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CROP PRODUCTION

# Influence of pollination on canola seed production in the Cerrado of Uberlândia, Minas Gerais State, Brazil

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**ABSTRACT.** The objective of this study was to evaluate the influence of pollinating insects on canola productivity for different commercial hybrids, Hyola 61 and Hyola 433, in Minas Gerais State, Brazil. The studies were conducted at the Fazenda Experimental Água Limpa of the Federal University of Uberlândia. To evaluate the influence of pollination by bees on the production of canola seeds, an experiment was carried out to exclude floral visitors and compare canola seed production following four treatments: (a) autogamy in Hyola 61, (b) autogamy in Hyola 433, (c) open pollination in Hyola 61, with insects having free access, and (d) open pollination in Hyola 433, with insects having free access. Flowers that were available for pollination had 25% and 18.8% heavier pods and 22.5% and 20.6% more seeds per pod in Hyola 61 and Hyola 433, respectively, than those bagged without pollination. The results show that the cross-pollination process, fostered by pollinator visits, positively influenced some parameters related to production, presenting an advantage in relation to canola autogamy in Cerrado Mineiro.

**Keywords:** *Apis mellifera*; *Brassica napus*; tropicalization; productivity.

## Serviços de polinização na produção de sementes de canola no Cerrado de Uberlândia, Estado de Minas Gerais, Brasil

**RESUMO.** O objetivo desse estudo foi avaliar a influência da polinização por abelhas na produtividade de canola em diferentes híbridos comerciais, Hyola 61 e Hyola 433, em Minas Gerais. Os estudos foram conduzidos na Fazenda Experimental Água Limpa da Universidade Federal de Uberlândia. Para avaliar os efeitos da polinização de abelhas na produtividade nas sementes de canola, realizou-se um experimento com exclusão dos visitantes florais, comparando a produção em quatro tratamentos: (a) autogamia em Hyola 61 e (b) autogamia em Hyola 433; (c) polinização aberta em Hyola 61 e (d) polinização aberta em Hyola 433 com acesso livre de insetos. As flores disponíveis para polinização apresentaram 25% e 18,8% sílicas mais pesadas e 22,5% e 20,6% mais sementes por sílica em Hyola 61 e Hyola 433, respectivamente, do que aquelas privadas de polinização. Os resultados apontaram que o processo de polinização cruzada, favorecida pela visita de insetos polinizadores, influenciou positivamente alguns parâmetros relacionados a produção, demonstrando-se vantajosa em relação à autogamia da canola no Cerrado Mineiro.

**Palavras-chave:** *Apis mellifera*; *Brassica napus*; tropicalização; produtividade.

## Introduction

Canola (*Brassica napus* L.) is an oleaginous plant of the Brassicaceae family that was developed by conventionally breeding rapeseed cultivars; rapeseed is a grain with high levels of erucic acid and glucosinolates (antinutritional substances). From its seeds, excellent quality oil for human consumption and for bioenergy is produced, and it is considered the third most produced oilseed in the world, surpassed only by the production of palm and soybean (De Mori, Tomm, & Ferreira, 2014).

Worldwide, the cultivation of canola covered an area of approximately 34 million hectares in 2014. The world's largest producers and consumers of canola grains are in the European Union, with production of 21.1 million tons for the 2013/2014 harvest and a forecast of 24 million tons for the 2014/2015 harvest. Consumption of canola grains was 24.5 million tons in 2013/2014, with grain imports of 3.4 million tons. The second largest producer and consumer is China, with production of 14.5 million tons for the 2013/2014 harvest and grain imports of approximately 5 million tons to supply the domestic market (USDA, 2016).

