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Introduction

The recent 30% average decline of managed colonies of western honey bees (*Apis mellifera*), mainly in the northern hemisphere, is a multifactorial problem believed to be caused principally by *Varroa destructor* and its vectored viruses (Torres, Ricoy, & Roybal, 2015), among a multitude of other stressors. Pesticides in the environment, particularly acaricides added directly into the hive, can accumulate in bee products, especially beeswax, and may affect bees' capacity to fight disease (O'Neal, Anderson, & Wu-Smart, 2018). Health risks to consumers should also be considered when residue carry-over from contaminated beeswax into stored honey can occur (Wilmart et al., 2016). Consumers can be exposed to residues when contaminated beeswax is used as a food additive, and as a pharmaceutical or cosmetic ingredient. Preliminary research has demonstrated the efficacy of heat on reducing *Varroa* populations (Bičík, Vagera, & Sádovská, 2016; Goras et al., 2015; Tihelka, 2016) and of ozone as a potential disinfectant and pesticide decontaminant of beekeeping equipment (James, 2011; James, Ellis, & Duehl, 2013). These two economical and eco-responsible emerging alternatives to conventional chemotherapy used to treat honey bees and equipment are reviewed in this article.

Hyperthermia as Pest Control

Hyperthermia as a method to fight *Varroa* has been documented since the 1970s. It is based on the fact that mites are more sensitive to high temperature than are bees. Temperatures from 40–47°C for different lengths of time lower *Varroa* populations without affecting the bee population (Ahmad, 1988; Harbo, 1994; Rosenkranz, Aumeier, & Ziegelmann, 2010). However, hyperthermia has since received little research attention, possibly because (1) most of the related experiments were made at the time when the decline of honey bee populations was less severe, (2) chemical products were cheap, quick, and simple to use, (3) problems of residue accumulation in beeswax were negligible, and (4) *Varroa*'s resistance to miticides had not reached a critical level. It could also be that early technology was complicated to handle and/or did not provide accurate temperature control. Currently, there are at least a dozen of commercially available heat devices marketed against *Varroa*. Although not all of them have had proper scientific validation, they all claim between 50 and 100% efficacy at reducing *Varroa* populations (Bičík et al., 2016; Goras et al., 2015; Tihelka, 2016).

It is also possible that hyperthermia can be used against other colony pests/pathogens, such as small hive beetles (SHB *Aethina tumida*) for example. Thermal sensitivity has been studied for SHBs (Meikle & Patt, 2011) and also the opportunistic pest, the greater wax moth *Galleria mellonella* (Warren & Huddleston, 1962). In fact, Hemmerlé (2017) demonstrated that a temperature of up to 47°C during over one hour effectively eliminated all development phases of the wax moth from the combs without compromising the waxy structure. Similar to *Varroa*, SHBs and wax moths are more thermosensitive than are bees (Table 1).

Bees are capable of achieving thermal homeostasis even during extreme temperatures. For example, the dark European honey bee (*Apis mellifera mellifera*), in its wide geographical range from the polar circle to southern France, tolerates temperatures from −40°C to 40°C (Ruttner, 1988). The endothermic heat production of the colony and the insulation of the breeding cavity allow the bees to regulate the brood nest temperature within the range of 32°C to 36°C and to survive cold winters (Stabentheiner, Kovac, & Brodschneider, 2010). Tolerance to extreme heat has been demonstrated by honey bees (Elekonich, 2009) and even on solitary bees (Erenler et al., 2016).

Endothermic heat production is a natural way to fight pests and demonstrated to be particularly developed in guard bees of the Asian honey bee (*Apis cerana*). However, given that all honey bees have evolved from the same origin, they share some common genetic and behavioral mechanisms that may influence colony defense (Nouvian, Reinhard, & Giurfa, 2016). This suggests that the *Apis* genus may have the capacity to utilize heat as a key defense strategy to fight different threats such as parasites, predators, and pathogens.

