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The expansion of agribusiness: harmful impacts of deforestation, pesticides and transgenics on bees

A expansão do agronegócio: impactos nefastos do desmatamento, agrotóxicos e transgênicos nas abelhas

Marcia Regina FAITA[1*], Adriana CHAVES[1], Rubens Onofre NODARI[1]

[1] Programa de Pós-graduação em Recursos Genéticos Vegetais, Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brasil.
[*] E-mail of contact: marcia.faita@gmail.com

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ABSTRACT: The benefits that bees offer the ecosystem are increasingly recognized, but not met by actions designed to conserve these pollinators. Therefore, the present work aims to describe the impact of three key characteristics of agroindustry that are currently damaging bees in Brazil: deforestation, use of pesticides and large-scale cultivation of genetically modified (GM) varieties. We hear of mass mortality and weakening and damage to hives, but no conclusive causes are announced. In particular, studies indicate that fungicides, herbicides and GM plants, even when considered harmless to bees, still promote physiological and behavioral changes in these insects. However, most of these studies do not indicate the real damage to the hives, which can only be observed in long-term studies carried out in the field. Even when risk assessments of fungicides and herbicides are conducted in the field on non-targeted organisms, the tests are considered valid for isolated individuals without regard to the eusocial behavior of bees in their colonies. Some studies present results from experiments performed on individuals in the lab, again not necessarily reflecting hive activity. Results of trials that consider the complexity of interactions among castes and different generations of bees are very scarce. Therefore, we herein take a comprehensive and detailed approach to three practices of Brazilian agribusiness that directly affect bee and colony health and survival, as well as their ecoservices.

Keywords: Fungicides; Herbicides; Monoculture; Landscape simplification; Chronic and acute damages

RESUMO: O reconhecimento sobre os benefícios que as abelhas oferecem para a manutenção da vida não está sendo revertido em ações efetivas para conservação desses polinizadores. Nesse sentido, o objetivo deste artigo é apresentar e discutir os impactos de três importantes características do sistema agrícola industrial, que

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vem causando danos às abelhas no Brasil: desmatamento, uso de agrotóxicos e cultivo em larga escala de variedades transgênicas. Os danos agudos têm sido frequentemente relatados, tanto em estudos científicos quanto pela imprensa, diante de eventos de mortalidade em massa. Igualmente relevante, os danos crônicos informam sobre enfraquecimento e mortalidade de colmeias, sem apresentar uma causa única ou conclusiva. Estudos têm evidenciado que herbicidas, fungicidas e plantas transgênicas, mesmo considerados inócuos às abelhas, promovem alterações fisiológicas e comportamentais nesses insetos. Porém grande parte deles não indicam os reais prejuízos às colmeias, que só podem ser observados em estudos de longo prazo, realizados a campo. Isso também revela uma falha grave nas avaliações de riscos de agrotóxicos e transgênicos sobre organismos não alvo, que consideram válidos testes feitos em indivíduos isolados de organismos que naturalmente vivem em colônias, como é o caso das abelhas eussociais. Resultados obtidos para um grupo de indivíduos em laboratório, que não necessariamente refletem o efeito na colmeia, são muito comuns. Contudo, resultados de ensaios que consideram a complexidade de interações entre castas e diferentes gerações desses insetos, são muito escassos. Assim constata-se que o princípio da precaução foi sumariamente ignorado em detrimento aos lucros financeiros que se concentram para poucos, enquanto os prejuízos ambientais e à saúde são repartidos entre todos. Desse modo, apresentamos e discutimos os efeitos danosos de três das principais práticas utilizadas no âmbito do agronegócio brasileiro, que afetam de diferentes modos a saúde e sobrevivência das abelhas, assim como os serviços por elas prestados.

Palavras-chave: fungicidas; herbicidas; simplificação da paisagem; monocultivos; danos crônicos e agudos.

1. Introduction

Overall, investigators have reported a growing decrease in the population of pollinators attributed to agricultural expansion, namely deforestation, use of pesticides and cultivation of genetically modified plants. In the last decades, agricultural production has intensified. Large areas of monocultures have continued to increase the use of pesticides and promoted the simplification and fragmentation of landscapes (Freitas *et al*., 2009; Mullin *et al*., 2010; Cunha *et al*., 2014). These authors warn that this scenario has been impacting the populations of innumerable pollinator species on a global scale, causing untold economic and environmental losses.

Most pesticides, such as neonicotinoid insecticides, are not selective, but they are associated with the reduction of pollinating insect populations in different countries (Henry *et al*., 2012; Whitehorn *et al*., 2012; Di Prisco *et al*., 2013; Godfray *et al*., 2014; Woodcock *et al*., 2017). Consequently, their use was restricted by the EU in 2013 (Carneiro *et al*., 2015). In addition to insecticides, other pesticides damage bees that are considered the main pollinators. The effects on bees caused by herbicides and fungicides have not been intensively studied since they are not the intended target of these pesticides. Adjuvants are inert substances mixed with the active ingredients in commercial pesticide formulations, and they can also have toxic effects on bees and their colonies (Zhu *et al*., 2014; Mullin *et al*., 2016).

