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Benefits of Entomophile Pollination in Crops of *Brassica napus* and Aspects of Plant Floral Biology

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Abstract

Rapeseed (*Brassica napus* L. var. *oleifera*) is an oleaginous species of the Brassicaceae family, being the third most produced oleaginous in the world. Rapeseed can produce fruits and seeds from both self-pollination and cross-pollination. However, cross-pollination rate is approximately 30% and may suffer variations due to the abundance and diversity of pollinator insects, cultivar and meteorological conditions. Different researchers have reported that pollination by insects, especially *Apis mellifera* honeybee, on rapeseed flowers provides an increase in productivity, improving yield and contributing to the uniformity and initial pod establishment. It is estimated that the economic value of *A. mellifera* honeybees for rapeseed cultivation in Brazil is US\$ 8.2 million. The objective of this chapter is gathering data for a compilation of information regarding rapeseed culture and the importance of *A. mellifera* in *Brassica napus* pollination.

Keywords: canola, oil, pod, pollination, seed

1. Introduction

1.1. History and botanical origin

Rapeseed is a plant of the Brassicaceae family, belonging to the *Brassica* genus. This plant was developed by conventional genetic breeding of rapeseed, which allowed the reduction

of erucic acid levels and glucosinolates that are toxic to humans. That is why the word canola, a derivation of an English term “CANadian Oil Low Acid” that refers to the generic cultivars of rape that meet the specific standards regarding the levels of these substances. Rapeseed was registered in Canada in 1970, and today its name is used to designate three species of *Brassica*: *B. napus* or Argentine variety, *B. rapa*, also known as Polish and *B. juncea* or mustard [1].

Taxonomic studies carried out in the 1930s showed that *B. carinata*, *B. juncea* and *B. napus* are allotetraploid species formed by hybridization events between diploid parent species *B. nigra*, *B. rapa* and *B. oleracea*. Hybridization between *B. nigra* and *B. oleracea* resulted in the formation of *B. carinata*; between *B. nigra* and *B. rapa* in *B. juncea* formation and between *B. oleracea* and *B. rapa* in *B. napus* [2].

Haploid genomes of *B. rapa*, *B. nigra* and *B. oleracea* are designated A, B and C, respectively. Thus, *B. rapa* diploids have two copies of the genome A on 20 chromosomes (AA, $n = 10$, $2n = 20$), and *B. napus* diploids have two copies of both genomes A and C on 38 chromosomes (AACC, $n = 19$, $2n = 38$), see **Figure 1**.

Mitochondrial DNA and chloroplasts analysis suggested that *B. montana* ($n = 9$) might be closely related to the prototype that gave rise to both *B. rapa* and *B. oleracea* cytoplasm. Furthermore, results from phylogenetic analyses have shown that there are multiple origins of *B. napus* and that the most cultivated forms of this species derived from a crossing where a closely related ancestral species of *B. rapa* and *B. oleracea* was the maternal donor [3].