Heat Protects Bees from Varroa

The Asian honey bee (*A. cerana*) is the natural host of the ectoparasite *Varroa*. One mechanism this bee uses to combat mites is increasing the temperature of the worker brood to levels above 35.5°C, at which all the stages of the mite are unable to develop. However, the drone brood is not heated above 35.5°C by the bees, seemingly to protect drone fertility, resulting in them being the only real host for *Varroa* reproduction in *A. cerana* colonies (Erickson, Page, & Hanna, 2000). Other species of honey bees have not developed similar responses to *Varroa*. Nevertheless, there are examples where applied hyperthermia contribute to the control of the mites. In Pakistan, Ahmad (1988) demonstrated that when replacing the hive roofs of infested colonies with glass covered by a black cloth, significant *Varroa* mortality occurred after three, 30-min treatments. During those assays, the temperature inside the colony rose to 45–47°C within 15–20 min when the ambient temperature was 41–43°C. In European honey bees, Le Conte, Arnold, and Desenfant (1990) demonstrated that temperature spikes were unfavorable to the development of the mites, suggesting that the regulation of brood temperature by bees and occasional temperature spikes may be key factors of resistance of honey bees to *Varroa*. The optimal temperature for mite development is between 32.5 and 33.4°C. This corresponds to the brood temperature of honey bee nests. Above 36.5°C, *Varroa* populations decrease significantly. Above

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Table 1. Maximum temperature tolerance of the adult honey bee and its pests.

Honey bees[a]	Small hive beetles[b]	*Varroa*[c]	Wax moth[d]
~48 °C	~35 °C	~40 °C	~47 °C[@]
Proposed standard thermic treatment: 42–45 °C for 2–3 h*			

*Humidity, CO_2, and time of exposure are also important factors to consider.

[a, b, c]All stages affected. [@]Ideal temperature to eliminate all stages of the wax moth is 47 °C during one hour in unoccupied or stored beekeeping equipment, however, 45 °C can kill adult wax moths.

[a]Hoppe and Ritter (1986); [b]Meikle and Patt (2011); [c]Pätzold and Ritter (1989); [d]Warren and Huddleston (1962).

38°C, mites begin to die without reproducing (Le Conte et al., 1990).

Heating the colony affects the viability of phoretic *Varroa* similarly to that of heating *Varroa* while they are in the brood. For the brood, heat does not present an apparent effect on emergence rates but rather on the survival rate, with younger brood (9–10 days old) being the most sensitive to heat (Appel & Büchler, 1991). Several studies describe the varrocidal effect of heat; one in particular (Bičík et al., 2016) mentions a study on 50 colonies over the course of three years that demonstrated that hyperthermia is an equally successful alternative to chemical treatment against *Varroa*. The colonies remained vital and capable of generating good yields of high-quality honey.

Heat Protects Bees from Predators

Asian honey bees kill hornets by balling them (clustering tightly around them) and raising the temperature inside the "ball" (Ugajin et al., 2012). They possibly evolved this strategy because six species of hornets are known to attack them (Nouvian et al., 2016). This defensive mechanism is less known in non-Asian honey bees but seems to be present nevertheless. There are, however, new reports of honey bees that utilize heat as a defense mechanism to fight relatively new predators, as observed in France, where European honey bees have been shown to ball the introduced Asian hornet, *Vespa velutina*, when attacked (Monceau et al., 2018). French bees have been exposed to the Asian hornet since 2004, when it was first introduced in Europe (Arca et al., 2015). Thus, it could be that during these 14 years of exposure, French bees may have started to express thermal defensive responses against the Asian hornet. This is not surprising, given that there are reports of bee balling by the Italian bee, *A. m. ligustica*, of *V. crabro*, a native wasp predator (Baracchi, Cusseau, Pradella, & Turillazzi, 2010). *A. m. cypria* (the Cyprian bee) also thermally balls *V. orientalis*. Similar to Asian bees, Cyprian bees block the hornet's respiration by inhibiting the pumping movements of its abdomen while also increasing the wasp's temperature (Papachristoforou et al., 2007). Stabentheiner, Kovac, and Schmaranzer (2007) demonstrated increases in thorax temperatures of individual bees to 37°C after aggressive interactions with wasps and to 38.6°C following attacks on humans. Correspondingly, heat generation may be a result of honey bee defensive behavior, with the added benefit that it, too, may impact pests.

