Arthropod-Plant Interactions (2018) 12:57–64
DOI 10.1007/s11829-017-9553-1

FORUM PAPER

The immunological dependence of plant-feeding animals on their host's medical properties may explain part of honey bee colony losses

Erik Tihelka[1]

Received: 5 October 2015 / Accepted: 17 July 2017 / Published online: 31 July 2017

Abstract The honey bee (*Apis mellifera*) is an important pollinator of agricultural and horticultural crops, but also of wild flowers. The species has been facing declines in many areas of the world, the causes being identified as multifactorial. Recently, it has been theorised that some plant-dwelling animals may develop a dependence on the medicinal properties of their hots plant's secondary metabolites. Here, the question of honey bee self-medication using organic materials, namely propolis, nectar, honey, honeydew, pollen, wood, and algae for self-medication is addressed. Self-medication in honey bees is a largely unexplored area and thus a comprehensive overview of the field is provided. Prior studies suggest that recent honey bee colony declines are driven by decreased forage plant availability. The problem is expanded and it is suggested, that if honey bees developed a dependence on medical properties of some disappearing plants or materials, this could explain a part of the colony losses observed around the world. To date, convincing evidence points towards self-medication with honey and propolis. Bees also contact plant secondary metabolites, fatty acids, essential oils, and microorganisms that are active against the causative agents of American foulbrood, European foulbrood, nosemosis, chalkbrood, stonebrood, and varroasis. In the future, selected taxa of plants with medicinal properties may be planted to boost honey bee health without chemotherapy. Future directions of research are discussed.

Handling Editor: Guy Smagghe.

✉ Erik Tihelka
etihelka@seznam.cz

[1] The Prague British School, K Lesu 558/2, 142 00 Prague 4, Czech Republic

Keywords Immunological dependence · Non-immunological defence · Self-medication · Honey bee · *Apis mellifera* · Invertebrate medicine

Introduction

The honey bee (*Apis mellifera* L., 1758) is considered to be the most important pollinator of horticultural and agricultural crops (Abrol 2012). It has been estimated that the economic value of pollination of only agricultural crops represents €153 billion, or 9.5% of the total value of the global human food production (Gallai et al. 2009). In addition, honey bees produce commodities such as honey or royal jelly and provide a range of ecological services such as biodiversity maintenance that cannot be quantified economically. However, recently, this species has faced large scale declines (Ellis et al. 2010; Potts et al. 2010), the causes being challenging to identify (Neumann and Carreck 2010). It has been suggested that the reasons are multifactorial; however, pathogens and parasites probably play a key role. The most damaging pathogens include the bacteria *Paenibacillus larvae*, the causative agent of American foulbrood, *Melissococcus plutonius,* and some other bacteria associated with European foulbrood, several species of fungi that cause chalkbrood (*Ascosphaera apis*), stonebrood (*Aspergillus fumigatus, Aspergillus flavus, and Aspergillus niger*), and nosemosis (*Nosema apis, Nosema ceranae*). The ectoparasitic mite *Varroa destructor* is counted among the most important pests (Williams 2000). Most treatment methods today relay on chemotherapy that is, however, unsustainable from the long-term viewpoint, since it leaves residue in bee products (Mullin et al. 2010), and the treated pathogens or parasites may quickly develop resistance (Hubert et al. 2014).

Recently, a study describing host-specificity in the jumping spider *Lyssomanes viridis* was published. A higher hatching success on leaves of *Liquidambar styraciflua* than other sympatric species was demonstrated (Tedore and Johnsen 2015). The reason provided was that the jumping spider may be dependent on the medical properties of the secondary plant metabolites that assure a higher hatching success. The study thus theorised that some plant-dwelling animals become immunologically dependant on the medical properties of their host plant. Apart from that, the study also suggests that when the optimal host of *L. viridis* is removed, the species may face problems. Could a similar deprivation of medicinal plants occur in honey bees?

It is recognised that quality nutrition has a key effect on honey bee health (Somerville 2005). Malnourished bees have impaired immune system which can increase their susceptibility to disease (Alaux et al. 2010). They are also more susceptible to environmental stressors such as pesticide poisoning (Wahl and Ulm 1983). Both quality and diversity of pollen are required for optimal honey bee nutrition (Di Pasquale et al. 2013). The decrease in honey bee forage availability as a result of agricultural intensification has been linked to a honey bee declines (Naug 2009). Is it possible that honey bees are directly dependent on medical properties of the plants they forage on, similarly to the jumping spider *L. viridis*? If yes, could the higher honey bee colony losses correlated with the loss of cropland, pastureland, and rangeland (Naug 2009) be caused by the removal of plants that the bees are immunologically dependant on? While the role of adequate nutrition for honey bee health is relatively well-studied, much less is known about honey bee self-medication (Erler and Moritz 2015). Therefore, the first role of this article is to summarise our current knowledge on *A. mellifera* self-medication with plant-produced materials. The work then discusses how the removal of plants the bees may self-medicate on could lead to elevated colony losses.