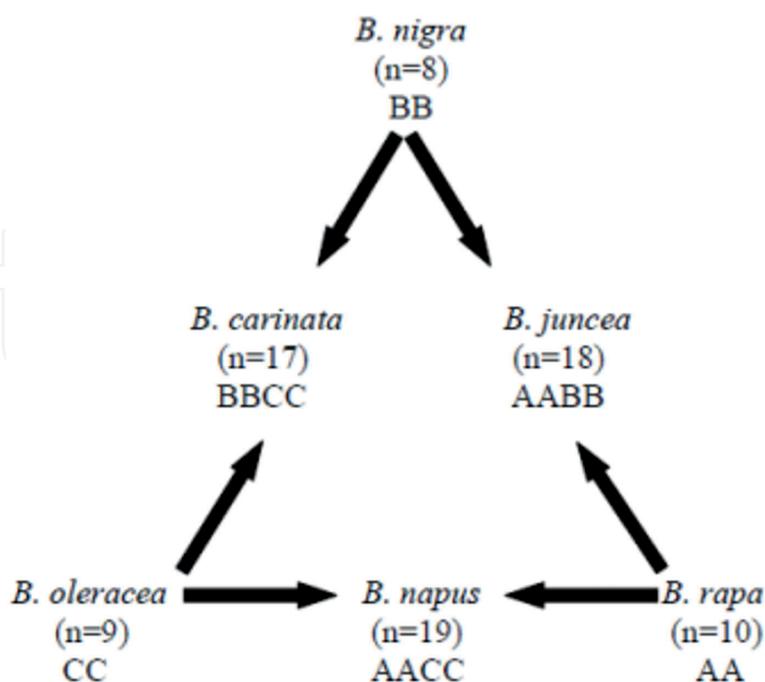


Figure 1. Nagaharu [2] triangle illustrating the genomic relations between Brassica species. The haploid genomes of the diploid species of *B. rapa*, *B. nigra* and *B. oleracea* are referred to as A, B and C, respectively.

2. Floral biology aspects

B. napus flowers are bisexual, have four sepals, four petals, four long stamens and two short stamens on the same flower. The anthers present longitudinal dehiscence. The ovary is superimposed, with parietal placentation, gamocarpelar and bicarpelar. There are nectaries located in the center of the flower, two between the ovary and the two short stamens, and two between the two long stamens and the petals [4].

Anthesis in rapeseed flowers can occur at any time of the day, but usually occurs early in the morning, around 9 am, when most of them are already open. The flowers remain open for up to 3 days, and at night they partially close and the opening occurs again the following morning. Winter rapeseed flowers remain open for one to 3 days, while spring rapeseed flowers remain open for 1–2 days. Flowering, which lasts from 22 to 45 days, depends on the weather conditions [5, 6]. For example, when the weather is cold and humid, flowers stay open longer for hotter and drier days [7].

In cultivars of the auto sterile *Brassica* genus, anthers of the long stamens release the pollen into the environment, and cross-pollination is essential. On the other hand, in some auto sterile cultivars, the release of the pollen begins even before the opening of the flower and continues until the end of the anthesis. In these cultivars, during the flowering period the stigma reaches the position of the long stamens, while the anthers initially release the pollen into the environment and then curl up for some pollen to be directed to the stigma of the same flower. In other cultivars, mainly those that produce yellow seeds, the pollen grains produced in the anthers are deposited on the stigma of the same flower, leading to self-pollination [5].

The stigmatic surface is receptive to the pollen for up to 3 days after anthesis. If the temperature is high, above 27°C, almost all pollen grains are released on the first day of anthesis, the time the flower becomes receptive to the pollen and the viability of the pollen is decreased. The most favorable temperature for rapeseed cultivation is around 20°C during the cycle. Fertilization occurs within 24 hours after pollination, and fertilization, the flower remains partially closed and the petals begin to peel (2 to 3 days after flower opening). The young pod becomes visible in the center of the flower 1 day after the fall of the petals [8].

Another important factor in rapeseed concerns the fertilization of the ovules, especially the percentage of ovules with complete embryo sacs in the opening of the flower. Even with a large deposition of pollen on the stigma, incomplete pollination may occur. In rapeseed, generally 30% of the eggs are sterile due to the absence of complete embryo sacs in the opening of the flower. The lower proportion of ovules with complete embryo bags will result in a non-fertilization of all the ovules of the flower and, consequently, a smaller number of seeds per pod [9]. In the terminal raceme, the lower proportion of fertile ovules due to the sterility of the ovules and the lower number of ovules per ovary in apical flowers are some of the causes for the smaller number of seeds per pod in the apical region compared to the basal region [10].

The life cycle of the rapeseed plant is divided into seven main stages: germination (stage 0), foliar development (stage 1), development of lateral branches (stage 2), stem elongation (stage 3), inflorescence emergence (stage 4), flowering (stage 5), seed development (stage 6)

and maturation (stage 7). Knowledge of the stages of development of the rapeseed plant is important for decision-making and crop management. However, the beginning of each stage of development is not dependent on the end of the previous step. From the beginning of flowering, each stage of growth is determined by analyzing the main flowering stem. The timing and occurrence of the different stages of growth will vary according to the conditions of growth, location and variety used in the crop [8].

