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Essential Oil Components and Volatile Organic Compounds as Pheromones

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Abstract

Studies (2000-2010) regarding (pest) insect and arachnid released pheromones, consisting of essential oil components of various plants or other volatile organic compounds, were compiled. Consequently, verbenol and verbenone were found to be an essential part of the bark beetle's aggregation pheromone, released with the intent to round up its conspecifics. Concerning plant lice, aphids use (E)-β-farnesene as an alarm pheromone to warn the other lice due to predators or enemies. Additionally, nepetalactol and nepetalactone, two essential oil components of the cat mint *(Lamiaceae)*, occur as an aphid released sex pheromone, emitted to induce mating behavior. On the other side, coccids, another family of plant lice, produce and recognize lavandulyl esters as their sex pheromone. Fruit flies or bees for example, have developed special mechanisms in pheromone production. Fruit flies incorporate phenylpropanoids of *Bulbophyllum vinaceum (Orchidaceae)* flowers to further produce their sex pheromone components out of the collected methyl eugenol. On the other hand, some bees imitate a linalool-containing orchid odor as their sex pheromone and thus allure their mating partners. Studies about termites reveal a large amount of different presumable pheromone compounds that also appear as essential oil components: neocembrene as a part of the trail pheromone of several *Prorhinotermes* species, (E,E)-α-farnesene as an alarm pheromone of *P. canalifrons* or several terpenes like γ-cadinene as primer pheromones of a certain *Reticulitermes* species, enforcing the effect of the juvenile hormone and therefore determining about caste development. A similar variety can be seen in stink bugs, where (4S)-cis-(Z)-bisabolene epoxide, (+)-α-curcumene, (-)-zingiberene, (-)-β-sesquiphellandrene and zingiberenol play a role among the different species. Furthermore, (E,E)-8,10-dodecadien-1-ol (codlemone) is released by a serious pest, the codling moth, as a sex pheromone. The vector of the American visceral leishmaniasis, the sand fly, consists of some further species which use derivatives of either α-himachalene, germacrene D or cembrene as a sex pheromone and thus can be distinguished. Concerning the pest insects, each time management strategies, conducted with pheromone traps, are presented or compared. Regarding arachnids, studies about spider pheromones are rare, whereas more information is available about mites, which release neral and geranial as an alarm pheromone in dangerous situations.

Zusammenfassung

Verschiedene Studien (2000-2010) über Pheromone von (Schädlings-)Insekten und Spinnentieren, die gleichzeitig als Bestandteile ätherischer Öle in verschiedenen Pflanzen vertreten sind oder bei denen es sich um kleine organische flüchtige Komponenten handelt, wurden zusammengefasst. Daraus wurde ersichtlich, dass der Borkenkäfer beispielsweise Verbenol und Verbenon als Aggregationspheromon verwendet, um seine Artsgenossen zusammenzutrommeln. Bei Pflanzenläusen konnte (E)-β-Farnesen als Alarmsubstanz der Blattläuse ausfindig gemacht werden, welches in gefährlichen Situationen ausgeschüttet wird, um die anderen Läuse zu warnen. Weiters gelten Nepetalacton und Lepetalactol, zwei Bestandteile des ätherischen Öls der Katzenminze *(Lamiaceae)*, als Bestandteile deren Sexpheromons. Schildläuse hingegen senden verschiedene Lavandulylester aus. Fruchtfliegen sowie manche Bienen haben spezielle Mechanismen bezüglich ihrer Pheromonproduktion entwickelt. So gewinnt die Fruchtfliege Methyleugenol aus der Orchideenspezies *Bulbophyllum vinaceum* und entwickelt aus diesem anschließend zwei Bestandteile ihres Sexpheromons. Auf der anderen Seite imitieren gewisse Bienen einen Linalool-hältigen Orchideenduft, um Partner für den Paarungsprozess anzulocken. Bei den Termiten werden zahlreiche ätherische Öl-Komponenten als verschiedene Pheromone freigesetzt: Neocembren als Bestandteil des Spurenpheromons verschiedener *Prorhinotermes* Spezies, (E,E)-α-Farnesen als Alarmpheromon bei *P. canalifrons* oder Terpene wie γ-Cadinen als Primerpheromone bei *Reticulitermes* Spezies, die das Juvenilhormon bei der Bestimmung über die Position im Kastensystem der Termiten unterstützen. Ähnlich vielfältig verhält es sich bei den Stinkwanzen, wo (4S)-cis-(Z)-Bisabolenepoxid, (+)-α-Curcumen, (-)-Zingiberen, (-)-β-Sesquiphellandren und Zingiberenol eine Rolle spielen. Weiters wurde (E,E)-8,10-Dodecadien-1-ol als Sexpheromon des gefürchteten Apfelwicklers identifiziert. Der Vektor der viszeralen Leischmaniose, die Sandfliege, kann in mehrere weitere Spezies unterteilt werden, die anhand ihrer unterschiedlichen Sexpheromone, nämlich Derivate von α-Himachalen, Germacren D oder Cembren, ausgemacht werden können. Bei den Schädlingsinsekten wurden jeweils verschiedene Studien bezüglich deren Bekämpfung mit Pheromonfallen angeführt oder verglichen. Bei den Spinnentieren liegen noch sehr wenige Studien über Pheromone, insbesondere bei den Spinnen, vor. Bei Milben hingegen bilden Neral und Geranial als Alarmpheromonkomponenten wichtige Bestandteile.