Honey bee self-medication

Honey bees collect nectar, honeydew, pollen, and resins on plants and thus are subjected to a diversity of bioactive substances throughout their life. As bees bring these supplies into the hive, bioactive substances are then contacted by other members of the colony and stored. This would theoretically provide a framework of an efficient non-immunological system that many colony members come into contact with. Social immunity is characterised as individuals collectively responding to an infection by actions that benefit the colony (Cremer et al. 2007). Self-medication is defined by Simone-Finstrom and Spivak (2012) as "an individual responding to infection by ingesting or harvesting non-nutritive compounds or plant materials". So far, self-medication and several other aspects of non-immunological defence have been confirmed across several insect taxa including the woolly bear caterpillars, armyworms, *Drosophila* fruit flies, and monarch butterflies (Parker et al. 2011; Abbott 2014). An increasing body of research currently focuses on self-medication in bees. The issue is discussed more broadly by Erler and Moritz (2015).

Self-medication with Propolis

In case of *A. mellifera,* self-medication was convincingly proven in the case of propolis. It has been demonstrated that propolis has vast antimicrobial actions (for example Ertürk et al. 2014). The role of propolis in the social immunity of the honey bee is well understood; it has been shown that bee colonies that had their nests treated with propolis invest less energy into immunity, because the propolis decreased the bacterial load on the bees (Simone et al. 2009). Simone-Finstrom and Spivak (2012) demonstrated that in response to infection by the entomopathogenic fungus *A. apis,* more bees forage on resin. This collective response to infection indicates self-medication behaviour.

Self-medication with nectar and honey

Nectar has been shown to have antimicrobial effects (for example Sasu et al. 2010) and so it would not be surprising if this plant excretion had a role in honey bee self-medication. Indeed, honey bees infected with *Nosema* prefer to feed on honeys with high antimicrobial activities, which suggest self-medication behaviour (Gherman et al. 2014). Other less direct evidence for self-medication with honey includes the work of Erler et al. (2014) that demonstrated that some honeys show strong inhibitory actions against *P. larvae,* the causative agent of American foulbrood and some other bacteria that are associated with European foulbrood. In addition, it has also been shown that with changing plant nectar sources, the concentration of *P. larvae* spores changes as well (Olofsson and Vasquez 2008). The concentration of *P. larvae* spores probably oscillates in bee colonies with ample stores of honey (Bzdil 2010), which could have important epidemiological implications. It is important to note that the changes in *P. larvae* spore load may be explained by seasonal changes in nectar sources and availability. Nevertheless, these changes may also be accounted for by the different antimicrobial properties of honeys produced during the different times of the year.

Similar interesting results were reached with bumbles bees. It was recently shown that when *Bombus impatiens* feeds on nectar, it also consumes a number of plant secondary metabolites, namely alkaloids, terpenoids, and iridoid glycosides. These secondary metabolites decrease the spore load of the intestinal parasite *Crithidia bombi* (Manson et al. 2010; Richardson et al. 2015). Additional studies revealed that if bumble bees consume medicinal plant secondary metabolites at higher than normal concentrations, it reduces or completely eliminates the parasite load without negative impacts on the host (Anthony et al. 2015). This holds true for other bumblebee species as well. *B. terrestris* with high pathogen loads preferentially feed on nectar containing nicotine. Nicotine is known to alleviate *C. bombi* infections (Baracchi et al. 2015). However, these interactions are not quiet unequivocal, since they have been shown to depend heavily upon the interactions between the secondary metabolites, parasites, and environmental conditions (Thorburn et al. 2015). Table 1 provides an overview of phytochemicals found in nectar that are known to beneficially affect pathogen load in bumblebees. Similar studies remain to be conducted with honey bees.

A number of honeys show high levels of antimicrobial activity (Al-Waili 2004). The antimicrobial effects vary between different honey varieties. The antimicrobial activities of honey are probably influenced by its hydrogen peroxide content, acidity, osmolality, flavonoids, phenolic acids, and in some cases by the level of non-peroxide factors (Molan 1992; Wahdan 1998). Importantly, in many cases, the antimicrobial activity of honeys has been linked directly or indirectly to their botanical origin (Molan 1992). In theory, by planting herbs that produce antimicrobial honey, it should be possible to stimulate honey bee health.

Self-medication with pollen

Less evidence exists for self-medication of bees with plant-derived substances other than propolis and honey. The pollen bees collect displays broad spectrum antimicrobial activities against human pathogens as well as fungi pathogenic to bees (Kačániová et al. 2012). Rinderer et al. (1974) put forward the idea that honey bees intentionally collect pollen that may be active against *P. larvae*, the causative agent of American foulbrood. Since then, our understanding of bee foraging behaviour on medicinal plants did not provide any conclusive evidence for self-medication.