Nonetheless, based on the technical recommendations issued by public and private agricultural research and extension institutions, it was promulgated by Law No. 7802, June 11, 1989, that food production could only be guaranteed through the use of pesticides. In addition, it establishes that pesticides, if used according to the manufacturers' recommendations, do not pose risks to non-target organisms and the environment. However, the results of scientific studies, including the present work, and field inspections demonstrate that just the opposite is true.

It should be noted that pesticides independent of classification and chemical constituents pose risks to a range of organisms. For example, scientific evidence has shown that herbicides not only damage plants, but also bees (Faita *et al*., 2018; 2020; Chaves *et al*., 2020). Therefore, the present study aimed to describe the impact of three key characteristics of agroindustry that are currently damaging bees in Brazil: deforestation, use of pesticides and large-scale cultivation of genetically modified (GM) varieties

2. Pollinators: importance of bees

Bees pollinate native and cultivated species that maintain natural balance of the ecosystem and contribute to the food supply (Potts *et al*., 2010). Bee products are important to the cosmetics, food, and pharmaceutical industries, but the contribution is small in comparison to their environmental services, since bees generate direct and indirect profits (Faita, 2018). Among insects, bees occupy a prominent place in pollination services, being considered the most important among pollinators (Malerbo-Souza & Halak, 2009).

Bees depend exclusively on floral resources for their food and survival, developing a relationship of reciprocal adaptations with angiosperms (Pinheiro *et al*., 2014). Most of them, especially trees, depend on pollinators to complete their sexual reproductive cycle, producing seeds and fruits (Vieira & Fonseca, 2014).

The economic importance of pollinators to agriculture has been recognized worldwide. *Apis mellifera* is considered the main pollinating bee, increasing productivity and product quality for many crops (Roubik, 2002; Nascimento *et al*., 2012; Toledo *et al*., 2013). Additionally, native bee species can contribute to increased productivity of cultivated plants (Heard, 1999; Bukovinszky *et al*., 2017), and, depending on the ecosystem, they are said to be responsible for 40% to 90% of pollination of plant species (Heard, 1999). A vast list of cultivated plants depends entirely on these animals or benefits from their visits. The absence of this service can negatively affect sexual reproduction and the genetic diversity of plants, in addition to compromising the production of food and related products (Klein *et al*., 2007).

According to Gallai *et al*. (2009), the global economic value of the pollination service by insects, mainly bees, was 153 billion euros in 2005 for the main crops that feed the world, corresponding to 9.5% of the total value of agricultural production (Potts *et al*., 2010). In the United States, the estimated pollination value for agriculture by *A. mellifera* bees alone was 9.3 billion dollars in 1989 and 14.6 billion in 2000, an increase of 36% (Morse & Calderone, 2000).

Chiari *et al*. (2008) studied the influence of *A. mellifera* on grain production and seed quality of *Glycine max* (L.) Merrill Roundup® Ready and conventional soybeans. For the studied cultivars, they identified a benefit in the grain yield of 37.84% when bee visits were allowed. In the cultivation of oranges, Malerbo-Souza & Halak (2009) reported the importance of entomophilia in the production of these fruits, as several pollination experiments in *Citrus* sp. proved the benefits in production, with an increase in the fruiting of flowers when bees are present (Malerbo-Souza *et al*., 2003). In addition, the production of larger and sweeter orange fruits with a greater amount of vitamin C occurs in the presence of bees when compared to treatment without bees (Gamito & Malerbo-Souza, 2006).

To estimate the economic contribution of pollination, Giannini *et al*. (2015b) carried out a review using data derived from three sources: (Klein *et al*., 2007), only species cultivated in Brazil (Giannini *et al*., 2015a) and data available on the website of the Brazilian Institute of Geography and Statistics (IBGE). According to these authors, the economic contribution of pollinators in Brazil amounts to almost 30% (approximately 12 billion dollars) of the total value of annual agricultural production of pollination-dependent crops (approximately 45 billion dollars). The authors used data for the years 2005 to 2012, including all plants of economic importance used as food, clothing, livestock, biofuel or for other uses. The dependence of plants on pollinators was reviewed, and the annual economic value of pollination was estimated for each crop. Out of 141 crop species analyzed, 85 depend on pollinators. Almost a third of these crop species were highly, or essentially, dependent on pollinators. However, information on pollinator dependence for some important crops has still not been obtained, showing the urgent need for basic research on reproductive biology and pollination ecology (Giannini *et al*., 2015a).

Additionally, it is important to reflect on the current state of environmental conservation, which directly interferes with the maintenance of the pollinator community and its services. From the optimistic viewpoint, the loss of pollination services for 29 of the main crops related to food production in Brazil would reduce production by 16.55 million tons, corresponding to 4.86 billion dollars per year. From the pessimistic viewpoint, these same values would be reduced to 51 million tons and 14.56 billion dollars per year. Such reductions would affect the country's gross domestic product (GDP), decreasing the agricultural contribution by 6.46% and 19.36% in the optimistic and pessimistic scenarios, respectively (Novais *et al*., 2016). The authors also warned that Brazil is vulnerable to a pollinator crisis since its economy is deeply grounded in agriculture and its production depends largely on pollinators.

3. Simplification of the landscape: consequences of deforestation and monoculture on bees

On August 28, 2020, MapBiomas released the estimated loss of native vegetation area between 1985 and 2019, which is equivalent to 10.25% of the national territory. The accumulated reduction is 87.2 million hectares, as shown in Collection 5 of MapBiomas (MapBiomas, 2020). The same study also showed that more than half of the loss of native vegetation in Brazil (44 million hectares) occurred in the Amazon.