In Brazil, canola cultivation started in the 1980s and currently involves only spring canola of the species *B. napus* var. *oleifera*, used in crop rotation systems mainly in the south of the country (Luz et al., 2012). The state of Rio Grande do Sul has been considered an outstanding state in terms of the production and planted area of canola, with values of 85.2% and 86.7%, respectively. The state of Paraná contributes 14.8% of the national production and 13.3% of the planted area of canola in Brazil (CONAB, 2016). Canola cultivation fits well with the crop rotation system adopted in Brazil, and it is rotated with soybean, both of which are sown before corn (Tomm et al., 2009a).

Although canola is a plant adapted to cold regions and cultivated mainly in temperate regions (latitudes between 35 and 55 degrees) (Tomm, Wiethölter, Dalmago, & Santos, 2009b), recent efforts and research have been carried out to introduce and adapt the crop to warmer climates at lower latitudes (tropicalization). Experiments and commercial crops in Goiás and Minas Gerais States have demonstrated that canola is a crop with a great potential to contribute to the expansion of Brazilian agribusiness, as it is perfectly suited to the crop production systems of the Central-West region of the country (Tomm, 2005). Moreover, this plant adapts to regions considered too adverse for the development of other cultivars (Ávila, Braccini, Scapim, Fagliari, & Santos, 2007).

Canola's flowers are hermaphrodites; they present both female (gyneum) and male (androecium) parts. They have a pistil with three parts: the ovary, which houses the eggs and which has 28 eggs per flower on average; the stylet, which is the medial portion of the pistil; and the stigma, which is the surface that receives the pollen grains. The androecium is formed by stamens. The canola flowers have six stamens, four long and two short. Each stamen contains a fillet that supports an anther. The anthers contain pollen grains, which are accessible after the opening of the anther and which constitute the male gametes of the plants (Witter et al., 2014b).

Canola is a self-supporting plant, and thus, both self-pollination and cross-pollination result in fruits and seeds. Flowers are more likely to be susceptible to self-pollination during the beginning of anthesis, when the anthers are inwardly facing the flower, and the stigma is below them, making it easier for the pollen to fall on the stigma. However, when the stigma exceeds the height of the anthers, it is not possible for the pollen of the flower itself to fall on the stigma. Self-pollinating by a pollinating agent, such as an insect, is still possible, but researchers believe that this is a mechanism that favors cross-pollination (Witter et al., 2014b).

Insect pollination, mainly by bees, increases the production of canola plants because these pollinators deposit more pollen grains on the stigma of the flowers compared to the amount of pollen grains deposited on the stigma by self-pollination (spontaneous) without the action of bees, as shown in the work done by Witter et al. (2015). Previous studies have shown that the presence of pollinating insects, especially *Apis mellifera* Linnaeus (Hymenoptera: Apidae), increases not only seed productivity (Delaplane & Mayer, 2000) but also the quality of the oil and thus the market value of the crop (Witter & Tirelli, 2014). In addition, despite autogamy, mechanisms for the occurrence of allogamy were found in the reproductive strategies used by this species, such as abundant pollen, nectar and aroma. These mechanisms are important for the genetic improvement of the species, favoring the occurrence of more adaptive characteristics (Mussury & Fernandes, 2000).

In an experiment conducted in Canada, Sabbahi, Oliveira, and Marceau (2005) demonstrated a 46% improvement in grain yield in the presence of three beehives per hectare compared to crops with no hives. In a study conducted in Australia, Manning and Boland (2000) observed that the number of pods per plant decreased with increasing distance between apiaries. In Brazil, Mussury and Fernandes (2000) demonstrated an increase of 31.9% in the number of grains per plant under natural pollination conditions when compared to autogamy conditions. For crops in Rio Grande do Sul, Blochtein, Nunes-Silva, Halinski, Lopes, and Witter (2014) verified that free visits of insects increased yield by 17% in the Hyola 420 cultivar and by approximately 30% in the Hyola 61 cultivar.

Considering the importance of canola cultivation and the recent efforts to expand its cultivation into low latitude regions in Brazil, there is a need to improve knowledge about the influence of pollination on canola seed productivity to optimize production. Thus, the objective of this study was to evaluate the influence of pollinating insects on two different commercial hybrids, Hyola 61 and Hyola 433, in the Cerrado biome of Minas Gerais.