Bees may nevertheless self-medicate on pollen since it is a source of beneficial microorganisms, antimicrobial fatty acids and perhaps also essential oils. Stored pollen contains lactic acid bacteria that play a key role in honey bee health (Forsgren et al. 2010). It has also been suggested that in periods of pollen dearth and when supplied with pollen substitutes, bees do not have access to important microorganism which decompose harmful substances of plant origin (Kaznowski et al. 2005). Some fatty acids found in the pollen of various plants (Manning 2001) are active against important bee bacterial pathogens (namely *P. larvae* and *M. plutonius*) (Feldlaufer et al. 1993a, b; Hornitzky 2003). Bees may come in contact with these simply by touch, ingestion but also when the pollen is stored in cells, the fatty acids may theoretically leak out and sterilise the six adjacent cells (Tadman 2014). Apart from fatty acids, a number of plant extracts and essential oils that are found in plants have been shown to have antibacterial (Alippi et al. 1996; Fuselli et al. 2006; Flesar et al. 2010), antifungal (Boudegga et al. 2010; Kloucek et al. 2012), and miticidal activities (Isman 2000; Gashout and Guzmán-Novoa 2009). Given the activities of pollen components and plant extracts against important honey bee parasites and pathogens, the role of pollen in self-medication, although not particularly well studied, is probably greater than we currently imagine. Since some plants contain more substances useful for bee health than others, some authors recommend that beekeepers grow these plants to stimulate their colonies' health (Manning 2001; Tadman 2014). An overview of such plants is provided in Table 2.

Self-medication on wood

Much research focuses on nectar and pollen as the dominant sources for bee nutrition (Brodschneider and Crailsheim 2010). However, it is often overlooked that foragers often visit, for no clear reason, other substrates such as dead wood or sawdust (Ingle 1988). Some foraging honey bees can also be seen burrowing in soil (E. Tihelka,

Table 1 Phytochemicals in plant nectar known to control pathogen load in bumblebees (*Bombus* spp.)

Phytochemical	Examples of plants	References
Anabasine	*Nicotiana glauca*	Richardson et al. (2015), Anthony et al. (2015), Thorburn et al. (2015)
Catalpol	*Chelone glabra*	Richardson et al. (2015)
Gelsemine	*Gelsemium sempervirens*	Manson et al. (2010)
Nicotine	*Nicotiana glauca*	Richardson et al. (2015), Biller et al. (2015), Thorburn et al. (2015)
Tymol	*Tilia europea*	Richardson et al. (2015), Biller et al. (2015)

Table 2 An overview of plants that may produce pollen containing substances useful for honey bee self-medication

Plant	Comment
Antirrhinum sp.	High content of palmitic acid (Manning 2001)
Arctotheca calendula	High content of linoleic acid (Manning 2001)
Borage (*Borago officinalis*)	High content of antimicrobial fatty acids including linoleic, linolenic, oleic, and palmitic acids (Tadman 2014)
Oregano (*Origanum vulgare*)	Contains substances effective against *P. larvae* (Tadman 2014). In vitro, oregano extracts cause a high mortality of *Varroa* mites (Gashout and Guzmán-Novoa 2009). Extracts also show inhibitory actions against the causative agent of chalkbrood (Kloucek et al. 2012).
Common poppy (*Papaver rhoeas*)	High content of antimicrobial fatty acids, namely linolenic acid (Manning 2001)
Scabiosa ssp.	High content of antimicrobial fatty acids such as linolenic acid (Manning 2001). Very attractive to bees
Common thyme (*Thymus vulgaris*)	Extracts from thyme suppress chalkbrood (Boudegga et al. 2010) and display antimicrobial and miticidal activities (Fuselli et al. 2006; Gashout and Guzmán-Novoa 2009). Feeding *Nosema* infected bees with thymol extended their life and decreased their spore load (Costa et al. 2010). Thymol is added into commercially produced varroacides such as Apilife Var, Thymovar or Apiguard

Compiled according to Tihelka (2016)

pers. observ.). More curiously, adult bees have also been observed on cattle dung (Crailsheim pers.com.). The reason for this behaviour remained unexplained, until Stamets (2014) suggested that bees collect beneficial fungi when visiting wood. Indeed, dead and decaying wood, alongside other abovementioned materials, is a rich source of various microorganisms (Stokland et al. 2012). These fungi play a key in increasing bee longevity. The fungi species *Fomitopsis officinalis, Inonotus obliquus, Fomes fomentarius,* and *Ganoderma resinaceum* have been shown to reduce the bee´s viral load (Stamets 2015). This may be another novel example of self-medication in honey bees.