Anthropic activity on natural landscapes, together with deforestation of native forests, causes changes in the set of components necessary for the survival of various organisms, in particular bees, both solitary and social. During the removal of vegetation for the implementation of economic activities of interest, bee colonies are eliminated, along with the trees that shelter them, contributing to the reduction of these insects (Santos, 2015). The expansion of agriculture, based on the principles and practices arising from the Green Revolution, had drastic consequences on different Brazilian ecosystems. Since its origins in the 1970s, it has promoted deforestation in large areas, growing at an impressive rate (Mesquita, 2009). Even with the effects already known, deforestation continues to occur.

Episodes of acute exposure, involving high bee mortality, have been reported frequently by inspection agencies and subsequently reported in the press. The disclosure of the death of "at least 500 million honeybees between October 2018 and March 2019 due to pesticides" is an example of this type of episode (Serb, 2019). Disclosed in this same report was the technical report of the National Agricultural Laboratory of Rio Grande do Sul (Lanagro-RS), Ministry of Agriculture, Livestock and Supply (MAPA), which found five types of pesticides in dead bees, honey, chicks and combs. In the northern part of the country, similar events are already happening because of soybean cultivation. The 2019 report indicated how meliponiculture and beekeeping face the poisoning of bees by pesticides (Amazônia Latitude, 2019). The use of pesticides, including herbicides in the vicinity of meliponaries has been increasing owing to the expansion of agribusiness in the region of Belterra-PA, in particular the cultivation of transgenic soybean varieties.

The transformation of natural environments to agricultural and / or urban areas may favor the isolation of bee populations and colonies as a result of habitat loss (Pinheiro-Machado *et al*., 2002). Changes in land use reduce nesting areas and the availability of floral resources (Ferreira *et al*., 2015; Kennedy *et al*., 2013), which can compromise the persistence of certain taxa (Pinheiro-Machado *et al*., 2002). The fragmentation and discontinuity of forests prevent the gene flow necessary for the survival of the colonies (Zayed, 2009). The isolation of species that occurs in these situations can cause inbreeding, contributing significantly to the manifestation of recessive genes, weakening the colonies that die gradually by eliminating the queen (Caires & Barcelos, 2017). In addition, Brazil has the greatest diversity of social stingless bee species in the world, housing 300 of the approximately 400 known species (Michener, 2007). What we know so far about the biology of these bees is insufficient to determine or identify their sensitivity or vulnerability to any type of human activity (Pinheiro-Machado *et al*., 2002).

Agricultural intensification also results in simplification of the landscape, threatening the provision of essential ecosystem services, such as pollination (Connelly *et al*., 2015). Activities such as livestock, extraction and exaggerated urban growth are also highly relevant in this process, driving deforestation and simplification of habitats (Fearnside, 2005). The continuous replacement of natural vegetation by planting crops and pastures further harms pollinators by the lack of floral diversity and resultant limited supply of nutrients to bees throughout the seasons (Blaauw & Isaacs, 2014).

Agriculture with deforestation practices, followed by monocultures, reduces the diversity of native plants that are sources of trophic resources for bees (Freitas *et al*., 2009). The richness and diversity of pollinator species existing in Brazil are affected by the expansion of areas of monocultures of species of economic importance, such as soybeans, limiting bees to a monofloral diet. With the intensive use of pesticides applied during the soybean, corn and cotton cultivation cycle, spontaneous plant populations are also eliminated. If deforestation reduces the habitat for bees, then monocultures, whether conducted in industrial or chemical agricultural systems, reduce the diversity of bee pasture in most of the area of large properties. Thus, after deforestation only forest fragments and small properties remain, where several varieties of domesticated species are cultivated that also become the source of food for bees.

During foraging, bees collect nectar, pollen and / or oils as a source of carbohydrates and proteins for all individuals in the nest (Faita, 2020). Social bees use collective behaviors to fight diseases at the colony level in a system called "social immunity" (Cremer *et al*., 2007). In this way, each bee can communicate and respond to the conditions of the hive, making individual choices that affect the colony. Thus, colonies function collectively as superorganisms (Moritz & Fuchs, 1998). Consequently, hives exposed to stressors that affect their ability to maintain or restore social immunity can become very weak and die (Archer *et al*., 2014).

Malnutrition and food scarcity can be highlighted as stressful events as well that reduce the immunity of bees (Negri *et al*., 2019; Zaluski *et al*., 2020). Thus, bees that forage monoculture areas have a poorly diversified diet and may not obtain all the necessary nutrients (Brodschneider & Crailsheim, 2010). Good nutrition depends on the diversity of floral resources present in the feeding of these insects (Negri *et al*., 2019), while the absence of these conditions can reduce the hive's population, compromise the physiological balance and the resistance to stress of bees, increasing their vulnerability to diseases and pesticides (Archer *et al*., 2014).

In addition to protein and sugars, bees find in pollen and nectar the phytochemicals they need to guarantee their individual or collective needs (Negri *et al*., 2019). Indeed, bees have developed the ability to identify the plants that supply the phytochemicals they need, starting to forage in a "self-medication" system. Storing food in hives also allows them to gain access to phytochemicals, even when the supplier plants are not in bloom (Erler & Moritz, 2016), implying that the health of hives depends fundamentally on their nutrition. This condition can be ensured by the existence of sites with preserved natural habitats and agricultural environments free of pesticides (Faita, 2020).

