



## REVIEW

## Honey Bee Colony Losses: Why Are Honey Bees Disappearing?

PETER HRISTOV<sup>1</sup>, ROSITSA SHUMKOVA<sup>2</sup>, NADEZHDA PALOVA<sup>3</sup>, BOYKO NEOV<sup>1</sup>

1 - Department of Animal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

2 - Research Centre of Stockbreeding and Agriculture, Smolyan Agricultural Academy, Bulgaria

3 - Scientific Center of Agriculture, Sredets, Agricultural Academy, Bulgaria

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#### Corresponding author

Peter Hristov  
 Department of Animal Diversity and Resources  
 Institute of Biodiversity and Ecosystem  
 Research, Bulgarian Academy of Sciences  
 "Acad. G. Bonchev" Str., Bl. 25,  
 Sofia, 1113, Bulgaria.  
 E-Mail: peter\_hristoff@abv.bg

### Abstract

The Western honey bee (*Apis mellifera* L., Hymenoptera: Apidae) is a species of crucial economic, agricultural and environmental importance. In the last ten years, some regions of the world have suffered from a significant reduction of honey bee colonies. In fact, honey bee losses are not an unusual phenomenon, but in many countries worldwide there has been a notable decrease in honey bee families. The cases in the USA, in many European countries, and in the Middle East have received considerable attention, mostly due to the absence of an easily identifiable cause. It has been difficult to determine the main factors leading to colony losses because of honey bees' diverse social behavior. Moreover, in their daily routine, they make contact with many agents of the environment and are exposed to a plethora of human activities and their consequences. Nevertheless, a number of different factors are considered to be contributing to honey bee losses, and recent investigations have established some of the most important ones, in particular, pests and diseases, bee management, including bee keeping practices and breeding, the change in climatic conditions, agricultural practices, and the use of pesticides. The global picture highlights the ectoparasitic mite *Varroa destructor* as a major factor in colony loss. Last, but not least, microsporidian parasites, mainly *Nosema ceranae*, also contribute to the problem. Thus, it is obvious that many factors are involved in honey bee colony losses globally. Increased monitoring and scientific research should throw new light on the factors involved in recent honey bee colony losses. This review focuses on the main factors which have been found to have an impact on the increase in honey bee colony losses.

### Introduction

The managed honey bees are the most important pollinators for many crops and wild flowering species. Many countries around the world, particularly in the northern hemisphere, rely on the Western honey bee, *Apis mellifera*, for commercial pollination of certain crops, but over the recent years some regions of the world have been suffering from an increase in losses in their managed honey bee colonies. The Colony Collapse Disorder (CCD) has been reported for the first time in 2006 in the USA (Neumann & Carreck, 2010).

Although some bee losses have also been reported in Japan and China, published data from various investigations have shown that honey bee colony numbers have been stable for the past ten years in these regions (Taniguchi et al., 2012; Liu et al., 2016). A significant rate of honey bee colony losses has been reported in South America as well. In a large-scale study in five countries, Argentina, Uruguay, Chile, Brazil, and Venezuela, the main factors leading to the honey bee losses have been described (Maggi et al., 2016). These include the ectoparasitic mite *Varroa destructor* and the widespread use of acaricides for its treatment, agriculture intensification,



unbalanced bee nutrition leading to honey bee-associated viruses (sacbrood disease in Brazil) and parasitic diseases (nosemosis in Uruguay and Argentina), etc. Other studies have highlighted local honey bee losses in different countries in South America. For example, during the period 2013-2014 a survey among bee keepers in Uruguay detected around 30% colony losses annually (Antúnez et al., 2017). The main factors for this reduction include queen failure, diseases and parasites, and widespread use of pesticides. In a 5-year online survey in Brazil (2013-2017) around 50% annual losses were observed, and pesticide exposures were suspected to be the main reason for the colony losses (Castilhos et al., 2019). In Argentina, coinfections with different pathogens (virus-fungi, mites-virus-fungi, and virus-mite) have been discussed as the main stressors for honey bees, but, according to some authors, there are much more complex reasons leading to honey bee population reduction (Garcia et al., 2019). The global picture has shown that there are no significant honey bee colony losses reported in Africa and Australia. In the Middle East, the high temperatures and droughts in the summer are the main factor leading to colony losses because many plants which are important sources for bee forage suffer from heat stress. Another factor aggravating the problem is the lack of comprehensive laws and legislations concerning the importation of bee families (Muli et al., 2014).

