20b	0.40	5.24c	0.40

Values designated by different letters are significantly different according to Tukey test ($p \leq 0.05$).
SD: standard deviation; Nº: number.

the lack of pollinator insects, in this way contributing to self sufficiency in pollen transport and cross pollination (Sabbahi, 2003).

In the yield component number of siliques per plant (Table 1), the free pollination treatment induced a mean of 291.17 siliques per plant, while the total and partial exclusion treatments induced means of 152.94 and 224.83 siliques per plant, respectively. According to Angadi *et al*. (2003) the number of siliques is the most important yield component in rapeseed canola production. According to Morrison and Stewart (2002) temperatures above 27 ºC increase flower sterility, while low temperatures negatively affect yield by decreasing the number and size of seeds per silique. In this respect the partial and total exclusion nets should be considered to be a factor which may modify the temperature and solar radiation received by the plants in the cages, affecting their development.

Significant differences occurred between treatments ($p \leq 0.05$). These differences underline the beneficial effect of insect pollination on this yield component, since the partial exclusion and free pollination treatments induced a higher number of siliques compared to the total exclusion treatment. Steffan-Dewenter (2003) confirms this situation, obtaining twice as many siliques per plant as reported in other studies.

The high standard deviation values would be explained by high plant density since a plant in a higher sowing density will only have ramification at the upper end and therefore the number of branches and siliques will reduce as the population density increases (Ozer, 2003), the number of siliques increase with the number of primary and secondary branches (Angadi *et al*., 2003). If the plants are isolated on the other hand, they will have ramifications from the lowest part of the stalk close to the ground, resulting in a considerable quantity of siliques per plant. Thus the yield will correlate significantly and positively with the principal and secondary branches and the height of the plant (Gupta, 2005). Other possible explanations for the variability presented by this component can be attributed to earliness differences, in earliness of flowering, floral abortion or varietal purity, since the seed used on this farm was not certified.

Pollination by bees in rapeseed produces an increase in productivity, improving the yield and contributing to the uniformity and early establishment of the pod (Abrol, 2007). According to Sabbahi *et al*. (2005) this results from the abundant production of flowers during the flowering period, which ensures proper pollination and thus a high and relatively early production of siliques. With respect to the lack of silique formation in the exclusion cages, the same authors indicate that although rapeseed is self-fertilizing, it does not produce a large number of mature pods in the absence of insect pollination. Therefore, the absence of cross pollination, which occurs when free plants in the presence of pollinators interchange pollen, is responsible for the low production of well developed siliques.

The 1000 grain weight component produced a mean of 5.20 g in the free pollination treatment, compared to 5.97 and 5.99 g for the total and partial exclusion treatments respectively (Table 1).

Significant differences ($p \leq 0.05$) were observed in the free pollination treatment as compared to the total and partial exclusion treatments, which were not statistically different. The free pollination treatment induced a reduction of 12.89 and 13.18% in this component as compared to total and partial exclusion treatments respectively. Similar results were obtained by Steffan-Dewenter (2003), who reported that the 1000 grain weight was highest in the total exclusion treatment (4.43 g) as compared to a treatment that had a high density of pollinators (3.87 g).

It was observed in this experiment that the seeds produced in the free pollination treatment were smaller than in the other two treatments. Manning and Wallis (2005) analyzed this component when pollination was performed mainly by honeybees, concluding that the lower seed weight was due to a higher number of fertile siliques, and therefore to more seed but lower in individual weight and size.

The percentage difference obtained in this experiment in the yield component 1000 grain weight was similar to the difference reported by Sabbahi *et al*. (2005), who detected a 13% decrease in a treatment with three hives compared to a treatment that had no hives.

In this research, free pollination treatment induced a mean yield of 5.24 t ha^{-1} (Table 1), which compares positively with a national mean yield of 3.6 t ha^{-1} (INE, 2008). Total and partial exclusion treatments induced mean yields of 3.47 and 4.70 t ha^{-1}, respectively. Similar results were reported by Oz *et al*. (2008), who also obtained the best yields in free pollination and partial exclusion treatments as compared to the treatment without pollinators.