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1. Introduction

Essential oils are known as ingredients of plants, providing them a characteristic and enjoyable odor. Monoterpenes and sesquiterpenes make up the major essential oil compounds. The release happens with the intent to allure insects and therefore enable the pollination. Furthermore, essential oils can protect the plants of various pests or diseases evoked through fungi or bacteria. In industry, the oils are extracted out of the plants and used for the production of perfumes or other cosmetic products. Moreover, they are applied in oil burners or added to foods to improve the flavor. In the medicine sector, essential oils are used for example as mucolytic or expectorant products (menthol, 1,8-cineole) or against gastrointestinal problems (essential oils of anise, caraway or fennel) or to encourage the blood flow (essential oils of mountain pine or common juniper). Further, they are utilized in aromatherapy. Less noted is the circumstance that many insects use certain essential oil components as main or minor parts of their numerous pheromones and thus draw the attraction of conspecifics to them. The core topic of this thesis deals with the occurrence of different essential oil constituents (monoterpenes or sesquiterpenes) or volatile organic compounds as pheromones of mostly pest insects and arachnids. Further insects such as bees, bumblebees, ants or butterflies will also be referred to because of their prominence and their interesting pheromones, e.g. the trail pheromone of ants. Pheromones of vertebrates will not be mentioned due to the fact that they mostly consist of lipid decomposition products. Research material was collected out of the timeframe from 2000 to 2010. In many examples, an interaction between plants and insects will be demonstrated. In some cases, plants serve as sources of pheromones, e.g. the fruitfly saps methyl eugenol out of a certain orchid species and consequently converts the substance into two different sex pheromone compounds. On the other side, bees imitate different orchid odors to enable mating activities. Furthermore, larvae of a certain gall wasp species force their host plants to change the composition of their scents and therefore signal their location to potential mating partners.

1.1. Pheromone definition

The word pheromone was derived by the greek terms “pherein” and “horman”, which mean “to carry” and “to excite”.[1] It was characterized by Butenandt and Karlson with the exploration of the silk moth’s bombykol. Earlier also known as ecto-hormones, today many authors avoid this description. The role of pheromones in humans or plants is not as important as in the realm of social insects. They vest the possibility for an intraspecies chemical communication and in some cases even for a communication in nearly related species. Although they similate hormones, pheromones cannot be counted among them. Whereas hormones are secreted into the blood, pheromones are excreted of the body. They are released from several different exocrine glands in a liquid form and later vaporize outside. Until now more than a hundred exocrine glands have been found in insects. The first two pheromones were identified in the 1960s with the queen substance produced by the honeybee and the already mentioned male attractant produced by female silk moths.

What distance can a pheromone lay back? That depends on several components: the wind, the compound, its steadiness in the air and of course a recipient with a good olfactory feature. Usually pheromones are small molecules, often lipids, with a molecular weight of maximum 250 atomic mass units.

To get a survey about pheromones it is necessary to distinguish the different existing types. First of all, one has to differentiate between releasers and primers. Releaser pheromones have a short effect and are responsible for an instant behavioral response when needed, while the long acting primers raise physiological changes in insects which lead to a response of their behavior. Releaser pheromones can again be distinguished in many further types, e.g. the sex pheromone, the aggregation pheromone, the trail pheromone, the territorial pheromone, the information pheromone, the alarm pheromone - even used in combination. Most relevance is given to the sex pheromone, the alarm pheromone and the aggregation pheromone.

1.1.1. Releaser pheromones

- Sex pheromones

Examples for sex pheromones in insects are the valeric acid in the sugar beet wireworm, the 9-keto-2-decenoic-acid in the honeybee, cis-7-dodecen-1-ol in the cabbage looper, cis-9-tetradecen-1-ol in the fall army worm, trans-10-cis-12-hexadecadien-1-ol in the silk worm moth, 2,3-dihydro-7-methyl-1H-pyrrolizidin-1-one, cis-vaccenyl-acetate and cetyl acetate in the male butterfly.

Those pheromones are released with the intention to find a partner for the mating. A female emporer moth for example releases such a pheromone, of which a male one takes notice with his palps even for a distance up to 11 kilometres.

- Alarm pheromones

Alarm pheromones are sent out in case of danger to warn the other members of the species. Taking notice of the pheromone, an insect tries to escape to a place with lower concentration of the alarm pheromone. So, the reaction depends on the concentration of the substance, a very small amount will not frighten an insect because it realizes that the danger is not near enough. Finding a dead member of the colony near the nest, an ant for example sends out a cloud of chemical substances to warn the others. Examples are heptan-2-one, tridecan-2-one, undecane, tridecane, isoamylacetate, 2-methylheptane-4-one, 4-methylheptane-3-one, 2,6-dimethyl-5-hepten-1-al, citral, citronellal or α-pinene.