Indeed, bee colony losses are not a new phenomenon and historical records show that extensive losses were not unusual in the past. Whilst recent problems may give the impression that there has been a massive decline, global research on honey bee colonies has shown that numbers actually increased between 1961 and 2007, mostly in Asia (426%), Africa (130%), South America (86%), and Oceania (39%) (FAO, 2009). In Europe, on the other hand, an extensive study involving 18 European countries during the period 1965-2005 found out a decline of honey bee colonies of about 16.1% (Potts et al., 2010). Moreover, honey bee losses in Europe have revealed a geographic pattern. For example, the number of bee colonies has been decreasing in Northern and Central European countries, and, likewise, in these countries a falling number of beekeepers has also been noted (Potts et al., 2010). On the other hand, in the Mediterranean and many South European countries the number of managed bee colonies is increasing (Potts et al., 2010).

It has been difficult to establish a common pattern for the colony losses, but different investigations confirm that it is a phenomenon characteristic of the Western honey bee, while the Asiatic honey bee, present in Southern, Southeastern, and Eastern Asia, appears to be more resistant to various pests and diseases.

### Role of pests and diseases in honey bee colony losses

To understand what is causing the current decrease in honey bee colonies worldwide, it is important to shed light on the key pests and diseases affecting bee health.

Honey bees are affected by a number of pests and diseases including mites, honey bee-associated viruses, microsporidia, bacterial infections and fungal diseases. Due to the burden of infectious diseases and their agents, honey bee colonies may manifest significant weakness or even death. Only recently have scientists come to better understand the importance of the development and interactions of these pests and diseases.

#### *Ectoparasitic mites*

The hive of the honey bee is a suitable habitat for several mites (Acari), including nonparasitic, omnivorous, pollen-feeding species, and parasites. Out of different mite species associated with honey bees, *Varroa destructor*, *Acarapis woodi*, *Varroa jacobsoni* and *Tropilae clareae* are economic pests of honey bees and their infestation may lead to the destruction of the beekeeping industry in many cases (Sammataro et al., 2000; Dhooria, 2016). *V. destructor* is the most serious pest of honey bee colonies worldwide, and an obligate parasite which is able to attack different developmental stages and castes of *A. mellifera* (Shen et al., 2005). It is interesting to note that Varroa mites have been established in New Zealand since 2000, but yet, Australia remains Varroa-free (Iwasaki et al., 2015). For several decades in the 20<sup>th</sup> century *V. jacobsoni* was the sole cause of “Varroosis” in *Apis mellifera* (Rosenkranz et al., 2010). For this reason, *V. jacobsoni* was mentioned in all literary sources in the last century as the main cause of “Varroosis”, although in most cases *V. destructor* was involved. Based on sequence analysis of mitochondrial DNA (*cox1* gene), it became possible to distinguish the new mite species *V. destructor* as morphologically and genetically different from *V. jacobsoni* (Anderson & Trueman, 2000).

The European honey bee *A. mellifera* was introduced for the first time in Asia in 1877 in order to improve the productive qualities of *Apis cerana* (Sakai & Okada, 1973). Nearly 80 years after the introduction of the Western honey bee in Asia, it was observed that *V. destructor* had switched hosts from *A. cerana* to *A. mellifera* by 1957 in Japan (Sakai & Okada, 1973) and by 1963 in Hong Kong (Delfinado, 1963). Its range expanded quickly through global human-mediated honey bee trade – both legal and illegal – and most probably via shipping. Currently, *V. destructor* can be found all over the world, except in Australia, some extreme northern territories, and remote islands such as the Seychelles and Comoros archipelagoes (Locke, 2016; Roberts et al., 2017). In Africa, African honey bees survive despite the presence of *V. destructor*, as do the Africanized honey bees in South America. This increased resistance of the Africanized honey bees against *V. destructor* may be explained with their more aggressive behaviour compared to the Western honey bee (Medina Flores et al., 2014; Oddie et al., 2018).