## Material and method

The studies were conducted at the Água Limpa Experimental Farm at the Federal University of Uberlândia, located at latitude 19°05'48"S, longitude 48°21'05"W, with an altitude of approximately 800 m. The soil of the area is classified as typical

Dystrophic Red Latosol, a moderate, medium texture soil, and the area had tropical Cerrado subdeciduous vegetation and a mild wavy relief (Embrapa, 2006). The Aw climate, according to the Köppen classification, is marked by two well-defined seasons, one rainy and one dry (Rosa, Lima, & Assunção, 1991). The area contains 104 ha of preserved area, formed by complex vegetation that includes restricted Cerrado, dense Cerrado, Vereda and gallery forest and 151.72 ha with fruit and pasture (Neto, 2008).

A completely randomized block experimental design with two hybrids and two pollination treatments (open and closed to insect visitation) and 10 replications was used, totaling 20 plots. Each plot consisted of six planting four meters in length, with 0.20 m spacing between rows and a density of 18 seeds $m^{-1}$. The plots were separated by 0.5 m, totaling an experimental area of 121 $m^2$. The experiment was established on April 2, 2016, with both hybrids flowering after 55 days and presenting senescence after 97 days.

The hybrids used in this experiment were Hyola 61 and Hyola 433. The hybrid Hyola 61 has polygenic resistance to *Phoma lingam* Tode ex. Shaw. Desm., known as blackleg, and performs well under water deficient conditions and severe cold. This hybrid has the characteristics of a medium cycle with 123 to 155 days from emergence to maturation and presents great stability of yield when cultivated under varied conditions. Hyola 433 is a short-cycle hybrid suitable for highly fertile soils (Tomm et al., 2009a).

To control insect pests, mainly aphids of the species *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae) and *Myzus persicae* Sulzer (Hemiptera: Aphididae), two applications of Decis 25 EC® were performed on June 4 and 15, and one application of Acetox Nortox® was performed on June 26, prior to the beginning of the evaluations.

To evaluate the influence of bee pollination on the production of canola seeds, an experiment was conducted to exclude floral visitors, and canola seed production was compared among the following four treatments: (a) autogamy in Hyola 61, (b) autogamy in Hyola 433 (the branches were covered with a fine mesh fabric during the entire period of flowering, depriving them of insect visitation), (c) open pollination in Hyola 61, with insects having free access, and (d) open pollination in Hyola 433, with insects having free access. For each treatment, 10 replicates were made, with 8 plants in each replicate.

After the maturing of the pod, the marked branches were collected and submitted to a laboratory analysis evaluating the following parameters: (1) pod weight (n = 800 pod/treatment), (2) number of seeds per pod (n = 800 pod treatment$^{-1}$), and (3) weight of a thousand seeds (per treatment).

The residual normality, homogeneity of variances and additivity of the blocks were verified by Shapiro-Wilk's, Levene's and Tukey's tests, respectively, for each variable. After fulfillment of the assumptions, analysis of variance (ANOVA) was applied, and if a significant difference was detected, then the means were compared by Tukey's test. All tests were performed at 5% significance. The analyses were performed by SPSS and SISVAR® software (Ferreira, 2011)

## Result and discussion

When the influence of pollination was evaluated, there was a significant difference between the treatments with open pollination (OP) and autogamy (AT) for both hybrids for the following characteristics: the mean weight of the pod and mean number of seeds/pod. The flowers that were available for pollination presented 25% and 18.8% heavier pods and 22.5% and 20.6% more seeds per pod in Hyola 61 and Hyola 433, respectively, than those that were bagged without cross-pollination (Table 1).