- Aggregation pheromones

Most importance of the aggregation pheromones is given to the trail pheromones which lead other colony members to places further away. Bees for example sense them for hundreds of meters, terrestrial insects like ants notice them for at least several meters. Foraging ants lay trail pheromones to guide their nest-mates the right direction to the food source. Other purposes are nest emigration, mustering mates for repairing the nest or slave raiding. The trails laid by terrestrial insects are continuous or just minimally interrupted and get reinforced by other mates in case of leading to a very good food

source. On the other hand, bees only lay spots of pheromones. Examples for the honeybee's and the ant's aggregation pheromones are geraniol, geranial, neral, geranoic acid or nerolic acid or the bark beetle's 2-methyl-6-methylene-7-octene-4-ol, 2-methyl-6-methylene-2,7-octadiene-4-ol and cis-verbenol.

- Other pheromones

Territorial pheromones are part of a dog's urine to mark the ground or used by seabirds to mark their nests. Identification pheromones are characteristics of dogs, cats or rats to give information about their identity or get information about another's identity who sojourns in the same territory.

1.1.2. Primer Pheromones

Primer pheromones cause a change in development, what makes them different to releaser hormones, which cause a change in behavior. Probably the best example is the 9-keto-2-decenoic-acid in the honeybee. A normal beehive consists of only one queen with 30.000 to 80.000 of workers under her. If another queen arose, a deadly fight could mean the end of the current queen, so she is interested to avoid egg-production or nurturing of a new queen by their workers with the aid of 9-keto-2-decenoic-acid.

2. Pheromones of insects

Taking a look at the production of pheromones by insects, it gets obvious that there are not a number of specific enzymes being responsible for the output. In fact, insects have learned to develop appending altered types of enzymes being able to convert simple metabolic substances into pheromone components with unique stereochemical properties. Females of *Blattella germanica*, the German cockroach, produce their sex pheromone out of fatty acids, which later get decarboxylated, hydroxylated and oxidated. It is the same procedure in the *Coleoptera* species, which additionally uses the isoprenoid pathway or amino acid conversion to receive its aggregation and sex pheromone. On the other hand, there are insects like *Drosophila*, out of the *Diptera* species, where the fatty acid loses its carbonyl carbon. Females of many Lepidoptera species obtain their sex pheromones by fatty acids getting desaturated and shortened and a final carbonyl carbon reduction. The same procedure can be accomplished by carbon frames out of amino acids. Generally, three hormones are needed to grant pheromone production. Juvenile hormone III plays a role in the *Blattodean* and *Coleopteran* species for example. *Diptera* species use ecdysteroids out of the ovaries for sex pheromone production, having an effect on prolongating enzymes (elongases). So called "pheromone biosynthesis activating neuropeptides" modify enzyme work during the production of fatty acids in *Lepidoptera* species.[2]

2.1. Pheromones of ants

As a social insect, ants live in organized colonies with a caste system that makes a distinction between one or several queens, female workers, sometimes soldiers and male ants. The long-living winged queen is the only member responsible for reproduction. Winged male ants have a very short life with the single purpose to mate the queen. Workers are wingless and do jobs like foraging, brooding or building up and repairing the nest. With the help of pheromones the queen can be recognized by their workers. So what happens after a queen's death? That depends on the colony. Colonies like *Atta* and *Acromyrmex* also die because their egg producer is gone and no one is able to replace her. Studies on *Atta sexdens rubropilosa* have shown that the colony then has a probability of survival for about three months but they also reveal that the worker's

functions like foraging are independent of the queen's presence. Equally, the worker's communication did not change. So it seems as if the queen has no influence on external activities.[3]

Up to now more than fifty exocrine glands have been found in ants. Living on the ground, the trail pheromone takes a special role in the ant's life. Using the chemical trails produced by their nest-mates, ants find their way from their home to the food source and back. All the trails make up a network with crossings in which the colonies have to find the right orientation. Laying two tunnel bifurcations, one symmetrical and one asymmetrical, revealed that ants predominantly decided with the help of the trail pheromones, on the way to the forage as well as on the way back.[4]

Studies on the species *Temnotheras albipennis* show that ants are able to investigate their surroundings to plan out in advance. When their nest gets destroyed, there is a need to build a new one on a save place without enemies that is big enough and in a good hygienic status. In a study, a new nest with lower quality than the old one was built and the ants were put there for one week to get the chance to explore the less good shelter. Afterwards their good old nest got destroyed and another low quality-nest similar to the other (low-quality) one was built. The ants chose the new nest although the other one was quite familiar to them. Other studies had explored that this behavior did not show up when there were two high-quality nests, one familiar and one unfamiliar. In this case the ants had chosen the familiar one. When their pheromone marks and landmark cues were removed before the destruction of their old nest, they chose by accident between the two new nests. Consequently, the study showed that ants are able to remember and judge about the quality of new nest locations by using their trail pheromones.[5]