*V. destructor* has been present for many years in most countries and is currently considered the biggest threat related to colony losses, not just as a vector in the transmission of honey bee-associated viruses but also due to its detrimental

effects on honey bee colonies (Le Conte et al., 2010). In the USA, it has been reported that during the winters of 1995-1996 and 2000-2001 honey bee colony deaths reached 50 to 100% in many apiaries (Le Conte et al., 2010; Pettis & Delaplane, 2010). No less were the reported losses due to the *V. destructor* infestation in Europe (Le Conte et al., 2010; Moritz et al., 2010). In central Europe, a high number of colony losses was observed in the winter of 2002-2003 (Hendrikx et al., 2009) and in southern Europe – especially in the winter of 2007-2008 (Mutinelli et al., 2010), with most beekeepers reporting *V. destructor* as the main causative agent of mortality. Similar data for the role of *V. destructor* as a major threat to *A. mellifera* have been reported in South America (Maggi et al., 2016). In contrast to the data mentioned above, it seems that the savannah honeybee *A. m. scutellata* in Africa and *A. cerana* in Asia have the ability to maintain mite populations at low levels, which reflects the low impact on these honeybee populations (Strauss et al., 2015; Chantawannakul et al., 2016).

Typical control of *V. destructor* involves the use of fluvalinate, a pyrethroid, treated strips placed in the hive during times of no honey production. Intensive use of these strips has selected for resistance in some parts of Europe (Floris et al., 2001), the United States (Macedo & Ellis, 2002), Israel (Mozes-Koch et al., 2000), and Mexico (Rodríguez-Dehaibes et al., 2005). The spread of pyrethroid resistance in Europe roughly follows that of the initial spread of the mite according to bee movement, suggesting that resistance evolved once and spread thereafter (Büchler et al., 2010). Coumaphos, an organophosphate insecticide, was soon introduced for emergency use after control problems with fluvalinate, but resistance to coumaphos is now present in Florida (Elzen & Westervelt, 2002) and northern Italy (Spreafico et al., 2001). Resistance to both pyrethroids and amitraz, an amidine, has been reported in the United States (Elzen et al., 2000) and in Mexico (Rodríguez-Dehaibes et al., 2005). Thus, the increased resistance of *V. destructor* against various insecticides creates a precondition for additional difficulty in combating mites and seeking alternative approaches.

### Viral infections

About 24 honey bee-associated viruses have been identified in the Western honey bee (*Apis mellifera*) (Gisder & Genersch, 2015). Some of them generally persist in the bee's body, without causing a disease or manifestation of any clinical signs. In general, virus infestations were not considered to be a significant problem to honey bee health. On the other hand, some viruses are more virulent and infective, and thus may cause a significant loss in honey bee colonies as well as a decline in honey bees' health and production. Some viruses show pathogenicity only under certain favorable environmental conditions.

The mite *V. destructor* is considered to be the main vector of many honey bee-associated viruses: the Deformed

wing virus (DWV); the Acute bee paralysis virus (ABPV), the Kashmir bee virus (KBV), and the Israeli acute paralysis virus (IAPV) (Locke, 2016; Ramsey et al., 2019). Furthermore, there are three viruses for which Varroa seems to play no significant role in the transmission of, namely, the Chronic bee paralysis virus (CBPV), Sacbrood virus (SBV), and the Black queen cell virus (BQCV) (Tentcheva et al., 2004; Nielsen et al., 2008). It is interesting to note that about 40 years ago there was no increase in colony losses despite the presence of Varroa mites; such losses, however, have become more and more apparent over the last ten years. This fact allows us to think that Varroa mites alone are not the cause of honey bee losses. The negative influence of *V. destructor* results from its role as a reservoir and important vector of some honey bee-associated viruses (Shen et al., 2005); the mite promotes replication of honey bee viruses like the DWV (Levin et al., 2016). Due to its feeding behavior, the Varroa mite directly injects viruses in the hemolymph, which has been associated with oral or sexual transmission of these viruses (Francis et al., 2013).