Heat Protects Bees from Pathogens

Western honey bees have been shown to be capable of raising their nest temperature as much as 20% in response to pathogen infection. This fever-like response has been demonstrated against chalk brood, *Ascosphaera apis* (Starks, Blackie, & Seeley, 2000). In this case, the fever response was apparently preventing infection, suggesting that either honey bee workers detect the infection before signs of disease are visible, or that larvae communicate the ingestion of the pathogen. Similarly, experimental *N. ceranae* infection in honey bees resulted in low thoracic temperatures, with infected bees preferring to inhabit the densely populated central part of the nest, where the temperature was approximately 3°C warmer than at the edges (Campbell, Kessler, Mayack, & Naug, 2010). However, it was not clear if these temperature changes were an active response of the host to the pathogen or, in contrast, pathogen-driven. Altogether, those reports may provide an indication that hyperthermia could be an appropriate strategy to help honey bees cope with various biological threats they face.

Ozone as a Sustainable Sanitation Technology

Given the powerful oxidizing capacity of gaseous ozone, and the fact that it can easily diffuse and spread throughout many surface types (unlike aqueous disinfectants, which are restricted to the surfaces to which they are applied), it is being used with increasing frequency as a disinfectant and decontaminant in the food industry and in medicine (Tsukamoto et al., 2016). Ozone is often more effective than other disinfectants, while not leaving any harmful residues (Martinelli, Giovannangeli, Rotunno, Trombetta, & Montomoli, 2017; Wysok, Uradzinski, & Gomolka-Pawlicka, 2006). The mode of action of ozone is very simple. Being an allotropic form of oxygen, atoms are separated after an electrical discharge, gathered in unstable trios, thus becoming a natural oxidant that can act as a total biocide capable of killing arthropods, microorganisms, and spores, as well as reducing chemical residues. After being generated, ozone completely reduces to O_2 again, therefore not posing a risk to the environment (Figure 1). Gaseous ozone is capable of reaching surfaces such as the wax comb cells and has been tested as a hive decontaminant with varied success (James et al., 2013).

Ozone as a Disinfectant

Ozone technology has been used to reduce exposure to infective agents and control pest problems in a variety of settings. Common examples of ozone use are water decontamination and treatment of fruits and vegetables to increase their shelf life by killing food-borne pathogens and pests. Water enriched with ozone is often used instead of traditional washing and disinfecting agents (Wysok et al., 2006). Ozone fumigation can reduce pathogen loads in used combs, given that gaseous ozone ensures full surface sterility (James, 2011). James (2011) further demonstrated the efficacy and practical application of ozone in eliminating pathogens that can persist for years on beekeeping equipment and in hives as dormant spores. She showed that ozone destroyed 99.95% to 100% of chalkbrood spores after one to three days of exposure, using 3,200 mg O_3/m^3. The American foulbrood bacterium (AFB, *Paenibacillus larvae*) required a higher ozone concentration of 8,560 mg O_3/m^3 during three days of exposure, along with 50°C and high humidity levels of 90% RH. During those experiments, ozone showed potential as a fumigant for

