

# The chemical ecology of host-parasite interaction as a target of *Varroa destructor* control agents

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**Abstract** – Honey bees and their ectoparasite *Varroa destructor* communicate through chemical signals among themselves, but they also eavesdrop on each other's chemical cues. We summarize semiochemicals of honey bees and *Varroa*, and their roles in honey bee-*Varroa* interactions. We also give an overview of current *Varroa* control methods, which can be classified into three categories: (1) chemical control methods with acaricides, (2) biotechnical intervention, and (3) bee breeding programs. Widely used synthetic chemical acaricides are failing due to the emergence of resistant mites. Therefore, new methods are being sought for *Varroa* control, and methods that target the semiochemical interactions between bees and mites are among the candidates. We review our discovery of compounds that alter the host choice of *Varroa* mites (from nurse to forager) in laboratory tests. Any semiochemical-based methods are still in the experimental stage and need validation in the field.

*Apis mellifera* / *Varroa destructor* / mite control / chemical senses / semiochemical

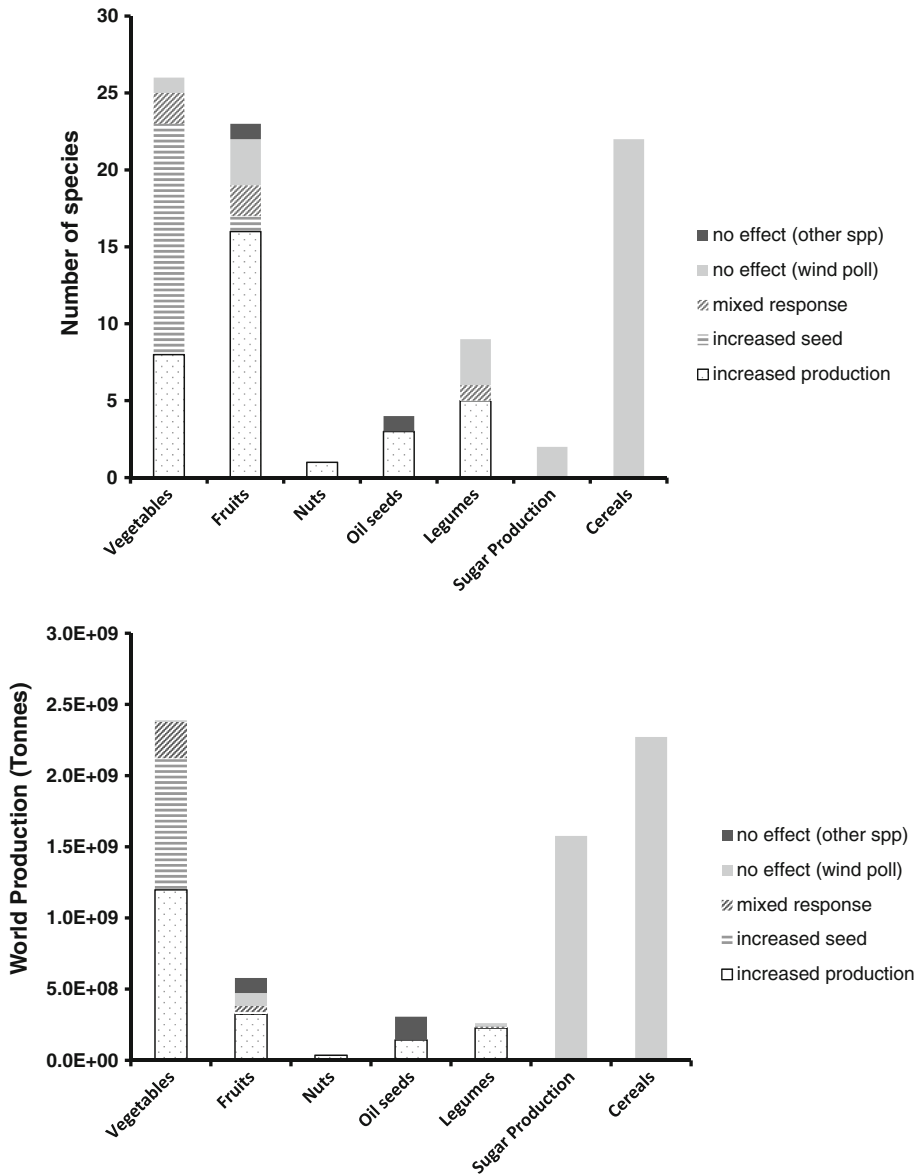
## 1. INTRODUCTION

Honey bees (*Apis mellifera* L.) are central to modern agricultural practices because they pollinate many crops, for which fruit set depends on insect pollination. Additionally, honey bee pollination improves quality and yield of many other crops that do not fully depend on insect pollination. Figure 1 depicts the number of plant categories (species or groups of species) that are raised for food in more than  $4 \times 10^6$  tons/year, and the total amount produced in each category (Klein et al. 2007). Honey bees provide ~90 % of insect pollination required in the production of these most prevalent food crops consumed by humans (Klein et al. 2007). The economic value of honey

bee pollination in food production has been estimated at €138 billion, and the surplus losses in human food crops if all insect pollinators were to disappear have been estimated at €191–310 billion (Gallai et al. 2009).