Significant differences ($p \leq 0.05$) were observed among treatments; the highest yield was induced by the free pollination treatment. Plant density in this experiment ranged from 25 to 54 plants m^{-2}. The yield increase induced by the free pollination treatment was 50.34% over total exclusion and 11.46% over partial exclusion, suggesting that high densities of pollinator, especially honeybees, increase the seed weight per plant and thus the yield (Steffan-Dewenter, 2003). Smith (2002) also reported a yield increase of 18% in cv. Karoo pollinated by honeybees; Manning and Wallis (2005) observed a similar effect with a yield increase of over 20% in comparison with rapeseed plants located more than 20 m from the bee hives, while Sabbahi *et al*. (2005) reported that rapeseed yield increased by 46%.

Relationship among response variables

Correlation indexes show high variability among response variables (Table 2), all significant ($p \leq 0.01$) as determined by Pearson's correlation indexes.

Yield was not correlated with the component grains per silique (0.144); yield and grain weight had a low negative correlation, -0.482 (Table 2). Yield, however, was positively and significantly correlated (0.735) with the component number of siliques per plant, which therefore became the main yield component in this experiment. Sabbahi *et al*. (2005) reported that the presence of pollinating honeybees in a rapeseed crop increases principally the number of siliques per plant and therefore the yield.

This study used a density of 6.5 bee hives ha^{-1}, inducing a yield increase of 50.34% as compared to the yield in the absence of honeybees and other pollinators. The information allows the conclusion to be drawn that the bee hive density utilized was adequate to obtain higher yields. However, an optimal bee hive density for rapeseed pollination needs to be determined.

CONCLUSIONS

Honeybees had a significant influence on rapeseed canola yield, increasing it by 50.34% (1.76 t ha^{-1}), compared to the yield in the absence of honeybees. However more tests are needed on this subject in different locations to establish the effects observed in this study, since the data refer to a single cropping season and site.

The presence of *Apis mellifera* at a density of 6.5 bee hives ha^{-1}, did not significantly increase the number of seeds per silique, and induced a significant decrease in 1000 grain weight. However, honeybee pollinators significantly increased the number of siliques per plant, as well as the yield, allowing a better expression of the yield potential of the cultivar.

RESUMEN

Evaluación de parámetros de rendimiento del raps (*Brassica napus* L.) polinizado por abejas (*Apis mellifera* L.). La reciente introducción de variedades híbridas plantea la interrogante de la contribución que pueda tener la presencia de abejas (*Apis mellifera* L.) como agentes polinizadores para desarrollar en pleno el potencial productivo del raps (*Brassica napus* L.). Con el objetivo de evaluar el rendimiento alcanzado por *B. napus* cv. Artus polinizado por *A. mellifera*, se realizó un ensayo en la localidad de Freire, Región de La Araucanía, Chile. Éste consistió en aislar o excluir las plantas de raps de los polinizadores mediante el uso de jaulas excluidoras. Los tratamientos consistieron en la exclusión total (T1), exclusión parcial (T2) y libre polinización (T0) con una densidad de 6,5 colmenas ha^{-1}, con el fin de determinar los siguientes componentes del rendimiento: granos por silicua, silicuas por planta, peso de los 1000 granos y rendimiento. El diseño experimental utilizado fue de bloques completos al azar con tres tratamientos y tres repeticiones. Los resultados obtenidos mostraron que el parámetro menos afectado por la intervención de la abeja fue la variable granos por silicua. En contraste, los parámetros silicuas por planta y peso de los 1000 granos experimentaron diferencias significativas contribuyendo a una producción que superó 5 t ha^{-1}, lo que significa un aumento de un 50,34% comparado con el tratamiento sin presencia de abejas. Estos resultados permiten concluir que la inclusión de las abejas en los cultivos está plenamente justificada como una herramienta de producción.

Palabras clave: colmenas, polinización.

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Table 2. Pearson's correlation indexes according to response variables.

Variables	Grains per silique	Siliques per plant	1000 grain weight	Yield
			g	t ha^{-1}
Grains per silique	1			
Siliques per plant	-0.048	1		
1000 grain weight, g	0.164	-0.387	1	
Yield, t ha^{-1}	0.144	0.735	-0.482	1

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