3. Pollination requirements in rapeseed flowers

In many plant species, pollination is essential for sexual reproduction. Many floral characteristics are adaptations to promote cross-pollination and have evolved to reduce the negative impact of inbreeding depression and increase the likelihood of male success [11].

The flowers pollinated by animals usually have attractive petals and offer floral reward such as nectar or pollen [5, 12]. However, visits by pollinators may sometimes not involve plant needs and seed production may be limited by the amount of pollen deposited on the stigma [13].

In plants with hermaphrodite and self-compatible flowers, such as *B. napus*, self-pollination can offer reproductive guarantee when there is a shortage of visits of pollinating agents. Mixed mating systems, which include self-pollination, are therefore adaptive [14], although there may be detrimental consequences due to inbreeding depression [15].

However, in rapeseed, pollinator insects, especially *Apis mellifera* honeybees, may play an important role in pollination and are believed to be involved in pollen transfer over long distances [16]. Honeybees combined with other bee species may result in better pollination than any single insect population [17].

Rapeseed has flowers capable of both self-pollination and cross-pollination, although under field conditions the fertilization of the ovules of their flowers usually results, for the most part, from self-pollination [1, 16]. The cross-pollination rate, which in canola is approximately 30%, may suffer variations due to the abundance and diversity of pollinator insects, cultivar and meteorological conditions [1, 18].

Despite higher self-pollination rate compared to cross-pollination in rapeseed, higher seed production has been reported when pollinated by bees [19–23]. Rapeseed flowers secrete large amounts of nectar and are very attractive to *A. mellifera* honeybees and other pollinating insects [5, 6].

In addition, in rapeseed, in spite of the autogamy, mechanisms for occurrence of allogamy were found from reproductive strategies used by this species, such as abundant pollen, nectar and odor. These mechanisms were important for the genetic breeding of the species, favoring the occurrence of more adaptive characteristics [4].

Although data are still conflicting and divergent among rapeseed varieties on the benefits of entomophile pollination, there is evidence that insects can qualitatively and quantitatively increase crop production. The interdependence of bees and the *Brassica* genus are manifested

in the fact that their pollen is very sticky and there is a need for pollen insects for their transfer. For *Brassica spp.* plants, bees are co-evolved in pollen transfer mechanisms [24].

Self-pollinating can promote seed production, but for this, viable pollen must contact stigma when it is receptive. The degree of self-pollinating would be greatest when the stigma is pushed up through the anthers. However, if time, pollen availability or stigma receptivity is not synchronized, seed production will not occur [25].

Entomophile pollination efficiency process depends mainly on the climatic conditions, as it also affects the crop as well as the pollinators [24]. High temperatures in the pre-anthesis may cause pollen sterility, and in the anthesis, delayed growth of the pollen tube [26], as well as high temperatures and low relative humidity may lead to a decrease in stigma receptivity [27] and degeneration of flower ovules [28]. Thus, understanding the role of pollinators and factors affecting insect-plant interactions may be of great importance for increasing grain yield in rapeseed.

In addition, it should be considered that most *Brassica* species are self-incompatible; therefore, insect visits are necessary for seed production [6, 29]. In *Brassica* species with auto incompatibility (AI), incompatible pollen grains barely germinate on stigma and, when germinated, their pollen tubes fail to grow on papillary cells on the stigmatic surface [30]. Self-incompatibility is a widespread mechanism in flowering plants that prevent self-fertilization. Self-pollen recognition is located at the *S* locus. Pollen rejection occurs when the same *S* allele is expressed both by the pollen and the pistil tissues. This suggests that the *S*-locus encoding the male determinant and another encoding the female component of the Self-incompatibility reaction [31].