(Z)-9-hexadecenal, the trail pheromone of the Argentine ant, was recently used to enable the production of a micro-encapsulated spray to combat invasive ants in fields. In studies of the year 2008, researchers used single pheromone spots as well as the pheromone spray in a small field area. Studying the ant's reaction admitted three conclusions. First of all, the usually used trails were not preferred to other trails now. Furthermore, the way down from a wall to the ground was not found anymore because of the pheromone's distraction. Thirdly, the amount of ants grew in the pheromone manipulated sectors. The spray formulation was constructed with the aim of carnuba wax-coated quartz sand and showed up a half-life time of 30 hours. Sprayed in four

different concentrations in Hawaiian fields, the number of ants decreased as well as the foraging activities for two days.[6] Current studies of the year 2010 presented similar results. In bioassays, the researchers used treated filter paper that helped to perceive the presence of the pheromone and within 14 days the number of visible trails was reduced as well as the number of foraging worker ants on prepared food-lures. So it was established that the formulation helped to disrupt the trails of the Argentine ant.[7]

Another study with the Argentine ant's trail pheromone (Z)-9-hexadecenal was conducted to trial whether it had an influence on attraction and consumption of a liquid sucrose solution. Therefore, a blend of 50 microliters of a 10% sucrose solution with 20 microliters of the pheromone solution (10µg/ml) conduced as a trap. The quantity of ants baited by the blend was more than 150% higher. A field test with 50ml vials containing (Z)-9-hexadecenal and the sucrose solution showed similar effects. A plastic membrane provided with little holes was put around the vials to give the opportunity to drink out of them after turning them around. 50% of the vials were manipulated with 1µg of the trail pheromone before. For the time of four hours, the ants were observed while consuming the blend of the pheromone-treated and the non-pheromone treated vials. The manipulated vials showed a 29% higher attraction to the ants than the normal vials. After 24 hours the number increased up to 33%.[8]

An important skill regarding all animals is the possibility to differentiate nest-mates from non-nest-mates or enemies. Therefore, insects have a CHC (cuticular hydrocarbons) profile consisting of long straight-chain saturated alkanes with or without methyl groups, with or without one or more double bonds, so that there exist hundreds of different CHCs. CHCs are produced in oenocytes and most of the species show a very complex profile. However, *Formica exsecta* show a very simple CHC profile, dominated by dimethylalkanes and Z-9-alkenes, but studies have shown that only the colony-specific Z-9-alkenes play a role in nest-mate discrimination and help the workers of these aggressive species to pretend their nest from non-nest-mates.[9]

The trail pheromone of the species *Gnamptogenys striatula* is produced by the Dufour's gland. Major compounds are esters of 4-methylgeraniol and of the bishomogeraniol isomer (2E)-3,4,7-trimethyl-2,6-nonadien-1-ol. Bioassays proved that in a mix of synthetic racemates particularly esters of 4-methylgeraniol caused high attraction in the ants, while esters of the bishomogeraniol were unattractive. Thus, only the attraction of 4-methylgeranyl esters was tested against the attraction of the whole secretion and the

results showed noticeable lower reaction of the ants. On the other side, a blend of the racemic 4-methylgeraniol's octanoate, decanoate and dodecanoate, making up the majority of the Dufour's gland secretion, was as attractive as the whole secretion.[10]

The harvester ant, *Pogonomyrmex barbatus*, acts differently in laying trail marks compared to other ants. Their colonies are in funds of about eight different foraging paths and alternately use one of them each day. Patrollers chose the trail for their foragers using secretions of the Dufour's gland on a picked spot of the nest with a length of about twenty centimeters. This small track leads to the initial of today's chosen trail, which has to be taken only by the workers, without company of the patrollers. A study showed that without the patroller's attendance, the group of workers chose the same trail as on the day before. It was also shown that only the Dufour's gland's excretions had effect on the foraging ants, the poison gland's extract had not.[11]

Beside the significance of the trail pheromone, other noticeable effects of ant releases are pointed out with the following studies. Leaf cutting ants produce a secretion with antimicrobial substances to defend their nest from parasites and other micro-organisms. The major components citral, 4-methyl-3-heptanol, geraniol as well as hexanoic and octanoic acids, 2-heptanone, 3-octanone, β-citronellol, phenylacetic and indolacetic acids were tested on their efficacy against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Nearly all of them showed activity, but particularly citral, 4-methyl-3-heptanol, geraniol, hexanoic acid and octanoic acid were highly effective against *C. albicans*, so that there could be potential to antagonize the human candidiasis.[12]