In recent years, bee populations have experienced large fluctuations and regional declines, and this has been problematic for the production of crops that require insect pollination, as well as for the sustainability of beekeeping businesses (Naug 2009; Le Conte et al. 2010; Rosenkranz et al. 2010; Moritz and Erler 2016). Losses of bee colonies have been attributed to various factors: mites, viral diseases (vectored by mites) (Le Conte et al. 2010; Rosenkranz et al. 2010; Kuster et al. 2014; Guzmán-Novoa et al. 2010), microsporidial parasites (Wolf et al. 2014), exposure to agrochemicals (Samson-Robert et al. 2014; Simon-Delso et al. 2014), migratory beekeeping (Le Conte et al. 2010), very dense apiaries (Frey and Rosenkranz 2014), and changes in floral resources

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**Figure 1.** Contribution of insect pollination to the production of human food crops. The graphs were generated from data given in the appendices of reference 1. *Shading* (see legend) indicates the contribution of insect pollination: *black* = insect pollination has no effect but other animals are the pollinators; *gray* = wind pollination; *diagonally hatched* = insect pollination has a mixed effect on both increased seed production and increased crop production; *horizontally hatched* = insect pollination increases seed production; *white dotted* = insect pollination increases production of the crop.

(Naug 2009; Donkersley et al. 2014; Sponsler and Johnson 2015). These factors are believed to be interdependent and to synergize in the losses of bee colonies. For example, viruses vectored by

*Varroa* appear to synergize with the mites themselves in bee immunosuppression and the development of increasingly virulent viral infection (Nazzi and Pennacchio 2014).

The ectoparasitic mite *Varroa destructor* (Anderson and Trueman) is at the top of the list of risk factors for honey bee colonies worldwide, except Australia where the mite has not yet invaded. *Varroa* attacks honey bees and weakens them; if left untreated, a *Varroa*-infested colony usually withers and eventually dies. *Varroa* mites parasitize both adult bees and pupae: they reproduce in recently capped brood cells and emerge with the adult bee (see “[Semiochemicals produced and detected by Varroa mites](#)” section below). These two stages in the *Varroa* life cycle are known as the reproductive and phoretic stages, respectively. *Varroa* transmits Deformed Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV), among other viral diseases. DWV reduces the worker life span during overwintering, such that insufficient workers are available to build up the colony in the spring. ABPV kills the bees more quickly than DWV, thereby leading to more acute disease and faster colony losses (Francis et al. 2013; Kuster et al. 2014). Mite infestation, along with the transmitted viral diseases, has been shown to be the major cause of colony losses during overwintering in northern latitudes, e.g., in Canada (Guzmán-Novoa et al. 2010).

Since *Varroa* mites switched from their original host, the Asian honey bee *Apis cerana*, to the European/African honey bee species, *A. mellifera*, various mite control methods have been implemented (Rosenkranz et al. 2010). These control methods can be divided into three groups: (1) chemical treatment, (2) biotechnical intervention, and (3) bee breeding. The most widely practiced control method in commercial apiaries is chemical treatment with miticidal compounds (see below). One problem with some of the most *Varroa*-effective agents used, such as the pyrethroid-fluvalinate, the formamidine-amitraz, or the organophosphate-coumaphos, is that the mites have developed resistance to these agents (see below, “[Chemical control methods](#)” section). Therefore, there is a worldwide effort to find new compounds and strategies for controlling or mitigating *Varroa* infestations in commercial honey bee colonies. Some of the new strategies being researched include (1) small inhibitory ribonucleic acid (RNAi) intervention, (2) *Varroa* pathogens, and (3) chemosensory mite disruption.

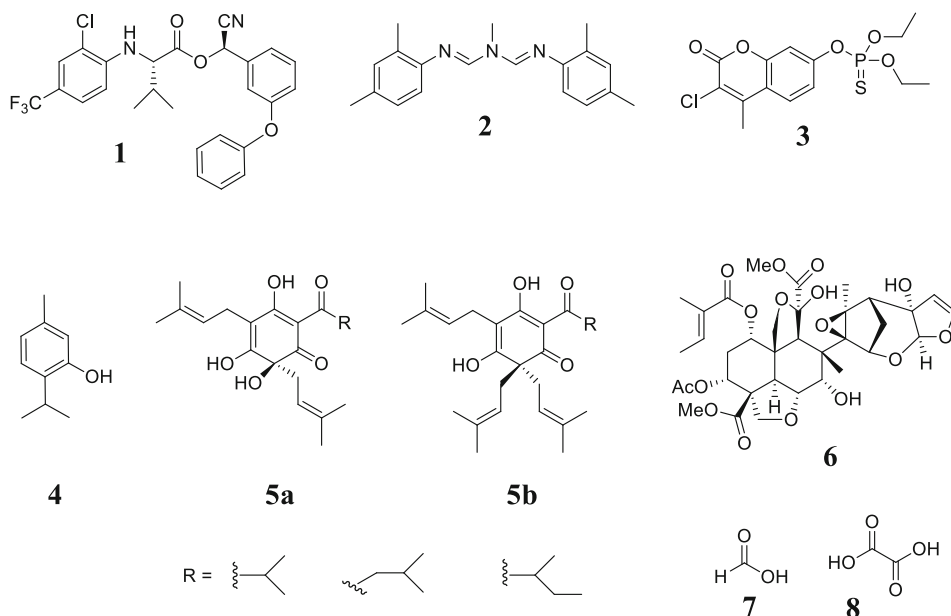
Honey bees and *Varroa* mites both depend on a myriad of chemical signals to communicate among their conspecifics and to sense the other species. Therefore, chemical communication is a good target for the development of new *Varroa* control tactics. *Varroa* mites have a specialized chemosensory organ on the forelegs, and bees have chemosensory structures (sensilla) on their antennae which they use to detect odors (see “[Chemical senses of arthropods](#)” section). In this article, we will summarize the known relevant chemical communication vocabulary of honey bees and *Varroa* mites, and how *Varroa* mites mimic and eavesdrop on honey bee signals (“[Semiochemicals and chemoreception of honey bees and Varroa mites](#)” section). We will start with an overview of the various control methods and tactics employed or being developed to control *Varroa* infestation in colonies (“[Varroa control methods and the need for new approaches](#)” section), to provide a sense of where semiochemical methods might fit into an integrated pest management scheme for *Varroa*.