Before the genesis of modern beekeeping techniques some 200 years ago, bees had a relatively easy access to beneficial fungi for self-medication. Colonies lived several metres above ground in gradually decaying tree cavities (Seeley and Morse 1976), which would expose them to beneficial fungi on a daily basis. Moreover, before the intensification of forestry (Kokeš 1982), bees would be able to forage freely for logs of dead wood. However, today dead wood is so scarce that the existence of a number of species of saproxylic insects is threatened (Speight 1989). Most modern beekeepers today keep bees in well-ventilated beehives (Büdel 1957, Grziwa 1957), some are even made of plastic and other artificial materials instead of wood. In addition, placing beehives on the ground level profoundly impacts the colonies' mycobiota. Bee bread of colonies kept on the ground level had a lower load of *Aspergillus* spp. and *Penicillium* spp. that inhabit the growth of the pathogen *Ascosphaera apis*, the causative agent of chalkbrood, than colonies kept approximately 2–4 m above ground in semi-natural cavities (Royce et al. 2015). In, addition, the widespread use of fungicides in modern agriculture may also threaten the ability of bees to self-medicate on beneficial fungi (Yoder et al. 2013). All these factors would deprive bees of their natural source of self-medicaments.

Self-medication with honeydew

The self-medication potential of honeydew remains unexplored regarding honey bees. This is a pity, since in many regions, honeydew provides a major honey flow. Also, since with modern forestry practices the likelihood of honeydew contamination with chemicals harmful to bees seems minimal (Zahradník 2016) it can serve as a stable supply of pesticide-free feed. Nevertheless, some fragmented evidence suggests honey bee self-medication may be possible with honeydew. Some galls associated with aphids, the primary producers of honeydew, show antimicrobial activities (Yoram and Inbar 2011). In addition, dark honey produced from honey dew has a higher antimicrobial activity than other honeys (Molan 1992; Vorlová et al. 2005).

Self-medication with algae

Vigilant beekeepers have observed that their bees often don´t generally forage on water provided to them in clean containers but rather seek water from "dirty" ponds, ditches, swimming pools, or fountains. Often, these are overgrown with algae. As the bees take in water, they directly contact the algae biomass. The relationship between bees and algae remained unexplained until very recently.

Prášil et al. (2016) report that during several past years, some bees hived in apiaries throughout Třeboň, Czech Republic have been bringing green powder from their foraging flights. A closer examination showed that this green powder was in fact dried *Chlorella* algae the bees were collecting in a nearby biotechnology facility. The bees were actively foraging for the dried algae and storing it in their combs similarly to pollen. Subsequently, when the biotechnology facility was repaired, the bees were prevented from having access to the dried algae. This has drastically reduced the honey yield of the colonies. The authors suggest that algae may have acted as an important source of nutrients for the bees. Preliminary studies have also shown that the algae of the genus *Chlorella* are active against *P. larvae,* the causative agent of American Foulbrood and a major cause of colony mortality in the region. It remains to be investigated whether honey bees are able to self-medicate on algae under natural field conditions.

Honey bee declines as a result of forage loss

As eusocial insects, honey bees rely more heavily on social immunity and have a less developed immune system on the individual level (Riessberger-Gallé et al. 2015). This is supported by the fact that the honey bee genome only contains one-third of genes encoding for immune defence effector proteins when compared to *Anopheles* or *Drosophila* (Evans et al. 2006). That implies that if honey bee social immunity was somehow limited, this could have significant negative effects on the colony health. The decline of bee forage and dead wood in agricultural landscape (Williams and Carreck 2014) may have a negative effect on the bee's ability to gather enough resources for self-medication. Indeed, honey bee colonies located in areas with more bee forage have been shown to have a higher honey yield (Naug 2009) which may be linked to their health status, as is discussed above.

It is known that diverse pollen sources affect bee physiology, tolerance to parasites (Di Pasquale et al. 2013), immunity (Alaux et al. 2010) and the colony health overall (reviewed in Brodschneider and Crailsheim 2010). In here, it is suggested that with the disappearing bee forage, the bees have less possibilities to self-medicate and thus are more susceptible to infections. Further research will of course be needed to verify these assumptions.

Conclusion and discussion

Self-medication using various plant materials has been demonstrated in several insect taxa (Abbott 2014). In honey bees, an increased number of bees forage on plant resins to fight infection with entomopathogenic fungi (Simone-Finstrom and Spivak 2012). Bees infected with gut parasites also preferentially feed on honeys that decrease the parasite load (Gherman et al. 2014). Apart from these two examples, direct experimental evidence lacks for honey bee self-medication on other plant-derived materials such as pollen or honeydew although there is considerable evidence suggesting that this occurs. Self-medication also seems likely in bees foraging for wood and algae. Because honey bees rely heavily on social immunity (Evans et al. 2006) and their forage is disappearing as a result of changes in agricultural practices (Naug 2009; Williams and Carreck 2014) it is herein suggested that this may lead to negative effects on honey bees. If the ability of honey bees to self-medicate dropped due to limited forage availability this would lead to a decreased ability of bees to fight infections.