Along with the simplification of landscapes, another ecosystem service negatively affected is the control of pests by natural enemies in agricultural cropping areas. Monocultures, in general, generate ecological imbalance because they increase the supply of food for pests, causing an accelerated reproduction of pest insects or fungi in these disturbed environments and a correspondingly greater use of insecticides and other pesticides. Therefore, this simplification of landscapes, accompanied by the pressure from pests, also increases production costs. In a study carried out in 2007 in the Midwest region of the United States, the cost of combating pests was estimated at 69 million dollars in the cultivation of soybeans, corn, and wheat (Meehan *et al*., 2011). In addition to contributing to the reduction of natural enemies and the increase of insect pests, the authors reported that the simplification of landscapes was associated with lower crop yields.

4. Use of pesticides in Brazil and the effects on bees

Pesticides consist of multiple classes and subclasses of components and are commonly classified according to the target organism (herbicides, insecticides, fungicides) or according to their chemical class (organophosphates, triazine, among others) (Bhalli *et al*., 2006). Since 2008, Brazil has been the largest consumer of pesticides in the world (Carneiro *et al*., 2015) with a consumption of more than 300 thousand tons per year of formulated products, representing more than 130 thousand tons of active ingredients consumed annually. An important stimulus to consumption comes from the decrease in prices and the generous exemption from taxes on pesticides, causing farmers to use an even larger amount per hectare (Pignati *et al*., 2011).

In the period between 2000 and 2010, sales of pesticides grew by 190%, while the planted area increased by 30%. These data demonstrate the intensification of the use of these products, increasing their consumption per hectare planted, according to data from the Brazilian National Health Surveillance Agency, Agência de Vigilância Sanitária (ANVISA, 2011). The increase in the average consumption of pesticides in relation to the planted area went from 10.5 liters per hectare in 2002 to 12 liters per hectare in 2011 (Pignati *et al*., 2011). This number is the result, in part, of the monoculture practices of transgenic cultivars, the development of resistance of spontaneous plants, fungi and insects and agricultural mechanization (Gupta, 2004, 2007; Franco *et al*., 2010). In addition, the prevailing tropical climate in Brazil, together with climate change, favors the proliferation of diseases and pests (Ghini *et al*., 2011), which may contribute to the high consumption of agricultural inputs. However, this does not diminish the problems that these products cause to the environment and non-target organisms.

In Brazil in 2006, the most spent on pesticides involved cropping based on monocultures (simplified environments) of soy and sugar cane (Porto & Soares, 2012). In 2015, soybean, corn and sugar cane monocultures together accounted for 76% of the planted area in Brazil and 82% of the total pesticides used in the country. Soy was the crop that most used pesticides, representing 63% of the total, followed by corn (13%) and sugar cane (5%) (Pignati *et al*., 2017). Therefore, it is evident that products from agribusiness based on extensive monocultures are responsible for the highest consumption of pesticides in Brazilian agriculture.

The intensive and extensive commercialization and application of pesticides in crops has generated discussions in most countries regarding the damage they cause, considering the cytotoxic potential of these products on non-target organisms (Guillén *et al*., 2012), including humans. In Brazil, Pignati *et al*. (2017) evaluated the correlation between the indicators of environmental quality and human health for the municipalities with a higher consumption of pesticides. These authors observed that the consumption of pesticides increases along with the the average coefficient of acute, subacute (fetal malformation) and chronic (child-juvenile cancer) exposure.

In addition, many other studies have demonstrated the harmful effects of pesticides on non-target organisms, such as vertebrates. Among them, the changes in the respiratory and hepatic system stand out (Santos Filho *et al*., 2003; Kesavachandran *et al*., 2006). Physiologically, biochemical changes can trigger oxidative stress and cytogenetic damage (Tope *et al*., 2006; Jia & Misra, 2007). The damage caused to DNA by pesticides can trigger carcinogenic processes, morphological anomalies, and alterations in gametes, interfering with the fertility and survival of populations (Bolognesi, 2003), in addition to generating chronic adverse effects on the structure and dynamics of populations and communities (Nacci *et al*., 1996; Kendall *et al*., 2001). The reduction in population size and the possible occurrence of the selection of pollutant-resistant genotypes, in turn, act as a genetic bottleneck with consequent loss of variability (Theodorakis & Shugart, 1998).

The assessment of the risks of a pesticide on a given insect should initially consider its biology. Social insects present a division of labor among all members of the colony. Since the activities within the colony are related to the age and genetic factors of individuals, the colony's social organization should also be considered (Calderone & Page, 1992). The Apiformes group (Superfamily Apoidea) comprises seven families and more than 16 thousand species (Michener, 2007), each with different life cycles, behavioral, morphological, and physiological characteristics. Thus, studies that propose to determine the effect of pesticides on eusocial bees by evaluating individuals outside the colony must be approached with some skepticism. In other words, caution is needed when defining the risk of a product on non-target organisms, especially to social bees.