## 2. VARROA CONTROL METHODS AND THE NEED FOR NEW APPROACHES

### 2.1. Chemical control methods

Chemical intervention can be divided into three subgroups: (1) synthetic acaricides, (2) natural product acaricides, and (3) organic acids. All chemical methods aim to reduce mite populations by selectively killing mites.

Synthetic acaricides, such as the pyrethroid fluvalinate **1** (Figure 2), have been used for a long time because they are effective and relatively selective toward mites (i.e., have a low toxicity toward bees) (Lindberg et al. 2000). Due to widespread resistance of the mites against fluvalinate (González-Cabrera et al. 2013), other synthetic acaricides (amitraz **2**, a formamidine, and coumaphos **3**, an organophosphate) have been used. Resistance against amitraz (Elzen et al. 2000) and coumaphos (Maggi et al. 2009) has also been documented, though amitraz is still used successfully in many places. A major disadvantage of synthetic acaricides is that they are hydrophobic



**Figure 2.** Structures of compounds used to control *Varroa* mites.

and accumulate in wax, leading to accelerated development of resistance, as well as sub-lethal effects on bees (due to continuous low-level exposure) and contamination of hive products (Frost et al. 2013; Hillier et al. 2013).

The natural acaricide thymol **4** is widely used in beekeeping, in various formulations (Imdorf et al. 1999; Lindberg et al. 2000; Le Conte et al. 2010). The compound is selective for mites, but irritates the bees (by binding to dopamine receptors) and can affect the taste of honey if used in high doses (Mondet et al. 2011). Lately, symptoms for resistance to thymol have also been reported (Pietropaoli et al. 2015). Hops extract, which contains humulones **5a** and lupulones **5b**, is another natural acaricide that is useful in the shipment of bees and establishment of new colonies in the spring (DeGrandi-Hoffman et al. 2012, 2014). Neem oil, which contains many limonoids such as azadirachtin A **6**, has been tested for *Varroa* control. Neem causes decreases in mite numbers (Lindberg et al. 2000; Melathopoulos et al. 2000), but it also causes increased queen and brood mortality (Naumann and Isman 1996). Therefore, neem is not used commercially to manage *Varroa*.

Organic acids (formic **7** and oxalic acids **8**) are widely used for spring and fall treatments against *Varroa* (Rademacher and Harz 2006; Calderone 2010; Giovenazzo and Dubreuil 2011). They do not accumulate in wax while still permeating the hive. Formic acid being

much more volatile than oxalic is apparently able to penetrate the brood capping and thus affect both reproducing mites in capped cells and phoretic mites (which parasitize adult bees), but causes higher worker and queen mortality than oxalic acid (Giovenazzo and Dubreuil 2011). Although resistance to most natural acaricides or organic acids has not been reported, the potential for resistance evolution exists because all of these compounds reduce mite populations by acaricidal action.

## 2.2. Biotechnical intervention

The most widely practiced biotechnical intervention is drone brood removal. It is based mainly on two facts: (1) drone brood is ~10–12 times more attractive to female *Varroa* mites than worker brood (Boot 1994; Rosenkranz et al. 2010), likely because drone brood are larger than worker brood and produce more of the *Varroa*-arresting odors (see below, “Semiochemicals of honey bees” section, fatty acid esters **16a**, **16b**, **18a–18d**) just prior to capping, and (2) its development is longer than that of workers allowing production of more female mites per cell. Combs that encourage the building of drone cells are installed in the colony; the queen is allowed to lay unfertilized eggs and drone brood is established. If removal of the trap comb is timed correctly, mite numbers can be kept low by this approach (Maul et al. 1988; Calis et al. 1999; Calderone

2005; Wantuch and Tapy 2009). The disadvantages of the method are as follows: (1) it is labor intensive, (2) it needs to be timed with drone rearing in the spring, (3) it leads to loss of brood, and (4) the mite population can rebound (Maul et al. 1988).

Brood interruption by queen confinement is another method of decreasing the total amount of brood that is available for *Varroa* females to reproduce. The queen is caged on the comb during the late summer, for approximately 1 month. During this time, less and less brood becomes available for *Varroa* to reproduce (Lodesani et al. 2014). Moreover, in the absence of capped brood, phoretic mites can be more efficiently treated by compounds such as oxalic acid **8**. The disadvantages of the method are as follows: (1) it is labor intensive, (2) it needs to be timed not to interfere with honey production, and (3) it may cause queen loss.