Wax combs probably play a key role as a reservoir of substances bees may self-medicate on. It can be expected that plant secondary metabolites, fatty acids, beneficial microorganisms, and others accumulate in bee combs throughout time. This agrees with older studies that identified a diversity of chemical substances in beeswax including some fatty acids (reviewed by Tulloch 1970). Other studies demonstrated an inhibitory action of wax combs against *P. larvae* (Lavie 1960) and *Aspergillus flavus, A. fumigatus and A. niger*, fungi associated stonebrood (Kačániová et al. 2012). As such, bee colonies with older wax combs should be less susceptible to disease. This is in contrary to the prevailing beekeeping practices where old combs are frequently replaced with new ones. However, the wax comb is a dynamic environment that is often contaminated with agrochemicals and miticides (Mullin et al. 2010) and so relations between comb age and colony health may not be straightforward. Further studies should determine the persistence of fatty acids and other plant metabolites in combs and their interactions with pesticide residues.

A finding of great significance is that some plants may be more useful for self-medication than others. The antimicrobial effects of honey are significantly affected by its botanical origin (Molan 1992). Also, pollen of some plants has a higher content of antimicrobial fatty acids effective against the causative agents of some dangerous bee diseases, namely American and European foulbrood (Manning 2001; Manning and Harvey 2002). This may not be a mere coincidence, since it was shown that honey bees feed preferentially on plants whose pollen has a high lipid content (Singh et al. 1999). This has inspired some authors to create lists of plants on which bees can restore their health (Manning 2001; Tadman 2014) (Table 2). If future research verifies the importance of these taxa for bees, they could then be planted for bees to self-medicate on. As such, the beekeeping industry would no longer need to rely so heavily on chemotherapy. Cultivating diverse flower mixtures would not only benefit bees but also other invertebrates (Haaland et al. 2011).

Future research will also need to not only look more closely at the beneficial effects of honey bee forage but also its negative influences. Some minerals may, under certain circumstances, lead to honey bee paralysis (Horn 1985). Also, anecdotal observations indicate that palmitic acid (shows antimicrobial actions against the causative agents of bee diseases) is toxic to the queen bee when over 75 ml is fed to a colony (Somerville 2005). In addition, a few species of plants produce toxic nectar (Adler 2000) that may under certain circumstances lead to the production of toxic honey (Goodwin 2013). It is especial noteworthy, that while the phytochemicals in the nectar of the yellow jasmine (*Gelsemium sempervirens*) can reduce the pathogen load in bumble bees (Manson et al. 2010), it can also be toxic to *A. mellifera* (Brown 1879). It is clear that honey bee forage does not only have positive influences.

Some may suggest that the most prominent cause of colony collapse is the loss of food sources as a whole. By restricting the access of bees to food, their immunity is reduced which may arguably have a much greater impact than the mere absence of materials important for self-medication. This claim seems to be supported by the fact that relatively little plant taxa are known that contain substances important for self-medication (Tables 1, 2). However, this may be more because only a handful of taxa were studied in this regard. Eusocial insects may come into contact with hundreds plant species each year, and any (or every) of these plants may be crucial for self-medication.

Given the results of the studies cited in the present text, it is very likely that the bees at least partially rely on plant-derived products and other materials for self-medication. It now remains to be experimentally tested whether bees react to infection by increased collection of these substances. It is equally possible that honey bees simply do not self-medicate on substances other than propolis and honey. If nothing, the present work describes a testable hypothesis and a further avenue of research that promises new interesting discoveries in the field of pollinator-plant interactions and invertebrate medicine.

Acknowledgements I am grateful to four anonymous reviewers for their comments that considerably improved the manuscript.

Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

References

Abbott J (2014) Self-medication in insects: current evidence and future perspectives. Ecol Entomol. doi:10.1111/een.12110

Abrol DP (2012) Honey bee and crop pollination. In: Abrol DP (ed) Pollination biology, 1st edn. Springer, Dordrecht, pp 85–110

Adler LS (2000) The ecological significance of toxic nectar. Oikos. doi:10.1034/j.1600-0706.2000.910301.x

Alaux C, Ducloz F, Crauser D, Le Conte Y (2010) Diet effects on honeybee immunocompetence. Biol Lett. doi:10.1098/rsbl.2009.0986

Alippi AM, Ringuelet JA, Cerimele EL, Re MS, Henning CP (1996) Antimicrobial activity of some essential oils against *Paenibacillus larvae*, the causal agent of American foulbrood disease. J Herbs Spices Med Plants. doi:10.1300/J044v04n02_03