Bees are considered bioindicators of environmental quality (Matin *et al*., 2016) since their foraging activity exposes them to different contaminants that can be identified in bee products, such as honey and pollen (Silva & Paz, 2012; De Oliveira Diniz *et al*., 2020). In addition, bees have fewer genes that encode proteins when compared to other insects, such as *A. mellifera* (Claudianos *et al*., 2006; Evans *et al*., 2006). The most pronounced differences occur in three superfamilies that encode detoxification enzymes for xenobiotics, which may represent greater sensitivity of bees to pesticides (Claudianos *et al*., 2006). This characteristic inherent in bees reveals the importance of studies on the effects of pesticides on these insects, especially those that address the synergy between combinations of xenobiotics, which represent interactions that need to be considered in risk assessments (Al-Waili *et al*., 2012).

The continuous contact of bees with pesticide residues present in the pollen and nectar of cultivated or spontaneous plants promotes the contamination of honey (Sanchez-Bayo & Goka, 2014). This contamination can also occur from the substance present in the water consumed by the bees to keep their body temperature and the swarm under control (Schmaranzer, 2000). The residues present in these media may be below the lethal dose, but they persist in honey, pollen and wax for indefinite periods, causing chronic effects (Desneux *et al*., 2007; Goulson, 2013; Sánchez-Bayo & Goka, 2014). Thus, if forage bees are contaminated, so are other individuals in the hive (Prado *et al*., 2019). As a consequence, such "cross-contamination" can promote changes in the cognitive capacity of individuals in the colony, impacting geolocation, impairing the collection of food, and compromising the maintenance of the colony (Sánchez-Bayo *et al*., 2016).

TABLE 1 – Active ingredient, mode of exposure, life stage of insects and effect of herbicides and fungicides on bees of native and exotic species from studies developed in Brazil.

Class	**Active ingredient**	**Species**	**Contact mode**	**Life stage**	**Observed effect**	**Reference**
Herbicide	Glyphosate	*Apis melli-fera*	Oral	Adult	Reduction in the amount of royal jelly produced and weakening of hives exposed to the herbicide with reduced adult bee population, brooding area and food supply after 130 days of exposure.	Chaves *et al.* (2020)
	Glyphosate	*Apis melli-fera*	Oral	Adult	Increased mortality of bees contaminated by Nosema spp.	Faita *et al*. (2020)
	Glyphosate and 2,4-D	*Melipona scutellaris*	Topical and oral	Adult	In combination, herbicides have increased mortality; upon oral exposure, both at the recommended field dose and at the double dose, bees reduced longevity.	Nocelli *et al*. (2019)
	Glyphosate	*Apis melli-fera*	Oral	Adult	Changes in the cellular organelles of the hypopharyngeal glands, promoting the early degeneration of these structures.	Faita *et al*. (2018)
	Paraquat	*Apis melli-fera*	Oral	Adult	Increased mortality and expression of detoxification genes in bees on a protein-free diet.	de Mattos *et al*. (2018)
	Paraquat	*Tetragonisca angustula and Tetragonisca fiebrigi*	Topical	Adult	Changes in the pattern of expression of isoenzymes.	Fermino *et al*. (2011)

	Pyraclos-trobin	*Apis melli-fera*	Oral	Adult	Reduction in the expression of royal jelly proteins.	Zaluski *et al*. (2020)
	Pyraclos-trobin	*Apis melli-fera*	Oral	Adult	Morphological changes and positive marking for cell death in the midgut of bees, which may have its nutrient absorption functions compromised.	Batista *et al*. (2020)
	Dipheno-conazole	*Melipona scutellaris*	Topical and oral	Adult	Caused mortality, as well as accumulation of this product in the tissues of insects.	Do Prado *et al*. (2020)
	Dipheno-conazole	*Apis melli-fera*	Topical	Adult	Low survival of exposed bees, along with the observation of adverse behavioral changes, such as agitation and changes in motor coordination.	Leite *et al*. (2018)
	Pyraclos-trobin	*Apis melli-fera*	Oral	Larvae	The fungicide alone did not alter larval longevity; however, in association with the insecticide Clotianidin, it reduced the longevity of bees.	Tadei *et al.* (2018
Fungicide	Pyraclos-trobin	*Apis melli-fera*	Oral	Adult	Morphological impairments reduced royal jelly secretion by nurse honeybees, which hampered colony maintenance.	Zaluski *et al*. (2017)
	Pyraclos-trobin	*Apis melli-fera*	Oral	Adult	Beehive survival was reduced and the hepatonephrocytic system was overloaded.	Domingues *et al*. (2017)
	Methyl thiophanate and chloro-thalonil	*Apis mellifera e Partamona helleri*	Oral	Adult	Mixture of the two fungicides caused toxicity with a high mortality rate in both species.	Tomé et *al*. (2017)
	Captan	*Bombus terrestris*	Oral	Adult	No significant effects of treatment noted on microcolony longevity, individual worker, pollen or sugar syrup consumption, number of discarded larvae, number of drones produced, or time to first oviposition.	Malone *et al*. (2007)

In general, most studies have focused on the effects of insecticides on bees, while few studies have addressed the effects of herbicides and fungicides, which have sublethal effects and are, therefore, less evident. Examples of Brazilian studies on the adverse effects of herbicides and fungicides are listed in Table 1.

A large part of the work carried out in Brazil uses the exotic species *A. mellifera* as a biological model (Table 1). This is a bee of great economic importance, and it is managed to produce bee products and directed pollination (Souza *et al*., 2007). However, in Brazil, about 1700 species of native bees are registered; of these, approximately 300 belong to the Meliponini tribe (Moure *et al*., 2007), presenting eusocial behavior, but few studies have reported the effects of herbicides and fungicides on bees.