### 2.3. Bee breeding

Bee breeding to increase *Varroa* tolerance is a promising strategy for sustainable control of *Varroa* infestation. Two approaches are used: (1) breeding bees known to have survived *Varroa* infestation for longer than a decade without intervention (Locke et al. 2012; Rinderer et al. 2014a, b), and (2) selecting bee lines that exhibit *Varroa*-specific hygienic (VSH) behavior (Tsuruda et al. 2012; Danka et al. 2013; Nicodemo et al. 2013; Rinderer et al. 2014a, b). VSH bee lines have been shown to remove significantly more *Varroa*-infected pupae than control lines. This removal seemed dependent on a threshold signal accumulating up to 5 days post-capping, generally from *Varroa* progeny developing in a cell (Harris 2015). Colonies bred for hygienic behavior show suppression of mite reproduction (SMR) and have been shown to contain significantly fewer phoretic and reproducing mites than the wild-type colonies (Ibrahim et al. 2007; de Guzman et al. 2015). It is important to note that VSH may be only one of several behavioral traits in colonies that suppress mite reproduction and, therefore, are more resistant to *Varroa* (see “Interaction of *Varroa* with *A. cerana* and Africanized honey bees (AHB)” section).

Grooming behavior of bees is another trait that is being explored in breeding programs (Currie and Tahmasbi 2008). Workers groom phoretic mites from their bodies, often damaging the mites in the process and causing them to fall. A recent linkage map revealed some candidate genes (many of them central nervous

system genes) associated with grooming behavior (Arechavaleta-Velasco et al. 2012).

## 3. SEMIOCHEMICALS AND CHEMO-RECEPTION OF HONEY BEES AND *VARROA* MITES

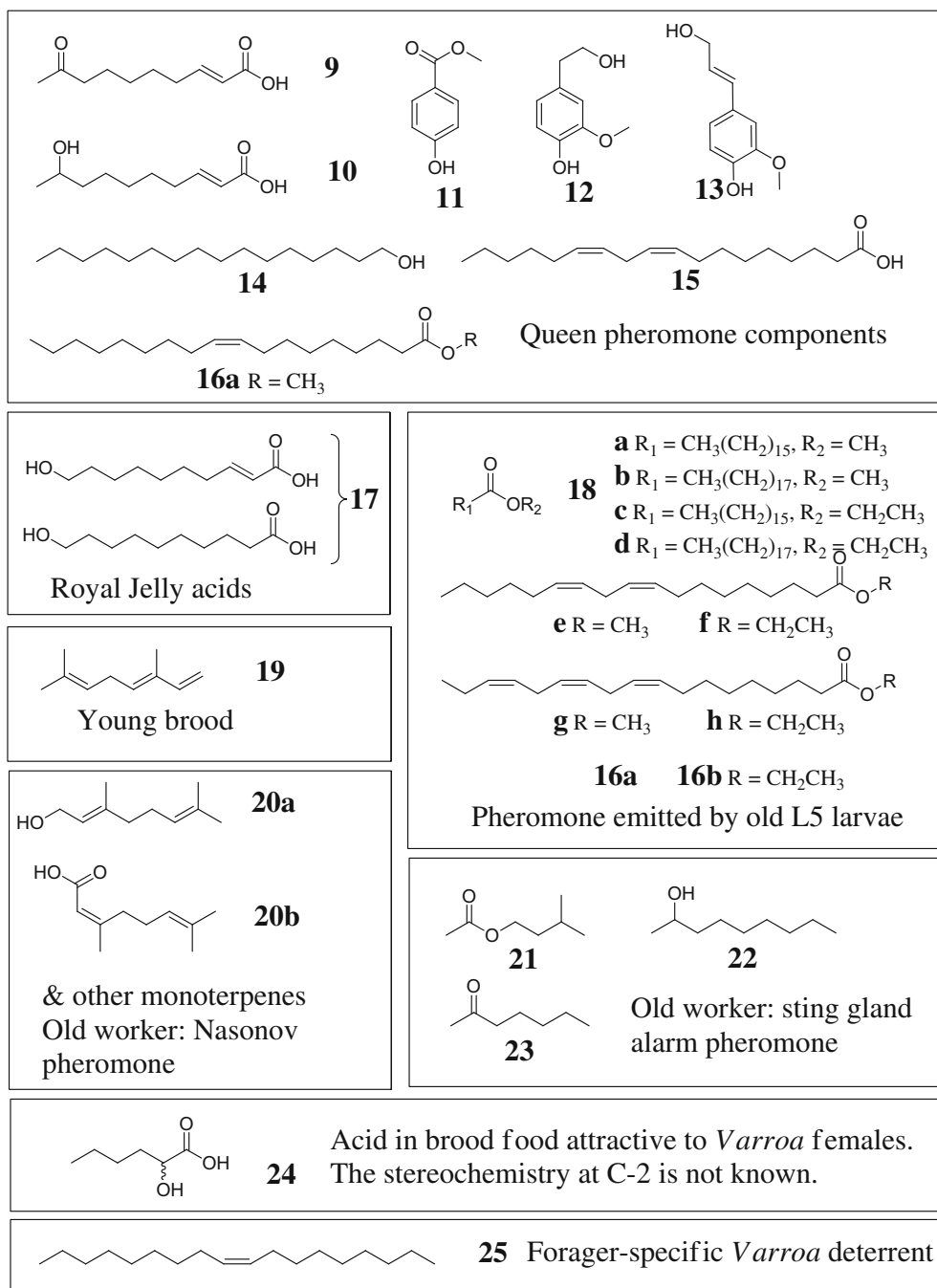
### 3.1. Semiochemicals of honey bees

In a honey bee colony, chemical cues are the principal mode of communication and social coherence (Keeling et al. 2004).