Al-Waili NS (2004) Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. J Med Food. doi:10.1089/1096620041224139

Anthony WE, Palmer-Young EC, Leonard AS, Irwin RE, Adler LS (2015) Testing dose-dependent effects of the nectar alkaloid anabasine on trypanosome parasite loads in adult bumble bees. PLoS ONE. doi:10.1371/journal.pone.0142496

Baracchi D, Brown MJ, Chittka L (2015) Weak and contradictory effects of self-medication with nectar nicotine by parasitized bumblebees. F1000Research. doi:10.12688/f1000research.6262.3

Biller OM, Adler LS, Irwin RE, McAllister C, Palmer-Young EC (2015) Possible synergistic effects of thymol and nicotine against *Crithidia bombi* parasitism in bumble bees. PLoS ONE. doi:10.1371/journal.pone.0144668

Boudegga H, Boughalleb N, Barbouche N, Ben Hamouda MH, Mahjoub ME (2010) In vitro inhibitory actions of some essential oils on *Ascosphaera apis*, a fungus responsible for honey bee chalkbrood. J Apic Res. doi:10.3896/IBRA.1.49.3.02

Brodschneider R, Crailsheim K (2010) Nutrition and health in honey bees. Apidologie. doi:10.1051/apido/2010012

Brown JPH (1879) Bee forage in the south. Am Bee J 15:500–502

Büdel A (1957) Fysikální pochody výměny vzduchu v česně. Odborné překlady 3:24–30

Bzdil J (2010) Occurrence of *Paenibacillus larvae* in bee debris and honey. Veterinářství 8:469–472

Costa C, Lodesani M, Maistrello L (2010) Effect of thymol and resveratrol administered with candy or syrup on the development of Nosema ceranae and on the longevity of honeybees (Apis mellifera L.) in laboratory conditions. Apidologie. doi:10.1051/apido/2009070

Cremer S, Armitage SA, Schmid-Hempel P (2007) Social immunity. Curr Biol. doi:10.1016/j.cub.2007.06.008

Di Pasquale G, Salignon M, Le Conte Y, Belzunces LP, Decourtye A, Kretzschmar A, Suchail S, Brunet J-L, Alaux C (2013) Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter. PLoS ONE. doi:10.1371/journal.pone.0072016

Ellis JD, Evans JD, Pettis J (2010) Colony losses, managed colony population decline, and colony collapse disorder in the United States. J Apic Res. doi:10.3896/IBRA.1.49.1.30

Erler S, Moritz RFA (2015) Pharmacophagy and pharmacophory: mechanisms of self-medication and disease prevention in the honeybee colony (*Apis mellifera*). Apidologie. doi:10.1007/s13592-015-0400-z

Erler S, Denner A, Bobiş O, Forsgren E, Moritz RF (2014) Diversity of honey stores and their impact on pathogenic bacteria of the honeybee, *Apis mellifera*. Ecol Evol. doi:10.1002/ece3.1252

Ertürk Ö, Yavuz C, Sirali R (2014) The antimicrobial activity of propolis from Ordu province of Turkey. Mellifera 14:11–16

Evans JD, Aronstein K, Chen YP, Hetru C, Imler JL, Jiang H, Kanost M, Thompson GJ, Zou Z, Hultmark D (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Mol Biol. doi:10.1111/j.1365-2583.2006.00682.x

Feldlaufer MF, Lusby WR, Knox DA, Shimanuki H (1993a) Isolation and identification of linoleic acid as an antimicrobial agent from the chalkbrood fungus, *Ascosphaera apis*. Apidologie. doi:10.1051/apido:19930201

Feldlaufer MF, Lusby WR, Knox DA, Shimanuki H (1993b) Antimicrobial activity of fatty acids against *Bacillus larvae*, the causative agent of American foulbrood disease. Apidologie. doi:10.1051/apido:19930202

Flesar J, Havlik J, Kloucek P, Rada V, Titera D, Bednar M, Stropnicky M, Kokoska L (2010) In vitro growth-inhibitory effect of plant-derived extracts and compounds against *Paenibacillus larvae* and their acute oral toxicity to adult honey bees. Vet Microbiol. doi:10.1016/j.vetmic.2010.03.018

Forsgren E, Olofsson TC, Vásquez A, Fries I (2010) Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae. Apidologie. doi:10.1051/apido/2009065

Fuselli SR, de la Rosa SBG, Gende LB, Eguaras MJ, Fritz R (2006) Antimicrobial activity of some Argentinean wild plant essential oils against *Paenibacillus larvae larvae*, causal agent of American foulbrood (AFB). J Apic Res. doi:10.1080/00218839.2006.11101304

Gallai N, Salles JM, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ. doi:10.1016/j.ecolecon.2008.06.014