Known as a primary compound of the bark beetle's aggregation pheromone, 4-methyl-3-heptanol with its four stereoisomers also plays a role as a component of trail pheromones in ants.[13] Furthermore, the alarm pheromone 4-methyl-3-heptanone was found to be similar in the leaf-cutting workers of *Atta opaciceps* and *A. sexdens sexdens*. The rest of their CHC profile, primarily including oxygenated compounds, showed differences in quality and quantity. That fact might lead to the conclusion that the excretion of the alarm pheromone is mainly task of the workers.[14]

Male samples of the species *Leptothorax gredleri* lose their interest on their female partners after the mating and perceive them as unattractive. Unmated or freshly mated female individuals reveal a CHC profile with a higher rate of branched alkanes and a lower rate of linear alkanes, which turns into the opposite within half an hour after the

mating. Female ants produce the new profile by themselves – and not only to look unattractive but also to help them finding shelter in a new reliable colony.[15]

2.2. Pheromones of beetles

2.2.1. Pheromones of bark beetles

The all over the world spread bark beetles are infamous for their wood damages. Whether leaf tree or conifer, no wood seems to be secure. Some species only attack one sort of tree, other species make no difference. In most cases, impaired or already dead trees are target of their attacks, but some especially dangerous species even destroy health wood and thus can bring whole areas to death. After drilling a hole, bark beetles prepare brood places for their descendants, in which the females lay their eggs. The most dangerous bark beetle of our sphere is the typographer with his attacks on spruces. Avoiding the involvement of bark beetles, it is important to timely recognize sick wood. An essential factor for their work is represented by the aggregation pheromone, which will be elaborated with the following studies.[**16**]

The differences in the composition of the species-specific aggregation pheromones could be pointed out by comparing 34 species of *Dendroctonus* and *Ips*. The studies revealed differences in related species as big as differences in non related species.[**17**]

Already known as an important compound of the ant's trail pheromone, 4-methyl-3-heptanol also plays a big role in the bark beetle's aggregation pheromone. Showing up four stereoisomers, (3S,4S)-4-methyl-3-heptanol could be determined as the major component of *Scolytus amygdale*, the almond bark beetle. Field tests lead to the insight that only the S,S-enantiomer – combined with (3S,4S)-4-methyl-3-hexanol working as a synergist – caused attraction in bark beetles. The other three stereoisomers did not show any effect.[13]

Dendroctonus valens LeConte, also known as the red turpentine beetle, determines huge harm by afflicting *Pinus tabuliformis* Carrière *(Pinaceae).* Trans-verbenol and cis-verbenol in conjunction with frontalin and exo-brevicomin make up the most important bark beetle pheromones. Studies using the single substances or a combination with

kairomones[1] were conducted to demonstrate the bark beetle's reaction. Female and male red turpentine beetles were attracted by all of the four components. However, (+)-3-carene as a host volatile caused more attraction than all of the four pheromone compounds. Studies conducted in the years 2006 and 2007 neither proved reaction to the single components nor to the combination. Frontalin as a single compound or in a blend with exo-brevicomin and trans-verbenol was found out to be responsible for decreasing attractiveness of (+)-3-carene in the study of 2006, whereas the study of 2007 could not approve this presumption.[18]

Studies with *Ips subelongatus* proved attraction to the conspecific substances ipsenol and ipsdienol - both are male-produced - as well as to heterospecific substances like cis-verbenol and verbenone and furthermore to host volatiles like α-pinene, β-pinene and p-cymene and non-host volatiles like geranyl acetone or alcohols. Racemic ipsenol was found out to be the only individual substance attracting males and females of *I. subelongatus*. Adding ipsdienol or 3-methyl-3-buten-1-ol or both together, no effect, nor higher attraction nor antagonistic activity, was apparent. On the other hand cis-verbenol and verbenone averted the reaction to ipsenol. The host volatiles α-pinene, β-pinene, p-cymene and even the non-host substance geranyl acetone did not cause attraction, but induced disruption in the bark beetle's pheromone reaction.[19]

The intent to explore the host selection of *I. typographus*, the spruce bark beetle, resulted in scanning 150 olfactory sensilla, which are used as sensory organs by insects. Pheromones, host-volatiles and non-host volatiles induced replying from 106 olfactory receptor neurons, the rest only showed low response. Out of the 106 replying olfactory receptor neurons, 45 were sensitive to pheromone components, 37 to host components and 24 to non-host components. Olfactory receptor neurons with high distinctive reaction were then classified into seventeen groups, of which seven showed main reaction to cis-verbenol, ipsenol, ipsdienol, 2-methyl-3-buten-2-ol, amitinol and verbenone. Further six groups were sensitive to α-pinene, myrcene and p-cymene plus 1,8-cineole and Δ^3-carene, the host volatiles. Non-host volatiles like 3-octanol, 1-octen-3-ol or trans-conophthorin were especially recognized by four groups.[20]

Cis-verbenol and 2-methyl-3-buten-2-ol represent the major compounds of the aggregation pheromone of *I. typographus*. Already more than ten years ago, in August

[1] In contrast to pheromones, kairomones enable an interspecies communication, through which primarily the receiver draws a profit from