The queen produces a series of  $\omega$ -1 functionalized 10-carbon fatty acids **9**, **10** (Figure 3) (Plettner et al. 1996), aromatic compounds **11**–**13**, hexadecane-1-ol **14**, linoleic acid **15**, and methyl oleate **16a** as part of her queen pheromone (Slessor et al. 1988; Keeling et al. 2003). Middle-aged workers produce a series of  $\omega$ -functionalized 10-carbon fatty acids **17** that are added to brood food and royal jelly. Older brood produce a blend of methyl and ethyl esters **18** that signal to nurse bees that the brood is ready for capping (Le Conte et al. 2006), whereas young brood produce (*E*)- $\beta$ -ocimene **19** to stimulate feeding from nurses (Maisonasse et al. 2009). Foragers produce ethyl oleate **16b**, a signal that (together with old brood pheromone, **18**) delays the transition of nurses into foragers (Alaux et al. 2009; Castillo et al. 2011). Foragers also emit a monoterpene blend pheromone (Nasonov, pheromone, of which **20a** and **20b** are components) at the hive entrance to attract foragers homing in (Williams et al. 1981). The sting glands of foragers contain an alarm pheromone (isopentyl acetate **21**, 2-nonanol **22**, and 2-heptanone **23**; Figure 2), which attracts other bees and increases their aggressiveness (Allan et al. 1987).

### 3.2. Semiochemicals produced and detected by *Varroa* mites

*Varroa* mites develop from eggs laid by the foundress (mated female) that entered a worker or drone brood cell shortly before its capping. Once a brood cell has been capped, the FA ester blend emitted by the pupa changes: ethyl esters **18c**, **18d**, **18f**, **18h**, and **16b** nearly disappear, whereas three methyl esters **18a**, **18b**, and **16a** remain



**Figure 3.** Selected compounds found in various honey bee pheromones.

high; these have been shown to trigger reproductive maturation of newly emerged *Varroa* daughters (Frey et al. 2013) by vitellogenin induction (Cabrera Cordon et al. 2013). However, these methyl esters cause artificially introduced phoretic

*Varroa* females to resorb their eggs and forego reproduction, likely as a signal that the pupa in the cell is too far developed for the next generation of mites to complete their development prior to bee emergence (Frey et al. 2013). Thus, *Varroa*

reproduction is timed very delicately with the signals from the bee brood.

The first mite to hatch is a male; all that follow (three to five) are females (Garrido and Rosenkranz 2003). The volatile signal in the capped cell, which contains **18a**, **18b**, and **16a**, also ensures that the first offspring of the foundress is male and, therefore, that the daughters mate and will be fertile (Garrido and Rosenkranz 2003). The young females emit a sex pheromone that attracts the male and elicits courtship and mating behavior (Ziegelmann et al. 2013a, b). This pheromone consists of three fatty acids (palmitic, stearic, and oleic) and their respective ethyl esters **18c**, **18d**, and **16b**. Older females no longer make this pheromone, so mating has to occur within a short window of time (Ziegelmann et al. 2013a).

After mating, the female mites continue their development, synchronized with that of the bee pupa. When the bee emerges, the female mites cling to the bee and become phoretic. During the phoretic phase, *Varroa* females parasitize adult worker bees, mainly nurses. Phoretic *Varroa* females detect and respond to several compounds produced by bees. The mites are arrested (i.e., stop moving about) by a blend of fatty acid esters **18a**, **18c**, and **18e** from old bee larvae (Calderone and Lin 2001) and by aliphatic alcohols and aldehydes of honey bee cocoons (Donzé et al. 1998). The mites are deterred and repelled by geraniol and nerolic acid **20a**, **20b** (Pernal et al. 2005), both components of the Nasonov pheromone which is produced in larger quantities by foragers than by nurses (Figure 3), and this may be one reason why phoretic *Varroa* mites prefer nurse bees over foragers. *Varroa* females are also deterred by the  $\omega$ -functionalized fatty acids from royal jelly **17** (Drijfhout et al. 2005). Brood food contains a volatile compound, 2-hydroxyhexanoic acid **24** (enantiomer not known), that is attractive to phoretic *Varroa* females (Nazzi

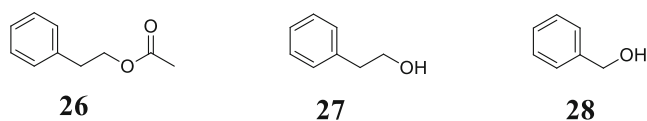
et al. 2004). Finally, foragers appear to have higher titers of (*Z*)-8-heptadecene **25** in their cuticular hydrocarbon blend than nurses, and this compound has been also shown to deter *Varroa* females (Piccolo et al. 2010).