**Table 1.** Mean weight of the pod (g), mean number of seeds/pod and weight of one thousand seeds (g) of the hybrids Hyola 61 and Hyola 433 with and without cross-pollination.

| Treatment | Average weight of pod (g) | Average number of seeds per pod | Weight of one thousand seeds (g) |
|---|---|---|---|
| Hyola 61 - OP | 0.076 ± 0.009 a | 14.38 ± 0.894 a | 2.28 ± 0.32 a |
| Hyola 61 - AT | 0.057 ± 0.006 c | 11.15 ± 1.133 b | 2.12 ± 0.35 a |
| Hyola 433 - OP | 0.069 ± 0.006 b | 14.88 ± 1.235 a | 1.94 ± 0.20 b |
| Hyola 433 - AT | 0.056 ± 0.004 c | 11.81 ± 0.659 b | 1.86 ± 0.15 b |

*Means followed by the same letters in the column do not differ as determined by Tukey's test at 5% probability. OP: open pollination; AT: autogamy.

Regarding the mean seed weight, Williams, Martin, and White (1986) and Adegas and Nogueira-Couto (1992) found an increase of 37.47 and 19.24%, respectively, in the productivity of canola plants with visits from *A. mellifera* when compared to plants with insect exclusion. In the present study, there was no difference in the weight of one thousand seeds for the pollination treatments (OP and AT) in both hybrids. However, Hyola 61 presented heavier seeds than Hyola 433 (Table 1)

In Rio Grande do Sul, according to Rosa, Blochtein, and Lima (2011), the induction of pollination in tests with free visits of insects to *B. napus* (Hyola 432) resulted in a 22.99% increase in grain weight in relation to autogamy. In Canada, Sabahhi et al. (2005) also recorded increases of up to 30% in seed weight with insect visits when compared to areas deprived of insects. Several factors have been discussed in the literature as determinants for full plant development and crop productivity, such as sowing density and spacing (Von Pinho, Gross, Steola, & Mendes, 2008), genotype (Silveira et al., 2010), cultivar (Krüger et al., 2011), time of sowing (Ungaro, Nogueira, & Nagai, 2000), harvest season (Marchiori et al., 2002) and environmental conditions (Mello et al., 2006). The absence of significant differences between the pollination treatments in this study emphasizes the need to study the adaptation and development of the crop in the Cerrado region and the need to perform new evaluations of canola crops in the presence of pollinators under climatic conditions that differ from those already studied.

There was a significant difference between the treatments for hybrids in relation to the mean weight of the pod. Hyola 61 presented a heavier pod, showing that it responded better to pollination, and was more affected by OP than Hyola 433. In the AT treatment, there were no significant differences (Hyola 61: AT = $0.057 \pm 0.006$ and Hyola 433: AT = $0.056 \pm 0.004$).

According to Gavloski (2012), the effect of pollination on grain yield depends not only on the density of pollinators in the field but also on the atmospheric conditions during the flowering period and on the canola variety. The hybrid Hyola 61 is characterized by having great yield stability when cultivated under varied environmental conditions. However, Hyola 433 is more affected by environmental conditions, especially highly fertile soils (Tomm et al., 2009a). These different profiles between the hybrids probably caused the different responses to the environmental conditions and resulted in different productivities. In addition, the availability of resources and the environment can determine the growth potential of specific genotypes and the number of flowers produced per plant. Consequently, the total number of seeds produced is susceptible to variations in not only different locations but also the seasons (Wiens, 1984).

The deposition (quantity and quality) and distribution of pollen grains on flower stigmas through wind and self-pollination, despite being sufficient for the promotion of fruit and formation of seed, are often not able to maximize the productive potential of the plant and ensure that all the seeds and fruits that the crop can potentially produce are in fact produced (Freitas & Nunes-Silva, 2012). In addition, it is necessary that at least 28 grains of pollen be deposited in the stigma of each canola flower since the ovary usually contains 28 eggs to be fertilized (Witter & Tirelli, 2014). The more pollen grains that are deposited on the stigma of the flower, the greater the chance that all the eggs will be fertilized and that all the possible canola grains will be formed. The amount of pollen grains deposited on flower stigmas depends on the number of pollinating insects present in canola crops (Marsaro-Junior et al., 2017).