Gashout HA, Guzmán-Novoa E (2009) Acute toxicity of essential oils and other natural compounds to the parasitic mite, *Varroa destructor*, and to larval and adult worker honey bees (*Apis mellifera* L.). J Apic Res. doi:10.3896/IBRA.1.48.4.06

Gherman BI, Denner A, Bobiş O, Dezmirean DS, Mărghitaş LA, Schlüns H, Erler S (2014) Pathogen-associated self-medication behavior in the honeybee *Apis mellifera*. Behav Ecol Sociobiol. doi:10.1007/s00265-014-1786-8

Goodwin M (2013) A New Zealand history of toxic honey. Lulu.com, Raleigh

Grziwa J (1957) Co se odehrává v česně v zimě. Odborné překlady 3:27–29

Haaland C, Naisbit RE, Bersier LF (2011) Sown wildflower strips for insect conservation: a review. Insect Conserv Diver. doi:10.1111/j.1752-4598.2010.00098.x

Horn H (1985) The causes of black disease of the honey bee. I. The influence of mineral content in honeydew honeys. Apidologie. doi:10.1051/apido:19850204

Hornitzky MAZ (2003) Fatty acids-an alternative control strategy for honeybee diseases: a report for the rural industries research and development corporation. Rural Industries Research and Development Corporation, Barton

Hubert J, Nesvorna M, Kamler M, Kopecky J, Tyl J, Titera D, Stara J (2014) Point mutations in the sodium channel gene conferring tau-fluvalinate resistance in *Varroa destructor*. Pest Manag Sci. doi:10.1002/ps.3679

Ingle S (1988) Mycotopia: Paul Stamets is perfecting the art of mushroom cultivation. Harrowsmith Magazine 3(15):68–73

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot. doi:10.1016/S0261-2194(00)00079-X

Kacániová M, Vuković N, Chlebo R, Haščík P, Rovna K, Cubon J, Pasternakiewicz A (2012) The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia. Arch Biol Sci. doi:10.2298/ABS1203927K

Kaznowski A, Szymas B, Jazdzinska E, Kazimierczak M, Paetz H, Mokracka J (2005) The effects of probiotic supplementation on the content of intestinal microflora and chemical composition of worker honey bees (*Apis mellifera*). J Apicu Res. doi:10.1080/00218839.2005.11101139

Kloucek P, Smid J, Flesar J, Havlik J, Titera D, Rada V, Drabek O, Kokoska L (2012) In vitro inhibitory activity of essential oil vapors against *Ascosphaera apis*. Nat Prod Commun 7:253–256

Kokeš O (1982) Vývoj lesního hospodářství na Benešovsku od konce 19. století. Sborník vlastivědných prací z Podblanicka 23:21–32

Lavie P (1960) Les substances antibactériennes dans las colonie d'abeilles (*Apis mellifica* L.). Ann. Abeille 3:103–183

Manning R (2001) Fatty acids in pollen: a review of their importance for honey bees. Bee World. doi:10.1080/0005772X.2001.11099504

Manning R, Harvey M (2002) Fatty acids in honeybee-collected pollens from six endemic Western Australian eucalypts and the possible significance to the Western Australian beekeeping industry. Aust J Exp Agr. doi:10.1071/EA00160

Manson JS, Otterstatter MC, Thomson JD (2010) Consumption of a nectar alkaloid reduces pathogen load in bumble bees. Oecologia. doi:10.1007/s00442-009-1431-9

Molan PC (1992) The antibacterial activity of honey 2. Variation in the potency of the antibacterial activity. Bee World. doi:10.1080/0005772X.1992.11099118

Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, Pettis JS (2010) High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS ONE. doi:10.1371/journal.pone.0009754

Naug D (2009) Nutritional stress due to habitat loss may explain recent honeybee colony collapses. Biol Conserv. doi:10.1016/j.biocon.2009.04.007

Neumann P, Carreck NL (2010) Honey bee colony losses. J Apic Res. doi:10.3896/IBRA.1.49.1.01

Olofsson TC, Vasquez A (2008) Detection and identification of a novel lactic acid bacterial flora within the honey stomach of the honeybee *Apis mellifera*. Curr Microbiol. doi:10.1007/s00284-008-9202-0

Parker BJ, Barribeau SM, Laughton AM, de Roode JC, Gerardo NM (2011) Non-immunological defense in an evolutionary framework. Trends Ecol Evol. doi:10.1016/j.tree.2011.02.005

Potts SG, Roberts SP, Dean R, Marris G, Brown JR, Neumann P, Settele J (2010) Declines of managed honey bees and beekeepers in Europe. J Apicu Res. doi:10.3896/IBRA.1.49.1.02

Prášil O, Urajová P, Krištůfek V, Frydrychová R (2016) Včely a řasy. Moderní včelař 13:23–24