1999, dose-dependent reaction was tested with the aim of traps releasing the two substances. Five different traps with consequently increasing amounts, going from a blank trap up to a Pheroprax trap (Pheroprax contains the pheromones of *I. typographus* and is used against it as an attractant in agriculture), were built up. In May 2000, a similar experiment with four trap-trees, again with increasing pheromone amounts, was accomplished. The higher the releasing-rate, the bigger was the attraction to the bark beetle. Wind effects led to random trap choices in the low-dose releasing traps. The high-dose trap with 15.2 mg/day and the Pheroprax trap were equally attractive. According to that, the potential of Pheroprax was approved and Pheroprax seems to work for distances up to fifty meters and more.[21]

Volatile substances spread by *Betula pendula (Betulaceae)* or *Picea abies (Pinaceae)*, which both do not apply as victims of the bark beetle, showed an effect in decreasing the reaction of the bark beetle *Pityogenes bidentatus* to cis-verbenol and grandisol, the main components of its aggregation pheromone. On the other side, also the volatiles of the host *Pinus sylvestris (Pinaceae)* caused a reduction of attraction. The same effect was shown by monoterpenes like α-pinene, β-pinene or terpinolene and Δ^3-carene of spruce and pine. Bark beetles of the species *P. bidentatus* in this manner have the possibility to timely differ a host tree from a non-host tree or an inappropriate one.[22]

Dendroctonus brevicomis LeConte, also known as the western pine beetle represents a big danger for *Pinus ponderosa (Pinaceae).* Up to now, no effective method could be found to protect the pine. *Dendroctonus* supspecies like *D. brevicomis* produce the antiaggregation pheromone verbenone, which is used as a pesticide against *D. ponderosa*, the mountain pine beetle and *D. frontalis* Zimmermann, the southern pine beetle. During a period of three years, *Pinus ponderosa* stands were treated with verbenone (50mg/d) to avoid damages caused by *D. brevicomis.* Unfortunately, the quantity of dead or attacked trees was nearly the same as in untreated trees. Investigations could not attest a degradation of verbenone or a defect of the verbenone-pouch. However, bioassays describe an effect of the verbenone-pouch for a distance of two meters. So it is necessary to wait for further results to make a decision whether to use verbenone as a bio-weapon.[23]

2.2.2. Pheromones of flea beetles

Flea beetles are characterized by their shimmering bodies and a big thigh bone on their hind legs, which is used for long-distance jumps. These jumping activities gave them the name "flea beetle". Female beetles place their eggs under the ground in spring or late summer. Later the larvae eat on the roots of the plants. As an adult, the beetles climb up the plants to eat the leaves. Because oft the foliage holes, especially left on young plants in the time of May to June, the flea beetle is considered as a pest.[24]

Phyllotreta cruciferae, the crucifer flea beetle, subsists on leaves of his host plant. Due to the notice that the male beetles are allured to their male conspecifics as well as to their female conspecifics, releases of both sexes, currently consuming some cabbage, were gathered in quest of an aggregation pheromone. Excretions were also gathered from *Aphthona flava*, *A. czwalinae* and *A. cyparissiae*, three further species, equally currently eating their host plants, serving as control. The male crucifer flea beetle showed six different components in its volatiles, which were also found in the releases of the other three species in addition to two further substances not being present in the crucifer flea beetle. (+)-Aryl-himachalene, (+)-trans-α-himachalene, (+)-y-cadinene were recognized as well as two himachalene hydrocarbon enantiomers being found in the firs *Abies nordmannia* and *A. alba (Pinaceae)*. Furthermore, a norsesquiterpene ketone, representing an analog of himachalene, and two himachalene alcohols were detected. Five of the named substances caused changes in electrophysiology. The acting of the substances as an aggregation pheromone could not be established but is likely.[25]

A further study investigated the effect of a trap consisting of four himachalene sesquiterpenes and (+)-γ-cadinene to the crucifer flea beetles in Hungarian fields. (+)-γ-Cadinene was obtained from plants, whereas the other four racemic substances were synthesized. The response of the insects was low and only the addition of allyl isothiocyanate, an alluring substance out of their hosts, led to successful results. Similar outcomes had been studied in North America too. Traps containing the racemic components were equally effective as the natural enantiomers, whereas the contrary enantiomers did not cause attraction. The current study revealed that the presence of only one substance was essential for attracting the beetles: (6R,7S)-2,2,6,10-tetramethylbicyclo[5.4.0.]undeca-9,11-diene. In the fields, also the nearly related species *Phyllotreta vittula*, *P. nemorum*, *P. ochripes* and *P. nodicornis* were trapped.