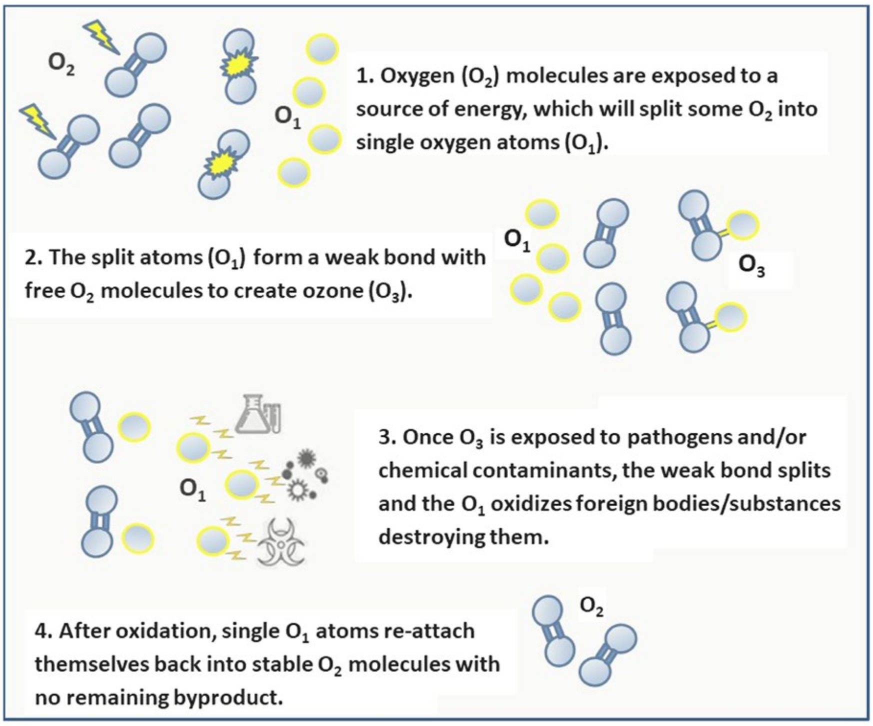

Figure 1. Mode of action of ozone as a disinfectant and decontaminant. Oxygen molecules are separated, gathered into unstable trios to oxidize, and act as a total biocide to disintegrate soon after.

bee nesting materials, but further research is needed to evaluate its acceptability and efficacy in the field.

James (2011) also showed that fumigating combs with ozone at concentrations of 920 mg/m^3 killed all life stages of the wax moth in one day. The egg stage was the most resistant to ozone, usually requiring two days of exposure. Therefore, several clean biotechnical alternatives, such as heat, freezing and ozone, exist that could replace the use of chemicals to control wax moths in stored combs effectively.

Ozone as a Decontaminant

The use of ozone fumigation for disinfection can simultaneously decontaminate beekeeping materials, at least partially, given that ozone acts on the comb surface only (James et al., 2013). A myriad of studies has demonstrated pesticide and metabolite accumulation in bee products (Daniele, Giroud, Jabot, & Vulliet, 2017; Mullin et al., 2010; Ravoet, Reybroeck, & de Graaf, 2015; Rortais et al., 2017; Wilmart et al., 2016). Traces of chemicals in recycled beeswax used to make foundation is a common problem, even in countries where lipophilic acaricides are no longer used, likely because many contaminants resist the wax melting temperature (Wilmart et al., 2016). Examples of liposoluble and non-volatile acaricides are coumaphos, fluvalinate, and cypermethrin that have accumulated after decades of their use as *Varroa* control agents (Ravoet et al., 2015). Bees contacting the acaricides distribute those substances throughout the whole hive within a short time, even if those acaricides are only added to limited areas of the hive. Commercially available beeswax from countries that use unauthorized products such as antibiotics is also a problem (Wilmart et al., 2016). Even when bees are allowed to build fresh comb and are treated with organic acids and essential oils that do not accumulate in the wax matrix, they do not escape crop protection products that are brought into the hive from foraging bees.

Mullin et al. (2010) conducted a broad survey of pesticide residues on hive matrices from the USA and Canada to find that ~60% of the 259 wax samples tested contained at least one systemic pesticide, and that almost all comb and foundation wax samples (98%) were contaminated with commonly used acaricides such as fluvalinate, coumaphos, and amitraz metabolites. In France, Daniele et al., (2017) analyzed hive products for residues of neonicotinoids, pyrethroids, and the fungicide boscalid. Neonicotinoids and boscalid were the most often detected pesticides regardless the matrix. The reduction of pesticides known to affect bees has been demonstrated after treatment with ozone in different products. For example, washing olives with ozonated water for 5 min reduces residues of chlorpyrifos, β-cyfluthrin, α-cypermethrin, and imidacloprid by 38, 50, 55, and 61%, respectively, during olive oil processing (Kırış & Velioglu, 2016). Ozone was shown to reduce levels of the neonicotinoids thiacloprid and imidacloprid within aqueous solutions (Černigoj, Trebse, & Polonca, 2007). Therefore, these studies may suggest that ozone can reduce pesticide residues in treated hives and hive equipment. James et al. (2013) showed that ozone degrades coumaphos, tau-fluvalinate, and several other chemicals used against *Varroa*. In experiments with glass vials containing residues of these mite-killing pesticides, ozone exposures of 500 ppm for 10 to 20 h degraded 93 to 100% of coumaphos and 75 to 98% of fluvalinate. However, higher concentrations and longer exposure times (10.7 mg O_3/l (5000 ppm^{-v}) for 96 h) were required to reduce pesticide concentrations in wax and comb samples.