A large number of studies reflect the relationship between honey bee-associated viruses and colony losses (Nielsen et al., 2008; Soroker et al., 2011; Cornman et al., 2012; Granberg et al., 2013; Li et al., 2014). Research conducted in this direction has shown strong indications for IAPV and ABPV (both are members of the ABPV/KBV/IAPV clade) being involved in winter colony losses (Cox-Foster et al., 2007; Genersch et al., 2010) and DWV being a key factor for overwintering colony losses in Germany (Genersch et al., 2010). The results from Cox-Foster et al. (2007) revealed that SBV, BQCV, DWV, and ABPV viruses were found in both CCD and non-CCD colonies, while IAPV and KBV were found only in the CCD colony. The obtained results allowed the authors to determine IAPV as a significant marker for CCD. These observations were subsequently confirmed by the study of Cornman et al. (2012), who established that viruses were significantly more abundant in CCD colonies, but in contrast to a previous study (Cox-Foster et al., 2007), they found no positive association between the presence or infection load of IAPV and CCD.

### Microsporidia

Microsporidia are fungal, obligate intracellular parasites infectious to honey bees. Microsporidia are possibly the smallest single-cell organisms with a true nucleus. The genus *Nosema* is a parasitic fungus that infects insects such as honey bees, bumble bees and silkworms. Two described species of microsporidia, *Nosema ceranae* and *Nosema apis*, parasitize on adult honey bees (Paris et al., 2018). It is well known that *N. apis* is specific for the Western honey bee, *Apis mellifera* L., whilst the Asiatic bee, *Apis cerana*, harbors *N. ceranae* (Fries et al., 1996). For a long time, it was believed that *N. ceranae* and *N. apis* were species-specific. Since the beginning of this millennium (mainly post 2003), many investigations have revealed that *N. ceranae* has switched hosts and has become

the dominant species in many countries (Klee et al., 2007; Paxton et al., 2007; Chen et al., 2008; Invernizzi et al., 2009; Stevanovic et al., 2011). Thus, it has been suggested that *N. ceranae* is possibly more virulent than *N. apis*.

It has been well documented that Microsporidia invade the midgut epithelial cells of worker bees, queens and drones (Papini et al., 2017). *Nosema* has adverse effects on the bee colony. The negative effect of nosemosis at the colony level relates to productivity and survival of honeybee colonies, including adult bee longevity, queen bees, brood rearing, bee biochemistry, pollen collection and other bee behaviors (Botías et al., 2013).

In contrast to *N. apis*, which rarely leads to the death of a diseased colony, since its emergence as a novel pathogen of the Western honey bee *A. mellifera*, *N. ceranae* has been generally associated with heavily diseased honey bee colonies (Vejsnaes et al., 2010). Considering *N. ceranae* as a potential factor in CCD, we may summarize that almost any given disease organism has to persist over time (i.e., there has to be an increase in larval / adult incidence of infection) before causing colony mortality, generally, *N. ceranae* acts simultaneously with other pathogens. Natural *N. ceranae* infestation can cause a sudden collapse of bee colonies and colony death in autumn or winter, and poor honey production and colony depopulation is six times higher in colonies infected with *N. ceranae* than in uninfected ones (Higes et al., 2008). A metagenomic survey for detection of various pathogens showed a prevalence of some *Nosema* spp. in CCD in contrast to non-CCD colonies (Cox-Foster et al., 2007). Moreover, the presence of more than one of the four pathogens – KBV, IAPV, *N. ceranae*, and *N. apis* – was observed in CCD colonies. The study on the connections between pathogens and CCD in collapsed colonies revealed an increase in the pathogen level in CCD colonies that was not observed in weak colonies (Cornman et al., 2012). The authors also found that in CCD colonies, *N. ceranae* loads were significantly correlated with the levels of DWV and KBV, which supports the association between *Nosema* spp. infestation and increased susceptibility to other pathogens.