Thus, (6R,7S)-2,2,6,10-tetramethylbicyclo[5.4.0.]undeca-9,11-diene, acting as the major attractant, and the other minor components seem to appear in the volatiles of the most *Phyllotreta* species.[26]

The odors of *Epitrix fuscula* Crotch, the eggplant flea beetle, were also collected when consuming its host plant. Analyzes of the male releases revealed six different components: (2E,4E,6E)-2,4,6-nonatrienal and (2E,4E,6Z)-2,4,6-nonatrienal representing the major compounds and further two himachalene sesquiterpene hydrocarbons as well as two himachalene sesquiterpene alcohols. The himachalene sesquiterpenes were the same as in the volatiles of *P. cruciferae* and subspecies of *Aphthona*. Regarding the retention time in gas chromatography, the NMR spectra and the mass spectra, the beetle-released substances and the synthetic versions showed same results. It was just the same with the himachalene sesquiterpenes and their appropriate synthetic substances. (2E,4E,6E)-2,4,6-nonatrienal and (2E,4E,6Z)-2,4,6-nonatrienal and three of the four himachalene sesquiterpenes caused attraction in GC-EAD tests. Blends consisting of the two first mentioned components were prepared in fields and the success in trapping the flea beetles was significantly higher compared to untreated traps. Due to these results, the aggregation pheromone could be useful in the fight against *E. fuscula* in aubergine fields.[27]

2.2.3. Pheromones of click beetles

Generally, click beetles are brown-black to black, lightly haired insects living in bushes. When being disrupted or feeling endangered, they let themselves fall down and feign death. When landing on their backs, a special breast joint helps them to escalate and land on their legs again. This activity causes a characteristic noise being responsible for the name “click beetle”. Their larvae are rated as pests, living in leaves, moss or under the bark of a dead tree and eating other insect’s larvae and plant roots.[28]

Volatiles of female click beetles out of the *Agriotes brevis* species were analyzed and geranyl butyrate and (E,E)-farnesyl butyrate were found to make up the main part of the beetle’s sex pheromone. A 20mg – 200mg blend consisting of the two substances in a 1:1 ratio was released out of vials and caused high attraction in the click beetles. The effect remained constant in the 73 days of investigation. Geranyl butyrate also

represents the major compound in the sex pheromone of *A. sputator*. However, the traps did not capture these click beetles in areas colonized by *A. brevis* as well as *A. sputator*, leading to the conclusion that (E,E)-farnesyl butyrate is responsible for this discrimination. Traps were construed to be entered either by flying in or by flying and creeping in. Traps with both access possibilities showed higher numbers of caught individuals than the other ones in the early season, thus, an appropriate trap design guaranteeing a high season-long success is needed.[29]

The identification of the components occurring in the volatiles out of the click beetle's pheromone gland led to the creation of pheromone traps with a high potential. *A. brevis*, *A. sordidus* and *A. rufipalpis* are three most common click beetle pests in the centre and west of Europe. As already mentioned, a blend of geranyl butanoate and (E,E)-farnesyl butanoate is responsible for attraction in *A. brevis*, whereas the single substance geranyl hexanoate represents the sex pheromone of the other two species. Using the knowledge about Russian species, the components for further traps in Europe were chosen in the following way: *A. lineatus* should be combated with a mixture of geranyl butanoate and geranyl octanoate, geranyl isovalerate should be used against *A. litigiosus*, *A. obscurus* should be baited with the help of geranyl hexanoate and geranyl octanoate, *A. sputator* should be trapped by geranyl butanoate and lastly, (E,E)-farnesyl acetate should be used in the fight against *A. ustulatus* (the black and the red form). Thanks to these outcomes, war can be declared against click beetles in a very efficient way in the centre and west of Europe.[30]

British Columbia served as location for field studies about pheromone traps containing a mixture of geranyl esters intended for the fight against click beetles, more precisely against male individuals of *A. lineatus* and *A. obscurus*. Regarding *A. obscurus*, a 1:1 blend of geranyl hexanoate and geranyl octanoate recorded the highest amount of captured pests. A mixture of geranyl butanoate and geranyl octanoate in a relationship of 1:9 or 1:10 was must successful in alluring *A. lineatus*. A usage of the examined pheromone baits could help trapping the pests in the north of America.[31]

The main sex pheromone components of another click beetle species, *A. proximus*, had been declared as (E,E)-farnesyl acetate and neryl isovalerate in former studies. Investigations conducted in Bulgaria and Portugal led to the result that high amounts of this species were caught by traps containing geranyl butanoate and geranyl octanoate,

the main compounds of the sex pheromone of *A. lineatus*. Therefore, further studies were accomplished in the fields of different countries in Europe, demonstrating a low response to the single presence of geranyl butanoate by *A. proximus* and an also low response to the single presence of geranyl octanoate by *A. lineatus*. The biggest success could be obtained by blending the two substances. Baits consisting of (E,E)-farnesyl acetate and neryl isovalerate did not capture a single individual of *A. proximus*. Additionally, geranyl octanoate was preferred to geranyl butanoate by both species in electroantennographic tests. Thus, geranyl butanoate and geranyl octanoate mixed in a relationship of 1:1 can be applied for locating or controlling the two species in the fields of Europe.[32]