A heat and ozone device is economical and simple to build. Different devices to heat-treat honey bee colonies against *Varroa* have been developed independently in different parts of the world. They can be categorized basically into thermal boxes, thermal hives, and thermal frames, which have been reported to provide various levels of efficacy (Tihelka, 2016). Standard hyperthermia protocols against *Varroa* have been published previously, where basic treatment temperatures that range between 42 and 45°C are reported to be fatal to *Varroa* while not causing evident detrimental impacts on the bees (Bičík et al., 2016; Goras et al., 2015). An example of a hyperthermia treatment device that is simple to build and use when mounting heat and ozone generators on a super of a hive is presented here (Figure 2). This prototype has been developed with an investment of under 100 USD, and although time consuming (approximately four hours per hive of preparation and treatment), it is easy to utilize. With this model, the treatment temperature is reached in approximately one hour, but this can vary depending on the external temperature and the capacity of the colony to thermoregulate. The temperature can be measured with two sensors, one placed in the center of the hive and the other in a corner. Sensors trigger a pulsing stop and restart of the heat generator each time the minimum and maximum temperatures of 42°C and 45°C are reached during the 2–3 h of colony exposure (Figure 2(A)).

For treatment with ozone, a generator of 10 g/m^3 capacity has been added to the device (Figure 2(B)) and can be mounted