Among bee species, variations in sensitivity to pesticides were evaluated in different studies and presented in a meta-analysis by Arena & Sgolastra (2014). Based on 150 studies reviewed by the mentioned authors, non-Apis bees are less tolerant to pesticides than Apis bees. Thus, reliable statements about the effects of pesticides on native Brazilian bee species cannot be made when using an exotic species as a basis of the studies.

Many species of solitary and social native bees nest in the subsoil and / or use mud to build their nests, exposing them even more to the pesticides present in this matrix. They are also more exposed during the larval period because, unlike honeybees that receive royal jelly and nectar, native bees are fed with unprocessed pollen which would have more pesticide residues when compared to nectar (Thompson *et al*., 2014) and other products of the hive (Calatayud-Vernich *et al*., 2018). In addition, when bees nest naturally, they cannot be moved temporarily during the spraying of pesticides, and in the case of solitary bees, the death of a female in nesting results in the end of the reproductive activity of that species (Arena & Sgolastra, 2014). This means that increased simplification of the landscape, substitution of native vegetation by cultivation of transgenic varieties, and, consequently, greater spraying of pesticides, when taken collectively, constitute real threats to different species of bees.

5. Examples of direct damage from the cultivation of transgenic plant varieties in bees

Since 1973, with the advent of recombinant DNA technology, later called genetic engineering, the necessary precautions for the development and use of the resulting products have been presented, among which we find transgenic, or GM, varieties. Such varieties produce new traits and alter the gene regulation of the host variety of recombinant or transgenic genes. The possible ecological risks were anticipated as far back as 1989, five years before commercial release of the first transgenic variety (Tiedje *et al*., 1989). Among the possible risks related to bees were (i) the production of substances that are, or could be, toxic to non-target organisms; (ii) the disruptive effect on biotic communities and the waste of valuable genetic resources, followed by contamination of native species, with characteristics originating from distant relatives or unrelated species, as well as adverse effects on ecosystem processes; (iii) the origin of toxic secondary substances after the in-complete degradation of dangerous chemicals; and (iv) the adverse effects on ecological processes. All these risks have caused real damage to bees, some of which are exemplified in this article (Table 2).

In the absence of official statistics, it has been estimated by Céleres, a Brazilian agribusiness consultancy, that the area cultivated in Brazil with GM varieties in the 2018/2019 crop season would have reached about 51.8 million hectares. In this area, GM varieties of cotton, corn, and soybeans with recombinant genes (derived from *Baccilus thuringiensis*), which are encoded to produce toxins with insecticidal function, occupied 72% of the area cultivated in the referenced crop (Céleres, 2019). Studies had already been carried out with native toxins from *Baccilus thuringiensis* (Bt) to predict the possible impacts of the recombinant toxins that were being developed on bees. Thus, when fed with *B. thuringiensis* spores or Cry protein, the bee mortality rate was higher than that in those not exposed to toxins (Vandenberg & Shimanuki, 1990; Vandenberg, 1990). Later, investigators discovered the deleterious effects of toxins produced by the *cry* genes, native or recombinant, on foraging activities, feeding behavior, and learning performance, all of which can impact the development of the hive (Ramirez-Romero *et al*., 2005, 2008).

In general, studies with social bees are carried out with individuals of the worker caste, which, under natural conditions, have an average survival of 35 days. In these cases, the bioassays are conducted in the laboratory under artificial conditions of humidity, temperature, and light, with a duration shorter than the survival period of worker bees (Table 2).

In addition to studies with isolated proteins, the effects of pollen containing recombinant Cry toxins also demonstrated the adverse effects of this group of toxins, which have insecticidal activity. The results of the analysis of bees directly on hives that were fed on Bt corn pollen, containing the *rcry1F* (recombinant *cry1F*) that codes for the Cry1F toxin, revealed a significant increase in the infestation of the Varroa destructor mite in adult bees, as well as a reduction in the hygienic behavior of colonies exposed to the Cry1F toxin, compared to control hives (Bizzocchi, 2014). Malone *et al*. (2007) found that the toxin produced by the protein reduced the survival time of bees fed a diet containing the protein Cry1Ab.

These results, combined with the inability of bees to distinguish GM from non-GM flowers (Malone & Pham-Dèlègue, 2001; Sabugosa-Madeira *et al*., 2007), clearly establish that laboratory bioassays do not correspond to the risk of damage to which bees are exposed in field conditions. Therefore, it is possible that the proximity and the scale of cultivation of GM varieties producing toxins with an insecticidal function cause more damage to bees than that revealed by laboratory experiments, as is the case of varieties carrying *rcry, rvir* and other genes isolated from *B. thuringiensis* and genetically altered *in vitro*, allowing even greater efficiency and amplitude in their toxic action.

The indirect effects of cultivating GM varieties are also critical for bees. Pollen from genetically modified organisms (GMOs) is also toxic to insects that cohabit in hives, such as the wax moths *Achroia grisella* and *Galeria mellonela* (Hanley *et al*., 2003; Oldroyd, 2007; Trevisan *et al*., 2013). It should be noted that dead colonies showing characteristic symptoms of Colony Collapse Disorder

(DCC) did not contain live wax moths or beetles of the species Aethina tumida in abandoned combs (Sabugosa-Madeira *et al*., 2007). When we combine this observation with the toxicity of pollen from GMOs, we have a case of indirect damage caused by the presence of transgenic pollen containing insecticidal toxins in hives.