Bicyclohexylammonium fumagillin, an antibiotic isolated from the fungus *Aspergillus fumigatus*, has been the only widely used treatment for nosemosis, or “nosema disease”, in western honey bees, *Apis mellifera* (Higes et al., 2011). The practice of periodic fumagillin treatment results in decreasing but nearly constant exposure of multiple generations of bees and pathogens to the drug. Although this practice appears to provide an environment conducive to selection of fumagillin-resistant *Nosema* strains, *N. apis* has evidently not developed resistance to the drug; however, studies have shown that *N. ceranae* can reestablish to pretreatment prevalence 6 months after treatments are terminated (Pajuelo et al., 2008). Some studies have indicated that protein profiles of bees fed fumagillin confirmed our hypothesis that fumagillin affects bee physiology at concentrations that no longer suppress *N. ceranae*. Thus, the use of fumagillin may

increase the prevalence of *N. ceranae* and is potentially a factor in replacement of *N. apis* by *N. ceranae* in USA apiaries (Huang et al., 2013). In addition to having negative effects on host physiology, fumagillin increases management costs, and residues may persist in the hive, posing risks to human health through honey consumption (van den Heever et al., 2015). Therefore, there is a need for alternatives to hard chemicals for *Nosema* spp. management.

### Synergistic effects of various diseases and parasites

The interaction of *Nosema* spp. and honey bee-associated viruses has been reported for bees co-infected with *N. ceranae*, CBPV or DWV. One study showed that co-infection of bees with *N. ceranae* and CBPV resulted in increased replication of CBPV but not mortality (Toplak et al., 2013). Costa et al. (2011) found a significant negative correlation between *N. ceranae* spore load and DWV titer in midgut tissues of workers bee.

An interesting study has evaluated co-parasitism with Varroa (*V. destructor*) and *Nosema* (*N. ceranae*/*N. apis*) on honey bees (*A. mellifera* L.) with different defense levels (Bahreini et al., 2015). The obtained results showed that high-mite-mortality-rate (high-MMR) of bees in the *Nosema* (-) group showed greater reductions in mean abundance of mites over time compared with low-mite-mortality-rate (low-MMR) bees, when inoculated with additional mites. However, high-MMR bees could not reduce mite load as well as in the *Nosema* (-) group when fed with *Nosema* spores. Mean abundance of *Nosema* spores in live bees and dead bees of both strains of bees was significantly greater in the *Nosema* (+) group. Molecular analyses confirmed the presence of both *Nosema* species in inoculated bees but *N. ceranae* was more abundant than *N. apis* and unlike *N. apis* increased over the course of the experiment. Collectively, this study showed differential mite mortality rates among different genotypes of bees, however, *Nosema* infection restrained Varroa removal success in high-MMR bees (Bahreini et al., 2015).

Other authors found significant colony level variation in infection levels, and subtle differences between the microbiota of colonies with high infection levels versus those with low infection levels (Rubanov et al., 2019). Two exact sequence variants of Gilliamella, a member of the honey bee “core gut microbiome” that has previously been associated with gut dysbiosis, were significantly more abundant in bees from colonies with high *Nosema* loads versus those with low *Nosema* loads.

### Stress of long-distance transportation of honey bee colonies for crop pollination

The stress of migratory beekeeping is also a risk factor for the health of bee colonies. For that reason, projects have been developed to test a year-round bee management scheme for large migratory and smaller non-migratory beekeeping

operations with an emphasis on the larger migratory operations that pollinate California almonds (almost half of all managed bees in the USA) (Pettis & Delaplane, 2010). For a more accurate analysis, observations need to be made in different regions because colony growth and disease epidemiology vary markedly in different parts of the country. The obtained data will provide an opportunity for evaluation of honey bee health between stationary versus migratory practices (Pettis & Delaplane, 2010).

It is well known that in most countries large numbers of hives are transported to multiple locations throughout the country by truck to pollinate seasonal fields and orchards. During transportation, colonies are challenged by a variety of stressors. The condition of a hive prior to transportation is often locally acclimated to ecological conditions which often differ greatly from those of the destination. They are moved between locations at interstate highway speeds and deployed in fields and orchards prior to the bloom. Changes in temperature, day length, and nutrient supplementation that bees experience after transportation can increase foraging activity and brood production earlier than would have occurred before relocation and in agricultural environments prior to floral bloom with low availability of resources (Fewell & Winston, 1992). Transportation has been described as a likely contributor to colony loss but the focus has been on changing forage quality and consistency, not stress endured during transportation (Oldroyd, 2007). Transportation stress has received less attention because of the difficulty of collecting data during shipping. Even though transportation lasts only a few days, colonies experience confinement, increased variation in temperature, air pressure, and vibration. During shipping, colonies experience a rapid progression of changing elevation and latitude. Proper ventilation is a primary concern because poorly ventilated colonies often die from overheating. The consequences of low-temperature stress are less obvious. A colony may experience extended periods of sub-lethal chill stress and loss of thermoregulation (LT) that affects long-term colony survival without proximate mortality by inducing developmental defects in new brood (Groh et al., 2004; Jones et al., 2005). Colonies have many possible locations on the trailer and may be oriented inward toward a center aisle or outward toward the road which may affect airflow, especially at interstate highway speeds.