Witter et al. (2014b) confirmed that there is a positive correlation between the density of insects (mostly bees) and the production of canola grains. In this study, both flowers deprived of insect visitation and those with free visitation were passive to anemophilia since the tissue used for the deprivation of floral visitors did not prevent the transfer of pollen grain between flowers of distinct plants, which was considered a common factor for both results.

The species *A. mellifera*, *Paratrigona lineata* Lepeletier, 1836 (Hymenoptera: Apidae) and *Trigona spinipes* Fabricius, 1793 (Hymenoptera: Apidae) were observed visiting flower buds during the canola flowering period, demonstrating the possibility of pollinating this crop in the Cerrado region. After verifying which pollinating bees are effective in a crop, an increase in the frequency of visits of these species to obtain direct benefits for increased productivity is recommended. Through direct management, the pollination services promoted by *A. mellifera* have been used to increase the production of several crops (Kremen, 2008).

In addition to the increase in seed productivity, there are also resources produced by the bees since canola crops offer large amounts of floral resources (pollen and nectar) (Witter, Nunes-Silva, & Lisboa, 2014a). In addition to honey production, which has been very valued by consumers, canola flowering stimulates the development and strengthening of bee colonies (Marsaro-Junior et al., 2017). In addition, beekeeping is a species conservation activity, one of the few agricultural activities that cover the three pillars of sustainability: the economic pillar, because beekeeping generates income for the farmer; the social pillar, because it is still mainly a family business; and the ecological pillar, because no deforestation is necessary to raise bees (Alcoforado-Filho & Gonçalves, 2000). Therefore, it is desirable for beekeepers and canola producers to strengthen their relationships and establish consortia, as both would benefit.

The protection of natural areas close to canola crops also guarantees pollination services from bees, which benefits productivity. Natural habitats are necessary for bees not only to as places to obtain food resources but also as places to nest and breed (Knight et al., 2009). Areas of peripheral vegetation, such as field borders, live fences, road margins, and riparian forests, also provide nesting sites and can provide corridors where bees and other beneficial insects can feed and migrate through the agricultural landscape (Witter et al., 2014a). At present, the relationship between the quantity and/or the distance from natural areas and the presence of bees (richness and abundance) in crop yields has been recognized in several studies worldwide and in Brazil (Schulp, Lautenbach, & Verburg, 2014).

Halinski, Dorneles, and Blochtein (2015), in experiments in Rio Grande do Sul, found that the productivity of canola seeds decreases as farms are located farther away from forest remnants. The average grain yield at 25 m from natural areas ranged from 3,368 to 4,656 kg $ha^{-1}$, while at 175 m, the variation ranged from 2,044 to 3,956 kg $ha^{-1}$. At 325 m, the productivity range was from 1,508 to 3,432 kg $ha^{-1}$. This finding is important to the sector involved in the production of canola (Blochtein, Witter, & Halinski, 2015) since the fields in southern Brazil are surrounded by homogeneous areas dominated by agricultural lands and scarce remnants of natural areas (Witter et al., 2014b). As a result of this landscape, there is a loss of favorable environments for pollinators and, consequently, a decrease in their permanence in the area. Thus, it is important to keep forest remnants close to crops, allowing pollinators to perform their services and increase grain yields and associated economic values.

## Conclusion

Canola shows an increase in the number of its seeds when pollinated by native bees and by *A. mellifera* in the Cerrado of Minas Gerais State. Canola flowers with free visitation by pollinators produce heavier pods and more seeds compared to those submitted to only autogamy. In relation to hybrids, when grown in the Cerrado, Hyola 61 presents heavier pods and seeds than Hyola 433 under both pollination treatments but has a smaller number of seeds per pod.

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