Another indirect effect involves the cultivation of transgenic varieties resistant to herbicides. In this case, the use of the GM variety is associated with the use of herbicides applied on plants of herbicide-resistant varieties. However, in this cropping system, the indirect effect results in decreasing the supply of resources to bees. Groups of bees and butterflies were similarly affected by the spraying of herbicides in crops of GM varieties of beet, sown in the spring, and canola in the winter, causing a negative impact on the abundance of pollinators and, possibly, on pollination (Bohan *et al*., 2005). Thus, during the growing season of GM plants, the authors found that the lower abundance of dicotyledons in the cropping system with the use of herbicides over the entire cultivated area suggested that bees, as well as other animals that depend on dicotyledons, would not do well if this cropping system were widely adopted.

This widespread adoption of GM varieties resistant to herbicides in association with the increased use of these kinds of pesticides has occurred in Brazil in the last 20 years. In the 2018/2019 crop season, 34.7 million hectares of soybeans, 15.6 million hectares of corn and 1.44 million hectares of cotton were grown (Céleres, 2019). Of varieties of the three species mentioned in the same report, 7.5% contain genes that produce toxins with insecticidal function, 27.6% contain genes resistant to herbicides, and 64.5% contain both characteristics (Céleres, 2019). In addition, two varieties of GM soy have genes that increase drought tolerance, but the cultivated area has not been reported. More recently, a variety of sugarcane, a variety of beans and a variety of eucalyptus, containing, respectively, toxin production genes, resistance to a virus and wood volumetric increase, were approved for cultivation in Brazil.

Well before commercial approval of transgenic plants, scientists and beekeepers expressed their concerns about the possible effects of their cultivation. The first concern is associated with the direct or indirect toxic effects on bees. The second concerns the contamination of non-GM varieties by GM varieties, which could be mediated by gene flow with the aid of bees. In fact, this has occurred between varieties of canola and corn. Although pollen can be carried from one crop to another by the wind, bees are floral visitors in conventional or transgenic varieties of corn (Figure 1) or soybean (Figure 2).

In addition to contaminating the landraces used for organic and agroecological production, GM corn pollen carried to the hives contaminates the honey produced. The detection of pollen from GM corn in honey in samples from Mexico (Gálvez-Mariscal, 2013), as well as pollen from GM soy in honey, occurred, among other discoveries, in Argentina (Gallez *et al*., 2005) and Mexico (Villanueva- Gutiérrez *et al*., 2014). Brazilian regulation (CTNBio, 2007) establishes a minimum distance of 100 meters between GM crops and non-GM corn, but this rule, even if obeyed, does not prevent bees or the wind from carrying pollen from one crop to another. The conclusion is unequivocal: there is no coexistence with crops that cultivate GM varieties without prejudice to producers of organic corn or honey.

FIGURE 1 – Bee collecting pollen from a transgenic corn variety.
PHOTO: Personal collection of authors.

FIGURE 2 – Bee collecting pollen from a transgenic soybean variety.
PHOTO: Prof. Márcio do Nascimento Ferreira.

TABLE 2 – Recombinant toxin, mode of exposure, life stage of bees and effect of the toxin expressed by transgenic plants on bees.

Gene	Product	Species	Exposure mode	Life stage and conduct of the bioassay	Effects compared to control treatment	Author
rbar	Provides resistance to the herbicide Glufosinate ammonia in plants	Many	Reduction of food (pollen / nectar) for three years	Adults	Reduced diversity and abundance of bees.	Bohan *et al*. (2005)
ncry1Ab	Toxin	*Apis mellifera*	Ingestion of toxin in the syrup for 10 days	2-day old larvae, in cages for 15 days	Increased time for food consumption, syrup contaminated with protein Cry1Ab, similar to time for consumption of syrup with imidacloprid, and impaired learning performance.	Ramirez- Romero *et al*. (2008)
rcry1Ac +cpTI	Toxin + Trypsin inhibitor	*Apis mellifera*	Ingestion of pollen containing toxin for seven days	Larvae Laboratory	Increase (50%) in the mortality rate, similar to that caused by imidacloprid, and less consumption of GM pollen and containing imidacloprid residue.	Han *et al*. (2010)
cry1Ac	Purified Toxin	*Apis mellifera*	Diet intake containing Cry1Ac for five days	Larvae <24h up to 18 days, in an incubator with biochemical oxygen demand (BOD).	The addition of water to the diet to the Cry1Ac toxin caused a significant increase in larval mortality but did not affect pupal development time.	Lima *et al*. (2010)
rcry1Ah	Toxin expressed in *B. thuringiensis*	*Apis mellifera e A. cerana*	Syrup intake containing rCry1Ah for nine days	Larvae <12 h, Cages for 21 days	There was no difference in survival, pollen consumption and hypopharyngeal gland mass.	Dai *et al*. (2012)

rcry1F	Toxin	*Apis mellifera*	Ingestion of pollen containing the toxin for three weeks	Larvae 10-14 days in hives	Reduction of hygienic behavior of the colonies and increased infestation of the mite V. destructor in adult bees.	Bizzocchi (2014)
cry1F	Toxin	*Melipona quadrifasciata*	Ingestion of artificial food containing the toxin	Larvae; Laboratory	No effect on survival rate, but it did shorten the feeding period.	Seide *et al.* (2018)
rcry2Aa	Toxin	*Melipona quadrifasciata*	Ingestion of artificial food containing the toxin	Larvae; Laboratory	Increased survival rate, but delayed development.	Seide *et al.* (2018)