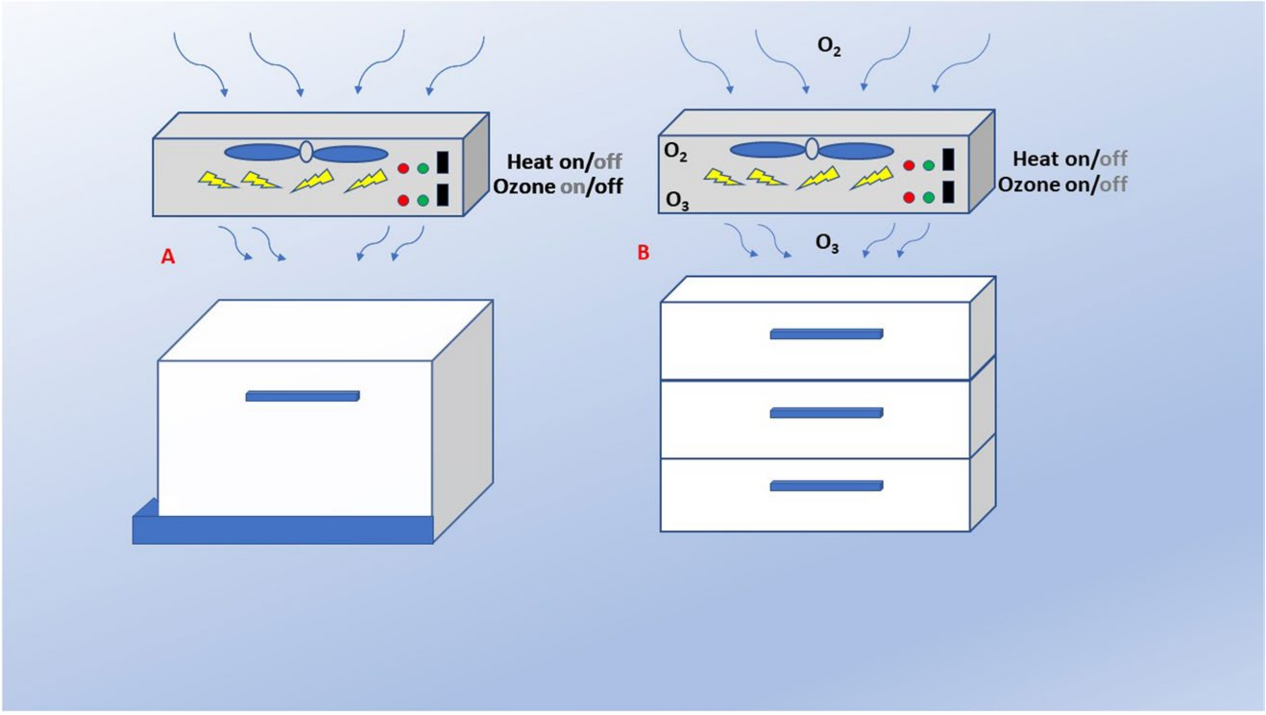

Figure 2. Schematic representation of an individual colony of honey bees where heat alone is added as antiparasitic treatment (A). The O_3 function can be switched on to disinfect and decontaminate hives/supers with previously used comb before reuse (B).

on top of uninhabited hives and components. Ozone fumigation from the top of the bee-less hive allows better penetration into the comb, given the natural upward tilt of the wax cells. It is important to prevent escape of ozone to avoid risk of respiratory tract irritation to the operator; therefore, the fitting of the device and the hive should be airtight during the treatment.

Discussion

Hyperthermia should be reconsidered as a possible good alternative to synthetic and natural acaricides since it is fatal to all phases (phoretic and reproductive) of *Varroa* (Bičík et al., 2016; Goras et al., 2015). Furthermore, it does not leave any residues and is unlikely to result in mite resistance (Tihelka, 2016). However, concerns exist that heat treatment may affect honey bees physiologically. A potential detrimental effect of hyperthermia is the risk to drone sperm and sperm stored in the queen's spermatheca (Stürup, Baer-Imhoof, Nash, Boomsma, & Baer, 2013). In order to circumvent this problem, the queen can be removed temporarily from the hive during the 2–3 h heat treatment and the treatment can be made at the end of the summer since there are only few drones left at this time. Package bees may be also treated with hyperthermia before the introduction of a queen to start a new colony with a limited amount of *Varroa* and without any exposure to chemical acaricides. All these steps are time consuming and require organization; therefore, this method is useful for beekeepers who manage a small number of colonies, although the technology may someday exist to treat large number of hives. Another concern may be the fact that some fallen mites may recover from heat exposure and re-infest bees with a risk of the development of heat-resistant *Varroa*. Therefore, treated hives should be outfitted with a screened bottom board so that the mites fall out of the hive, or an adhesive substance to prevent fallen heat-treated *Varroa* from reinfesting bees (Goras et al., 2015).

There is no known way to disinfect and decontaminate a beehive and its combs completely. Even gamma irradiation cannot fully eliminate pathogens from combs (Simone-Finstrom, Aronstein, Goblirsch, Rinkevich, & de Guzman, 2018). Additionally, gamma irradiation can be costly and impractical since irradiation has to be done in a regulated facility. Heat and ozone technologies have progressed enough to develop an efficacious method of treating honey bee colonies and equipment that does not require chemicals. In addition, the use of ozone as a disinfectant could prevent the need to burn hives and empty frames to avoid AFB reinfection (James, 2011), which has a great financial impact on beekeepers. Savings on transport and storage costs of disinfectants can be made since ozone is obtained from atmospheric oxygen; it is thus produced at the site of use. Although ozone fumigation is a relatively simple method that beekeepers can implement themselves, exposure to ozone should be avoided since high concentrations in the air can be irritating and even poisonous to humans. However, when used under controlled (sealed) conditions, it is an effective and safe disinfectant (Steigert & Franke, 2000). The permissible average concentration of ozone in the air should be no more than 0.1 ppm. Ozone likely is only useful as a surface disinfectant; therefore, cells with honey, pollen, or brood cannot be disinfected. Correspondingly, only empty honey combs should be treated. Also, since ozone is an oxidant, no metal tools should be disinfected using it.

Our knowledge of hyperthermia and ozone use in beekeeping practices is far from field-ready. Research with updated technology is needed to provide guidance on safe application protocols and reveal benefits and possible risks of using hyperthermia and ozone. It should be emphasized that bees and brood should not be exposed to ozone as it is toxic to both. In conclusion, although heat and ozone methods are effective, can be safe, and are simple to use, they need to be developed further before they can be implemented widely.

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