In rapeseed, the stigma can accumulate pollen in the absence of pollinating insects from the pollen of its own anthers by spontaneous self-pollination by mechanical contact caused by flower collisions or anemophilic pollination, as well as by cross-pollination [32]. In lower pollinator densities, rapeseed seed production occurs through the spontaneous auto-pollination mechanism in the flower and the mechanical contact caused by collisions between flowers or anemophilous pollination.

On the other hand, in moderate bees densities, non-insect pollination modes and entomophile pollination also contribute to seed production. Even at the highest levels of pollinator abundance found in crop fields, spontaneous self-pollination accounts for almost a third of production [32]. In this context, the dynamics of pollen transfer mediated by bees must be considered to be dependent on the amount of pollen available, as well as on pollen removal rates from anther and deposition on the stigma by specific pollinators [33].

Pollinators with high removal and low pollen deposition (HRLD) on flower stigmas will benefit a plant species when there is no better pollinator available. Pollinators with high pollen removal and high deposition (HRHD) on stigmas may have a decrease in total pollen transfer as a result of visits by HRLD pollinators. HRLDs parasitize plants by diverting the grains that would be delivered by HRHDs. In situations where two visitors remove equal amounts of pollen, the one with the highest deposition rate will always be a more efficient pollinator; if it removes different amounts, which is better not only depend on deposition rates, but on other variables such as visitation frequency for deposition [33].

4. Impact of pollination by honeybees on *B. napus*

Commercially grown rapeseed hybrids are predominantly auto-fertile, but the degree of cross-pollination is still uncertain [34]. In cultures that are poorly dependent on pollinators, such as rapeseed, introduction of honeybee colonies is generally not recommended [35]. However, as long as the flowers of these hybrids are attractive to the pollinators, the introduction of 3–5 colonies of *A. mellifera* or 5–8 colonies of *A. cerana* uniformly distributed per hectare may be ideal for increasing production and higher oil content of the seeds [33]. There is evidence that pollination by honeybees in rapeseed provides increased productivity, improving yield and contributing to the uniformity and initial pod establishment [24, 36].

In *B. napus* the number of pods per plant may decrease by 16% in plants located at a distance of 1.000 m from the apiary [20]. Manning and Wallis [37] found grain yield 20% or 400 kg.ha⁻¹ higher in plots located close to the apiary than those located at a distance of 200 m from the apiary. Pordel et al. [38] reported that pollinating insects, especially *A. mellifera*, more abundant in rapeseed crops, could increase grain yield by 53%.

The influence of honeybee density on rapeseed production in nine agricultural fields with three honeybee densities: 0, 1.5 and 3.0 colonies/hectare was evaluated. The results of this experiment indicated an increase in the seed productivity of 46% in the presence of three colonies per hectare in relation to the absent area of pollinators [21].

Araneda-Durán et al. [22] evaluated *B. napus* cv. Artus production pollinated by *A. mellifera* from an experiment that consisted of three treatments: exclusion of pollinators from rapeseed plants, partial exclusion and free pollination with a density of 6.5 colonies/hectare. The results evidenced increase of seed productivity induced by the treatment with free pollination of 50.34% on the total exclusion and 11.46% in relation to the partial exclusion.

On the one hand, in the Hyola 433 and 61 rapeseed cultivars it was observed that insect pollination was higher for the variables number of pods per plant, number of seeds per pod and average pod weight, respectively, in the condition of autogamy [23]. In CTC-4 rapeseed cultivar, visits of honeybees collecting nectar and pollen contributed to increase pod production per square meter and mass of each grain. However, no influence was recorded on the total number of seeds per pod normal and abnormal seeds per pod, germination and oil content in seeds [39].

Therefore, divergent results were obtained in an experiment carried out with the CTC-4 rapeseed cultivar in Dourados, Brazil. In this experiment, there were no statistically significant differences when the free-pollination and autogamy treatments were compared for the variables pod size, number of seeds per pod and weight of 10 pods [4].

It should be considered that the productivity of rapeseed seeds is a function of population density, number of pods per plant, number of seeds per pod and seed weight. Besides, the numbers of pods per plant being the most important variable for increase in grain yield, especially in crops with low plant densities and non-uniform populations [40].