Supporting the importance of chemoreception in *Varroa* mites as they enter brood cells and switch from phoretic to reproductive, a recent study has found that the pheromone receptor transcription factor like (PRTF) is expressed much more abundantly in the forelegs of phoretic mites than of reproductive ones (Singh et al. 2016). Phoretic mites with experimentally reduced levels of PRTF (through RNAi) had weaker electrotarsogram responses to bee head space and took longer to find a host, but had induced vitellogenin levels (Singh et al. 2016).

It is important to note that several compounds (e.g., fatty acids or esters) have different roles or meanings in different contexts in the hive. Adding these multifunctional semiochemicals to a colony, e.g., to affect a particular behavior of *Varroa* or of the bees, may cause side effects elsewhere in the colony. This is why we decided to test synthetic odorants with *Varroa* and bees (see below, “Synthetic odors that affect mite behavior” section).

### 3.3. Odors produced by diseased brood

Chemosensory detection of diseased brood plays an important role in hygienic behavior. For example, in one study, specific odor signatures of chalkbrood-infested brood were identified as phenethyl acetate **26**, phenyl ethanol **27**, and benzyl alcohol **28** (Figure 4). Hygienic honey bee colonies uncapped cells that contained dummies impregnated with **26–28** significantly faster than cells with control dummies (Swanson et al. 2009). Similar odors from *Varroa*-infested brood have not yet been identified, but probably



**Figure 4.** Structures of compounds emitted by chalkbrood-infested bee larvae that induce hygienic behavior in worker bees.

exist as VSH behavior, and *Varroa* resistant lines show differential expression of genes that are likely involved in chemoreception (Parker et al. 2012; Guarna et al. 2015).

### 3.4. Synthetic odors that affect mite behavior

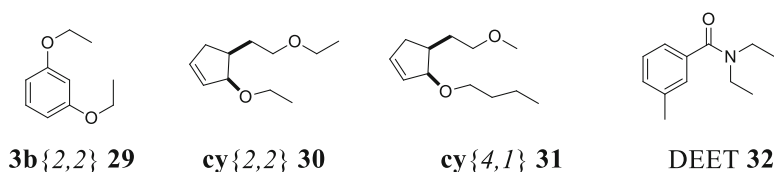
Our research has led to the identification of various synthetic compounds that affect the behavior of *Varroa* mites. Various aromatic and alicyclic ethers **29**–**31** (Figure 5) were screened by both electrotarsogram recordings from phoretic *Varroa* forelegs and behavioral assays, in which a phoretic *Varroa* female was left to choose between a freshly freeze-killed nurse and forager. Normally, phoretic *Varroa* females choose a nurse over a forager, and this preference matches the fact that a nurse bee is likely to take them to a brood cell where the mite can start the next cycle of reproduction. We found compounds that reverse this choice. These compounds also cause a long-term inhibition of the detection of nurse headspace odor in electrotarsogram tests. Compounds **29**–**31** did not affect the frequency with which a mite chose a nurse or forager bee host (Eliash et al. 2014). Chiral compounds **30** and **31** were active as racemates; the activity of individual enantiomers is being explored currently.

We have also found that *N,N*-diethyl-3-methylbenzamide **32** (DEET) decreases the frequency with which phoretic mites find a host bee in laboratory bioassays, but it does not alter the host choice the way the ethers **29**–**31** do (Singh et al. 2014). Thus, DEET acts as a host finding deterrent in laboratory bioassays, whereas the ethers **29**–**31** alter the host choice of phoretic mites. Electrophysiologically, DEET caused long-term inhibition of *Varroa* foreleg responses to nurse bee headspace, whereas it is sensed by

bees, and it caused transient (short-term) inhibition of honey bee antennal responses to queen bee head space. Behaviorally, DEET did not visibly affect the interaction of workers with the queen (Singh et al. 2014), even though it has been known as an effective bee repellent (Schroeder et al. 2007). On the other hand, **cy**{4,1} **31** showed long-term inhibition of *Varroa* foreleg responses to both nurse bee headspace and (*E*)- $\beta$ -ocimene, and it showed a short-term enhancement of honey bee antennae to (*E*)- $\beta$ -ocimene (Singh et al. 2015). Its effect on bee behavior remains to be tested. The effect of all *Varroa* chemosensory disruptive compounds remains to be evaluated at colony level.

### 3.5. Chemical senses of arthropods

Bees and mites detect the chemical signals described earlier through gustation and olfaction. Both chemical senses are located in sensilla, external cuticular structures that are innervated by sensory neurons. Olfactory sensilla are located mostly on appendages such as antennae, whereas gustatory sensilla are located on the mouth parts and are double-walled with a single pore at the top (Ryan 2002). Some olfactory sensilla are single-walled and multiporous (e.g., *s. basiconica*, *s. placodea*); others are double-walled (*s. coeloconica*) (Ryan 2002). Bees also have sensilla placodea on their antennae; these single-walled, multiporous plate-like structures are innervated by 5–35 olfactory receptor neurons (ORNs) (Akers and Getz 1992). Regardless of sensillum type, the dendritic end of each ORN interacts with odorants, and the axon of each ORN projects to a particular region of the antennal lobe in the brain, a glomerulus. ORNs tuned to the same odorants have their axons converge in the same glomerulus (Kelber et al. 2006).



**Figure 5.** Compounds discovered that disrupt host choice or decrease the frequency of host choice in phoretic *Varroa* females.