As pollen is present in honey, even in a minimal amount, it was considered, in the European Union, an ingredient in honey and not a natural component. Thus, by decision of the European Court of Justice on 6 September 2011 (Case C-442/09), honey contaminated with pollen from GMOs was included in the classification of foodstuffs requiring authorization to be placed on the European market (Lamping, 2012). Thus, whenever a bee collects pollen from a genetically modified plant, this can make every harvest of honey non-marketable (Lamping, 2012), representing socioeconomic damage to beekeepers.

In addition to the detection of DNA or protein from transgenes present in GM varieties, residues of glyphosate-based herbicides (HBG) in association with GM varieties resistant to herbicides have also been detected in honey. Rubio *et al*. (2014) studied honeys purchased in the United States and found that 45% of the 11 samples of organic honey and 62% of the 58 samples of non-organic honey had glyphosate residue. According to the authors, in general, glyphosate levels were lower in samples from countries that had not authorized the commercial cultivation of GM varieties.

6. Final considerations

The efforts of the scientific community have contributed significantly to elucidating the causes of the phenomenon of mass mortality of bees, as observed in different parts of the world. All the findings point to a set of factors that include, among others, the simplification of the landscape, the use of pesticides and large-scale monoculture of transgenic or GM plants. Together, these three practices promote nutritional deficiency in bees, weakening their immune system, and leaving them vulnerable to parasites and pathogens, in addition to reducing nesting sites.

Evidence on sublethal effects of herbicides and fungicides indicates that the damage to bees caused by these pesticides, which are said to be harmless by manufacturers, should not be ignored. In addition, most of the studies refer to bees of the species *A. mellifera*, considered the most effective pollinator, in addition to being a major producer of honey and other bee products. Only minimal information is available on the effects of pesticides on stingless bee species in Brazil, the diversity of which is the largest in the world (Michener, 2007) and is being lost, even before it is known in detail. Compromise of their natural habitats directly interferes with their health and survival.

As noted previously in this article, the arbitrary classification of pesticides into insecticides, fungicides, and herbicides, among others, is not reflective of the target organism of interest for each pesticide. The examples mentioned in this article and a wide range of studies have demonstrated that the target of an insecticide, fungicide, or herbicide comprises a large set of species outside the scope that the classification suggests.

In addition, in Brazil, nowadays, we are experiencing a dynamic of new and constant release of pesticides prohibited in other countries (Hess *et al*., 2021 - in this special issue), as well as the maintenance of tax incentives in the trade of these products. Meanwhile, however, insufficient resources make it impossible to conduct research on the long-term effects of pesticides on non-target organisms. We are also forced to live within the limits of permissiveness of residues that are not acceptable in other countries, creating a nefarious scenario for bees and other living beings. For decades, we have been warned about the risks of human activity to the biosphere. Over time, these warnings have been borne out when contextualized studies were carried out.

Based on the data presented in this article, the continuing damage caused to bees and other living beings by pesticides, transgenic varieties and deforestation can be avoided by following a basic precautionary principle that incorporates part of other concepts, such as justice, equity, respect, common sense, and prevention. Thus, the burden of proving the absence of harmful effects of a given technology would be borne by the proponent of the technology. However, the precept is currently not practiced in Brazil where it is still acceptable to evade robust risk assessments or hold manufacturers accountable for marketing products known to be contaminated. This calls into question the weaknesses in both legislation and in the performance of regulatory and inspection agencies; however, it is unacceptable that responsibility should be given only to farmers who use these products.

Pesticides comprise only one component of agribusiness, whereas the entire agribusiness model causes irreversible damage to the environment, including the destruction of native forests and biological diversity. While large companies profit from the sale of agrochemicals and transgenic seeds, Brazilian taxpayers bear the social and environmental cost of the adverse effects of this cropping system.

The precautionary principle is also related to the respectful and functional association of man with nature made concrete by low-impact agricultural systems, such as agroecology and organic production. These systems do not use transgenic (or GM) varieties or pesticides, but they do produce high biological quality organic food. The benefits of agroecology include the reduction of water and soil pollution and the conservation of biodiversity (Nodari & Guerra, 2015). These and other benefits derive from agroecological practices that also contribute to the recovery of watersheds and reduce dependence on external inputs. Finally, agroecological products promote health, as they have greater nutritional value and fewer residues of pesticides and heavy metals (Barański *et al*., 2014).

In the current scenario of agriculture, contextualized research contributes mainly in two ways. First, it can transform the state of ignorance and uncertainty into defined risks associated with a new product or activity, leading to effective risk management strategies and measures that include anticipatory actions to protect the health of people and ecosystems. Second, when carried out in a participatory manner with farmers, contextualized research contributes to the advancement of scientific knowledge that underpins agroecological principles and processes, which are appropriated and innovated by agroecological farmers themselves.

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