5. Situation and economic aspects

Rapeseed is a kind of cold climate; therefore, its commercial cultivation in the world is concentrated in temperate regions, mainly in latitudes higher than 35°C [41]. Air temperature and water availability are the most important environmental variables for its growth and development [42].

Most of the rapeseed produced in Europe is of the winter type, however, in Brazil there are only spring rapeseed and from *Brassica napus* L. species. This is because even in the coldest conditions in Brazil, as in State of Rio Grande do Sul, the number of hours required for winter cultivars is insufficient [43].

Its cultivation is mainly due to its seeds, which produce between 35% and 45% of oil. The main use of rapeseed is like cooking oil, but it is also commonly used in margarine. Rapeseed meal is produced as a by-product during the oil extraction from the seeds and used as a source of high protein content intended for animal feed [1]. In addition, rapeseed is an excellent alternative for crop rotation with grasses and vegetables, as well as being appropriately inserted in the cultivation systems that predominate in the South of Brazil [44].

Currently, rapeseed accounts for 15% of vegetable oil production, behind soybeans (28.6%) and palm (33.2%), as well as being the third largest commodity in the world [45]. The main world producers in the 2011/2012 harvests were the European Union, Canada, China and India. World production of rapeseed seed in the 2011/2012 harvests was projected at 60.93 million tons, at 33.76 million hectares of planted area [46].

In Brazil, rapeseed grains production in the 2011/2012 harvests was 52 thousand tons, in 42.400 hectares of planted area; with State of Rio Grande do Sul being the largest producer, followed by the State of Paraná [47]. Producers have harvested, on average, 20.44 sacks per hectare or the equivalent of 1226.00 kg.ha⁻¹, with production costs of R\$ 1310.00 per hectare. The price of the 60 kg bag of rapeseed marketed in August 2012 was R\$ 72.66 [48]. Therefore, the gross revenue of the crop can be estimated at approximately R\$ 62.9 million, with net sales of R\$ 7.4 million.

Bees are the most important and economically most valuable pollinators in the world. Many crops of economic interest, such as oilseeds, are dependent, at least in part, on pollination by these insects. The evidence for this is that in 2005, the world economic value of pollination services totaled € 153 billion, representing 9.5% of the economic value of world agricultural production used for human consumption [49]. In the United States alone, in 2000, the benefit of honeybee pollination services totaled US\$ 14.6 billion [50].

The economic value of *A. mellifera* honeybees for rapeseed cultivation in Brazil can be simulated in order to determine the contribution of the Africanized honeybees to the total economic value of the oilseed production. This estimation can be performed from the $V_{hb} = V \times D \times P$ Eq. [50, 51], where V_{hb} is the annual value of the crop attributed to *A. mellifera* honeybees; V is the value of the rapeseed production in grains in the 2011/2012 crop, published by [48]; D is

the culture dependence by pollinator animals of 0.25 [49]; P is the effective proportion of pollinating insects that are *A. mellifera* L., obtained by [50] of 0.90. Therefore, $V_{hb} = 62.9 \times 0.25 \times 0.90 = R\$ 14.2$ million or about US \$ 8 million.

Thus, the amount of R\$ 14.2 million attributed to honeybees represents that the pollination services of rapeseed deserve further investigation due to the lack of knowledge about the pollination requirements of the various hybrids commercialized in Brazil. In addition, the benefit generated by honeybees may change due to climatic conditions and the hybrid used, which indicates once again the importance of understanding the possible factors that may affect the pollination process performed by these insects.

In addition, the growing demand from the productive sectors increases the area cultivated with rapeseed in Brazil, despite the slight drop in production caused by unsatisfactory environmental conditions. Currently, most of the Brazilian crops occur in States of Rio Grande do Sul and Paraná, with some crops in States of Mato Grosso do Sul and Santa Catarina. Brazilian producers are improving technical knowledge on cultivation and harvesting, improving the final results of the harvest [45].

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