Mites do not have antennae; instead, they use their front pair of legs as chemosensory organs. The front legs contain a pit organ that has nine olfactory sensilla, shaped like sensilla basiconica (Dillier et al. 2006; Eliash 2012; Häußermann et al. 2015). Based on electrophysiological recordings, the mite's front legs clearly respond to volatile stimuli (Eliash et al. 2014; Singh et al. 2014).

Chemosensory sensilla contain several proteins that interact with chemical signals. The chemoreceptors on the neuronal dendrites are the sensors of chemical signals. There are two types of odorant receptors known for arthropods: (1) odorant receptors (ORs) with seven membrane-spanning regions and a co-receptor (ORCO) with which ORs pair (Wanner et al. 2007; Wanner and Robertson 2010), and (2) variant ionotropic receptors (IRs) that are ion channels and distant relatives of the glutamate receptors (iGluRs) (Croset et al. 2010). Each OR is paired with a conserved co-receptor that serves as chaperone and probably forms a ligand-gated ion channel with the receptor, a mechanism distinct from that of vertebrate G-protein coupled receptors (Wanner and Robertson 2010). To date, the OR/ORCO system has only been detected in insects, including honey bees (Wanner et al. 2007; Wanner and Robertson 2010). IRs do not appear to be paired with any co-receptor, but have to form multimeric structures to function as ligand-gated ion channels (Croset et al. 2010). Arthropods, nematodes, and mollusks have IRs, and insects have an expanded set of divergent antennal IRs (Croset et al. 2010). Recently, IR40a in *Drosophila melanogaster* was identified as the main target site of DEET (Kain et al. 2013), and crustaceans have been found to have IRs as their only olfactory receptors instead of OR/ORCO systems (Corey et al. 2013). Mites, therefore, will likely have some chemosensory IRs. Genomic data for *Varroa* are a valuable repository for the identification of chemosensory genes in these mites (Cornman et al. 2010).

The dendrite of an olfactory sensory neuron responds to odorant activation of the chemoreceptor (either OR or IR) by depolarizing. This initial depolarization is followed by neuronal spiking, a response mediated by voltage-gated channels. Voltage-gated cation channels that are distinct from the Na<sup>+</sup> channels in the central nervous

system have been detected in insect sensilla (Zufall et al. 1991). Surrounding the dendrites of chemosensory neurons is the lymph, an electrolyte solution rich in fatty acids and odorant-binding proteins (OBPs) or chemosensory-specific proteins (CSPs) (Honson et al. 2005). The function of OBPs is not well understood; it is believed that these proteins aid in diffusion of odorants through the lymph and also act as kinetic traps to prevent adaptation of the ORN (Gong et al. 2009). It is interesting to note that hygienic worker bees (bred based on the freeze-killed brood removal bioassay) detect and remove diseased larvae apparently by identifying specific odors from diseased brood (Swanson et al. 2009). Recent proteomic analyses have revealed that two bee OBPs (16 and 18) are more abundantly expressed in hygienic bee lines than in wild-type lines (Guarna et al. 2015).

Neurotransmitters, particularly octopamine, also play a role in controlling olfactory processes in insects. For example, hygienic bee lines were found to have more octopamine-containing neuronal clusters in the brain and these stained more intensely than those from wild-type lines (Spivak et al. 2003). Hygienic bees responded more strongly and with lower thresholds to diseased brood odorants (see below), and this response was diminished by an octopamine antagonist (Spivak et al. 2003). The differences in chemosensory mechanisms between bees and mites might be useful in the development of agents that alter the behavior of *Varroa*, while not affecting bee behavior, or that improve the detection of *Varroa* by bees.

### 3.6. Interaction of *Varroa* with *A. cerana* and Africanized honey bees (AHB)

*A. cerana*, the original host of *Varroa* mites, is more resistant to mite infestation than *A. mellifera* (Tewarson et al. 1992). Three reasons have been suggested for this resistance: (1) *A. cerana* workers groom more (both themselves and other workers), particularly if the mite is from another colony. (2) The mites reproduce only in drone brood; mites that emerge from worker brood are generally sterile because *A. cerana* workers have a shorter pupal development compared to

*A. mellifera* workers and their brood cells are smaller. (3) There appears to be more *Varroa*-triggered hygienic behavior in *A. cerana* than in *A. mellifera*; workers uncap cells with infested pupae and remove either the whole pupa with mites or the foundress, particularly if she is from another colony (Tewarson et al. 1992). The hygienic behavior appears to be elicited by the odor of the mites (Rosenkranz et al. 1993). *A. cerana* is similar to *A. mellifera* in its  $\omega$ - and  $\omega$ -1 functionalized fatty acid profile (Plettner et al. 1997); however, its brood-associated pheromones are not known. Given that the interaction between *Varroa* and *Apis* is subtly timed by the ontogeny of various bee semiochemicals, the pheromones of *A. cerana*, as well as *A. cerana*'s ability to detect mite odors, should be explored further.