### **Other factors influencing colony health and survival**

In addition to different diseases, there are some other factors that lead to colony losses. In certain cases, it is the interaction of these factors that leads to morbidity and mortality, and colony losses.

#### *Climate conditions*

Climate is a crucial factor affecting temperature and humidity. The humidity in the hives must be maintained as

low as possible, while the temperature of the brood must be maintained at 34 °C, and in winter the core temperature of the hive must not fall below 13 °C (Nürnberger et al., 2018). This is essential and honey bee colonies must have sufficient access to carbohydrates to maintain these temperatures and survive. Prolonged periods of cold or wet weather or the food source becoming depleted can also have a negative influence on honey bee colony health. It can inhibit the flying activity and interrupt nectar and pollen supplies to the hive. In contrast to low temperature, if the brood temperature rises above 34.5 °C, the bees display behavioral differences combined with learning and memory difficulties (Wang et al., 2016).

The effect of weather on bee colonies as a key factor in CCD has been reported in a survey of honey bee colony losses in the USA (VanEngelsdorp et al., 2010). CCD has been linked to changes in bee habitats and malnutrition, both of which are indirectly caused by climate change. In addition, climate change allows invasive species to take over bee hives, spoil stored food, and disrupt many processes within these hives, causing a further decline in bee populations (Memmott et al., 2007; Thomson, 2010).

#### *Habitat loss and landscape changes*

The loss and fragmentation of natural habitats resulting from urbanization or intensification of agriculture lead to the reduction of sources of alternative foraging for the honey bee, and the nesting places of wild bees – hollows and tree hollows, bushes, holes, caves and others (Goulson et al., 2008; Brown & Paxton, 2009). The process of reducing and degrading the terrain occupied by natural vegetation – grassy or tree, escalated in the 20<sup>th</sup> century and continues today. Growing honey bees in an urban environment is considered to have its advantages – alternative food sources throughout the season (parks, alleys, etc.) and a lower risk of pesticide intoxication. However, if the environment is highly urbanized, flowering vegetation may not be sufficient. In addition, there is a risk of man-made pollution as well as collision of flying bees with moving vehicles (Goulson et al., 2015). Pollinators, managed or wild, cannot escape the various and massive impacts of industrial agriculture: they suffer simultaneously from the destruction of natural habitats caused by agriculture, and, because pollinators' natural ranges inevitably overlap with industrial farming landscapes, the harmful effects of intensive agricultural practices (Kovács-Hostyánszki et al., 2016; Belsky & Joshi, 2019).

Fragmentation of natural and semi-natural habitats, expansion of monocultures and lack of diversity have a negative impact on honey bee health and survival (Patrício-Roberto et al., 2014; Rollin et al., 2016). Destructive practices that limit bee-nesting ability, and the spraying of herbicides and pesticides, industrial agriculture one of the major threats to pollinator communities globally. On the other hand, agriculture systems that work with biodiversity and without chemicals, such as ecological farming systems, can benefit

pollinator communities, both managed and wild (Földesi et al., 2016; Sponsler et al., 2019). By increasing habitat heterogeneity for bees, for example, ecological mixed-cropping systems can provide additional flower resources for pollinators. This emphasises the potential beneficial roles of ecological/organic agriculture methods.

Studies on the arrival of the bees to pollinate almond flowers in California orchards – primarily in five counties between Los Angeles and the San Francisco Bay Area – marks the start of a brief frenzy of activity. It is the world's largest pollination event. Depending on where your farm is located, some pollination strategies may be more appropriate than others. Wild bees are more often found in orchards near natural habitat (Potts et al., 2010; Lee et al., 2019). In these areas, maintaining natural habitat will be important. Growers with orchards far from habitat can diversify pollination strategies by using alternative managed bees, like the blue orchard bee, in addition to honey bees, and by adding flowering resources to support those managed bees and attract wild species.