Richardson LL, Adler LS, Leonard AS, Andicoechea J, Regan KH, Anthony WE, Irwin RE (2015) Secondary metabolites in floral nectar reduce parasite infections in bumblebees. P R Soc B. doi:10.1098/rspb.2014.2471

Riessberger-Gallé U, López JH, Schuehly W, Crockett S, Krainer S, Crailsheim K (2015) Immune responses of honeybees and their fitness costs as compared to bumblebees. Apidologie. doi:10.1007/s13592-014-0318-x

Rinderer TE, Rothenbuhler WC, Gochnauer TA (1974) The influence of pollen on the susceptibility of honey-bee larvae to *Bacillus larvae*. J Invertebr Pathol. doi:10.1016/0022-2011(74)90100-1

Royce L, Yoder J, Nelson B, Lorenz A (2015) Tree hive colonies. Bee Culture

Sasu MA, Wall KL, Stephenson AG (2010) Antimicrobial nectar inhibits a florally transmitted pathogen of a wild *Cucurbita pepo* (Cucurbitaceae). Am J Bot. doi:10.3732/ajb.0900381

Seeley TD, Morse RA (1976) The nest of the honey bee (*Apis mellifera* L.). Insectes Soc. doi:10.1007/BF02223477

Simone M, Evans JD, Spivak M (2009) Resin collection and social immunity in honey bees. Evolution. doi:10.1111/j.1558-5646.2009.00772.x

Simone-Finstrom MD, Spivak M (2012) Increased resin collection after parasite challenge: a case of self-medication in honey bees. PLoS ONE. doi:10.1371/journal.pone.0034601

Singh S, Saini K, Jain KL (1999) Quantitative comparison of lipids in some pollens and their phagostimulatory effects in honey bees. J Apic Res. doi:10.1080/00218839.1999.11100999

Somerville D (2005) Fat bees skinny bees—a manual of honey bee nutrition for beekeepers. Rural Industries Research and development Corporation, Goulburn

Speight MCD (1989) Saproxylic invertebrates and their conservation. Nat Environ Ser 42:5–56

Stamets P (2014) Report from the underground—Bioneers. Youtube https://www.youtube.com/watch?v=DAw_Zzge49c. Accessed 4 Nov 2016

Stamets PE (2015) U.S. Patent Application No. 14/641,432

Stokland JN, Siitonen J, Jonsson BG (2012) Biodiversity in dead wood. Cambridge University Press, Cambridge

Tadman J (2014) Medical herbs for bees—an experiment. The Australasian Beekeeper

Tedore C, Johnsen S (2015) Immunological dependence of plant-dwelling animals on the medicinal properties of their plant substrates: a preliminary test of a novel evolutionary hypothesis. Arth-Plant Int. doi:10.1007/s11829-015-9386-8

Thorburn LP, Adler LS, Irwin RE, Palmer-Young EC (2015) Variable effects of nicotine, anabasine, and their interactions on parasitized bumble bees. F1000Research. doi:10.12688/f1000research.6870.2

Tihelka E (2016) Teorie samoléčení včel: polní experiment let 2012–2020. VIII. setkání uživatelů Varroamonitoring systému, Brno

Tulloch AP (1970) The composition of beeswax and other waxes secreted by insects. Lipids. doi:10.1007/BF02532476

Vorlová L, Karpíšková R, Chabinioková I, Kalábová K, Brázdová Z (2005) The antimicrobial activity of honeys produced in the Czech Republic. Czech J Anim Sci 50:369

Wahdan HAL (1998) Causes of the antimicrobial activity of honey. Infection. doi:10.1007/BF02768748

Wahl O, Ulm K (1983) Influence of pollen feeding and physiological condition on pesticide sensitivity of the honey bee *Apis mellifera* carnica. Oecologia. doi:10.1007/BF00388082

Williams DL (2000) A veterinary approach to the European honey bee (*Apis mellifera*). Vet J. doi:10.1053/tvjl.2000.0474

Williams I, Carreck NL (2014) Land use changes and honey bee forage plants. In: Matheson A, Carreck NL (eds) Forage for pollinators in an agricultural landscape, 1st edn. International Bee Research Association, Milton Keynes, pp 7–20

Yoder JA, Jajack AJ, Rosselot AE, Smith TJ, Yerke MC, Sammataro D (2013) Fungicide contamination reduces beneficial fungi in bee bread based on an area-wide field study in honey bee, *Apis mellifera*, colonies. J Toxicol Env Health A. doi:10.1080/15287394.2013.798846

Yoram G, Inbar M (2011) Distinct antimicrobial activities in aphid galls on *Pistacia atlantica*. Plant Signal Behav. doi:10.4161/psb.6.12.18031

Zahradník P (2016) Může používání přípravků na ochranu rostlin v lesním hospodářství negativně ovlivnit včely? Moderní včelař 13:14–18