Africanized honey bees (AHB) are derived from *A. mellifera scutellata* taken to Brazil, where it has been noted that AHB are also more *Varroa* resistant than the European races of *A. mellifera* (EHB) (Calderón et al. 2010). As in *A. cerana*, mite reproduction on AHB is less successful than on EHB. AHB groom more, exhibit more hygienic behavior towards mite-infested nestmates, and they have shorter development times and smaller cells for worker brood than EHB (Calderón et al. 2010). Thus, AHB colonies have fewer fertile mites. AHB have similar mandibular gland semiochemicals to EHB (Plettner et al. 1993; Pankiw et al. 1996), but the subtleties of their other pheromones or of odors that elicit hygienic behaviors are not known. Thus, these AHB semiochemicals and their cognate OBPs, ORs, or IRs (see "Chemical senses of arthropods" section) should be studied and compared to EHB as potential molecular markers of desirable *Varroa* resistance traits in breeding programs.

## 4. NEW VARROA CONTROL AVENUES BEING RESEARCHED

### 4.1. Small inhibitory ribonucleic acid (RNAi)

RNAi involves the treatment of honey bees with small inhibitory, double-stranded (ds) RNA sequences that target *Varroa*-specific processes and do not affect the bees. For example, feeding

of bees with dsRNA against DWV caused the virus titer to decrease significantly and adult bee survival to increase significantly (Desai et al. 2012). In another example, mixtures of dsRNAs against multiple *Varroa*-specific genes were fed to bees and shown to be transferred horizontally to *Varroa*. One of these mixtures was very effective in reducing *Varroa* population by silencing the expression of selected genes in the mites (Garbian et al. 2012). The gene of a glutathione *S*-transferase (GST, a detoxification enzyme that couples electrophilic centers on hydrophobic substances to a hydrophilic peptide to increase excretion) has been successfully downregulated in *Varroa* through treatment of mites with RNAi. However, this GST-specific RNAi was not tested in bee colonies (Campbell et al. 2010).

Specific receptors or CSPs in the *Varroa* mites could be promising targets for RNAi intervention. For example, targeting the/a receptor responsible for detecting L5 larvae might result in a decrease in successful cell invasion by phoretic mites and, thus, a lower reproductive rate. Alternatively, targeting the/a receptor responsible for a foundress' decision to lay eggs may disrupt the delicate timing of this process and thereby also lower reproductive rates.

One challenge with this technique is that high doses of the dsRNA are needed, as RNA hydrolyzes easily while in the food or in the digestive tract of the bees.

### 4.2. *Varroa* pathogens

A recent survey of bacterial pathogens associated with phoretic *Varroa* females has revealed nine strains of *Bacillus thuringiensis* that cause high mortality to *Varroa* but do not affect the bees (Alquisira-Ramírez et al. 2014). Furthermore, several searches for entomopathogenic fungi have identified strains of *Metarhizium anisopliae*, *Beauveria bassiana*, and *Clonostachys rosea* that kill *Varroa* mites, thereby causing mite drops and decreased mite reproduction (Hamiduzzaman et al. 2012; Meikle et al. 2012; Piralí-Kheirabadi et al. 2013). These fungi also infect the bees, thereby triggering strong immune responses that seem to counteract the *Varroa*-induced immunosuppression (Hamiduzzaman et al. 2012). Some

challenges of entomopathogenic fungi include the formulation of fungal conidia preparations, the formation of potentially harmful mycotoxins upon fungal infection, and the mixed effects of bees (Meikle et al. 2012).

### 4.3. Chemosensory mite disruption

Recently, the mite sex pheromone has been identified and shown to cause mating disruption if applied to wax comb. Mating disruption and decreased *Varroa* reproduction have been observed by addition of oleic acid to combs, which caused reduced spermatozoa loads in *Varroa* females due to decreased mating (Ziegelmann and Rosenkranz 2014) or mistimed introduction of phoretic *Varroa* mites to capped brood cells (Frey et al. 2013). The challenge with the mite pheromone is that it consists of compounds that are also used for signaling by the bees; its widespread application in the hive may cause side effects to the bees.

Our recently discovered compounds 29–31 could be released in a colony to get phoretic mites to move from nurses to foragers and, therefore, be carried away from the brood thus exposing the *Varroa* to grooming bees and/or to soft acaricidal agents (Eliash et al. 2014). One potential problem with this strategy could be more drifting of mites riding on foragers between colonies in dense apiaries.

## 5. CONCLUSIONS

Acaricide-based *Varroa* control methods alone are not sustainable because of the rapid development of resistant mite populations and sub-lethal effects on bees. Therefore, new approaches for *Varroa* control are needed. Here, we have given an overview of the main chemical signals emitted and perceived by bees and mites. We have also summarized commonly used methods as well as new promising strategies for *Varroa* control being researched. Many of these old and new methods may be suitable for integrated *Varroa* management. For example, biotechnical control methods and organic acids have already been explored in combination (Lodesani et al. 2014).

Chemosensory methods likely will be implemented alongside other methods to achieve synergistic control. For example, our compounds 29–31 could be explored alongside other strategies, such as organic acids, VSH bees, and RNAi or biotechnical intervention, as part of integrated *Varroa* management.

**L'écologie chimique de l'interaction hôte-parasite comme cible des agents de lutte contre *Varroa destructor***

*Apis mellifera* / Acari / lutte anti-parasite / sens chimiques / médiateurs chimiques

**Die chemische Ökologie der Wirt-Parasit-Interaktionen als eine Möglichkeit der Bekämpfung von *Varroa destructor***

*Apis mellifera* / *Varroa destructor* / Varroabekämpfung / Chemische Sinneswahrnehmung / Pheromone

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