#### *Diet and nutrition*

Feeding bees is often insufficient, due to overcrowding of the hives or irregular foraging, and in the conditions of prolonged cold and rainy weather, it is lacking (vanEngelsdorp et al., 2009). Feeding is deficient in areas with intensive agricultural production, where the so-called stress from a monotonous or “monocultural” diet is observed (Goulson et al., 2015). This refers to the continuous foraging of bees on crops in mass flowering, grown in large areas, such as sunflower or rapeseed, as well as acacia, where the purpose is to produce honey or just pollination of plants. Other factors related to insufficient nutrition include low-nutrient pollen and nectar, plant species including crops, flowers that contain natural but toxic to bee substances. This is the case for the amygdalin glycoside found in almond flowers (London-Shafir et al., 2003; UNEP, 2010).

It is well known that large quantities of food are required for the development and health status of honey bee colonies. Undoubtedly, the most important characteristics of food sources are regularity, quality and quantity of the nectar and pollen (Decourtye et al., 2011). Within intensively farmed agricultural landscapes, nectar- and pollen-producing crops may provide a narrow window with mass flowering followed by a shortage and even a complete lack of pollen and nectar resources. A typical example is observed with oilseed crops such as rape (*Brassica napus L.*) and sunflower (*Helianthus annuus L.*), where nectar and pollen resources are usually abundant during the blooming, but only for a short period. The subsequent temporal dearth of resources requires the creation of additional vegetation such as field margins (strips bordering crop fields), hedgerows (linear scrub along field boundaries), woodlands, ponds, ditches, and fallow farm fields (Decourtye et al., 2010).

The role of nectar and pollen for honey bee health and survival is indisputable, so beekeepers now provide

supplements in the form of syrup or pollen in case of deficiency. These cannot fully replace natural compounds in terms of nutritive value. Consequently, making sure that honey bees have access to pollen and nectar at the right time in their natural environment, remains the best way to guarantee colony survival (Decourtye et al., 2011). The analysis of the nutritional composition of bee bread (mixture of pollen and nectar or honey) has shown a close relation with local land use and therefore available floral resources (Donkersley et al., 2014). The finding that bee bread protein content correlates with land use suggests that landscape composition may have an impact on insect pollinators, as poor nutrition may contribute to the widespread and ongoing pollinator population decline by increasing the vulnerability to various stresses.

The role of nutrition in the immune response to viral pathogens transmitted by *V. destructor* has also been analyzed (DeGrandi-Hoffman & Chen, 2015). According to this study, the role of the nutritional value of pollen and nectar and the relationship between diet and immunity are crucial in determining individual immune response. Doubtlessly, improved nutrition can optimize colony growth and immune responses to honey bee-associated viruses and Varroa infestation

The influence of pollen nutrients on bee health has also been investigated in healthy and varroa-parasitized bees through Digital gene-expression (DGE) (Alaux et al., 2011). The obtained results have shown that pollen-induced molecular mechanisms have a positive influence on some immune genes expression, thus affecting longevity and the production of some antimicrobial peptides, which helps to increase the immune defense of honey bees. However, the negative impacts of varroa on the bee metabolism and immune functions cannot be overcome by pollen feeding. This demonstrates that varroa infestation is extremely virulent and difficult to control, probably due to the influence of the multiple viruses vectored by the mite.

A similar study investigated the influence of varroa infestation on immunological and nutritional status of the honey bee (Aronstein et al., 2012). It was observed that protein content was depressed and free amino acid content elevated in Varroa-infested pupae, suggesting that protein synthesis is impaired, which affects growth in honey bee. The relationship between the values of nutritional and immune-related indices was more complex, and the effects on the colony showed the reduced weight of pupae in colonies with high Varroa abundance.

#### *Beekeeping practices*

Specific peculiarities of beekeeping can be the direct cause or a supplement to the complex of stressors that can contribute to colony breakdown. These include artificial, unilateral feeding, antibiotics, acaricides and insecticides applied in the hive, exposure to adverse temperatures and temperature fluctuations, infections and parasites, overexploitation of bee products, unreliable sources of bees

and queens (Schierow et al., 2012; Capri & Marchis, 2013). One-sided selection of the honey bee results in genetic erosion in the species population and a lack of resistance to infectious diseases, mites, beekeeping acaricides applied in hives, etc. (Capri & Marchis, 2013; Johnson & Corn, 2015).

### GMOs

Soybean and cotton varieties, followed by corn, with genetically incorporated genes for insecticide synthesis and herbicide tolerance, were first introduced in the United States in 1996. In 2007, 113 million hectares in different parts of the world (EU is among the exceptions) were sown with genetically modified crops (USDA-Biotech Crop Data, 2009). With the expansion of the area planted with these crops, concerns have arisen about the safety of bees and other pollinators. Researchers have conducted a number of studies, involving dozens of plant species carrying Bt genes of *Bacillus thuringiensis* for resistance to insect pests. The results have been summarized by Johnson et al. (2010) and Johnson (2015). No evidence of a negative effect of genetically modified plants on the bees feeding on them has been established.

### Exposure to pesticides

In the past decades, beekeepers have begun to use agrochemical pesticides not only for many crops, but in forests and other environments for the control of insect pests (Moritz & Erler, 2016; Hladik et al., 2018). The exposure of bees to pesticides is through ingestion of residues found in the pollen and nectar of contaminated plants (crop plants or the weeds around the fields), which is why they pose the greatest danger to bees (Pilling et al., 2013; Al Naggar et al. 2015).

In recent years, the application of a new generation of pesticides – neonicotinoids – has been broadly discussed among the scientific and beekeeping communities. They are used worldwide and are widely applied for plant protection (crops, vegetables, and fruits), veterinary products, and biocides for invertebrate pest control in fish farming (Simon-Delso et al., 2015).

A large number of studies reflect the role of neonicotinoids as a factor leading to colony losses (Blacqui re et al., 2012; Goulson, 2013; Godfray et al., 2015). In general, the effects of neonicotinoid insecticides exposure may be summarized as: (1) loss of reproduction (brood) which threatens the existence of the colony (Decourtye & Devillers, 2010), (2) as neurotoxic agents they affect the mobility of bees by inducing symptoms such as knockdown, trembling, uncoordinated movements, hyperactivity, and tremors (Medrzycki et al., 2003; Colin et al., 2004), (3) influence on olfactory learning and memory (Decourtye et al., 2005), (4) increased overwintering bee colony losses (Faucon et al., 2005; Wood et al., 2020) etc.

In addition to their individual negative impact, pesticides and various pathogens may interact to have stronger negative effects on honey bee colonies. For instance, the combination

of the fungicides chlorothalonil and pyraclostrobin has been found to increase more than twice *N. ceranae* infection rates in bees that consumed greater quantities of them, suggesting that some fungicides have a stronger impact on bee health (Pettis et al., 2013). The interaction between *V. destructor* infestation and neonicotinoids has resulted in significantly reduced survival of long-lived winter honeybees (Straub et al., 2019). The exposure to the formamidine miticide amitraz increases mortality associated with viral infections (Fine et al., 2017; O’Neal et al., 2017), while synergistic interaction when bee larvae are exposed to clothianidin or the organophosphate dimethoate, in combination, negatively affects the survival and cellular responses in American foulbrood-infected honey bee larvae (L pez et al., 2017).

### Concluding Remarks

The recent investigations have reported an increase in colony losses in some regions and have prompted investment in more co-ordinated monitoring of bees and research into how pests and diseases, bee diversity, beekeeping practices and bee foraging environment are affecting bee vitality. In addition, land management and environmental conditions affect the availability and quality of food sources as well as the conditions in the hive. Effective management of bee colonies under changing situations is dependent on beekeeping practices and bee selection/breeding. All of these factors can impact on bee vitality and bees’ ability to deal with pests and diseases.

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### Author’s contributions

All authors have equally contributed to the idea of this review. P.H. and B.N. prepared the original draft with considerable contributions from R.S. All authors have substantially contributed to the writing of the final text. Moreover, all authors have read and agreed to the published version of the manuscript.

### Conflicts of interest statement

The authors declare that they have no conflict of interest.

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