GC-MS analyzes of the gland releases out of female click beetles belonging to the *A. acuminatus* species, revealed neryl butanoate and 2,6-dimethyl-(Z,E)-2,6-octadien-1,8-diol dihexanoate as the only two sex pheromone components, appearing in a 1:5 mixture. Synthetic production of the two substances approved their structure. In the fields, male exemplars of the click beetle species showed high response to neryl butanoate. The only presence of 2,6-dimethyl-(Z,E)-2,6-octadien-1,8-diol dihexanoate did not attract a single individual, but its addition to neryl butanoate forced the reaction. Regarding the fact that by now all other species of *Agriotes* release geranyl or (E,E)-farnesyl esters, *A. acuminatus* is the first species showing neryl esters with a (Z)-2-configuration in its sex pheromone.[33]

The larvae of the click beetle *Elater ferrugineus* are known as predators of other insect larvae, especially those of *Osmoderma eremita*, the scarab beetle. Their preferred habitats are hollow trunks of trees. The main compound of the male scarab beetle's sex pheromone had been characterized as (R)-(+)-γ-decalactone. During a study, the utilization of this pheromone as a kairomone guiding the click beetles the way to their captures, was investigated. Lindgren funnel-shaped baits containing (R)-(+)-decalactone were placed in the fields as well as neutral traps. The pheromone manipulated baits caught a significantly higher number of click beetles than the other one. The presence of (R)-(+)-decalactone could not be assessed in the volatiles of *E. ferrugineus*, proving that it is not part of its own pheromone. Due to the fact that both females and males responded to the sex pheromone of *O. eremita*, (R)-(+)-decalactone seems to serve the click beetle as an indicator of the way to the habitat, where its conspecifics sojourn and prey can be found. Pitfall traps could not reach the number of captured click beetles

achieved by the Lindgren traps. Regarding the trapped amount of scarab beetles, both systems showed similar results. Therefore, traps containing (R)-(+)-decalactone could be used as a pest management against both click beetles and scarab beetles.[34]

Another study was concerned with the issue of the importance of the (R)-enantiomeric structure of γ-decalactone. Electrophysiological tests and field studies were conducted. Most olfactory receptor neurons showed reaction to both (R)-decalactone and the racemic blend. None of the receptor neurons favored the racemic version to the natural compound, thus (S)-decalactone responding receptors seem to be absent. Chiral division of the (R)- and the (S)-enantiomer inside a racemic blend approved the result by analyzing GC-linked recordings of a single sensillum. Moreover, an inhibitory activity of (S)-decalactone could be excluded because of a persistent attraction to (R)-decalactone in field traps also containing the (S)-enantiomer. Additionally, (S)-decalactone did not act as an antagonist in the click beetle *E. ferrugineus*. For economical reasons, the application of the racemic pheromone component could be a good alternative to the use of (R)-decalactone.[35]

Studies investigating the sex pheromone of *E. ferrugineus* showed that its main components are 7-methyloctyl 5-methylhexanoate (1 part), 7-methyloctyl octanoate (1 part), 7-methyloctyl 7-methyloctanoate (3 parts) and 7-methyloctyl (Z)-4 decenoate (3 parts).[36]

2.2.4. Pheromones of other beetles

All over Europe, *Anthonomus rubi*, the strawberry blossom weevil, counts as a feared pest in strawberry fields. Pheromone components of the insect were studied to enable the production of a pest management strategy without using insecticides. Analyzing the odors of males and females gathered out of the fields, showed three characteristic male produced components: lavandulol, (cis)-1-methyl-2-(1-methylethenyl)cyclobutane-ethanol and (Z)-2-(3,3-dimethylcyclohexylidene)ethanol, that were released in amounts of 0.82 µg, 1.2 µg and 6.1 µg per day in each individual. The latter two mentioned substances are also known as the boll weevil's aggregation pheromone components grandlure II and grandlure I. Small numbers of grandlure III ((Z)-(3,3-dimethylcyclohexylidene)acetaldehyde) and grandlure IV ((E)-(3,3-

dimethylcyclohexylidene)acetaldehyde) could be found too. The exact configuration of lavandulol could not be identified. Strawberry plants of the species *Fragaria ananassa (Rosaceae)* were figured out to emit higher amounts of the characteristic germacrene D when surrounded by the pheromone releasing pest. Pheromone emission of the males could only be noticed near to the strawberry plant. No release was shown in presence of *Matricaria recutita (Asteraceae)* or *Anthriscus sylvestris (Apiaceae)*, both serving as its food sources. Traps consisting of different mixes of the synthetic lavandulol and grandlure I-IV were placed in the fields. Higher amounts of weevils captured in the baits were achieved by a mixture of lavandulol, grandlure I and grandlure II compared to lavandulol absent traps or untreated control traps. Grandlure III and grandlure IV did not cause attraction. In the midst of the season, the number of captured females was higher than the number of captured males. However, afterwards it was the other way round. Furthermore, horizontal orientated baits were more successful than vertical placed ones.[37]

In Korea, *Platypus koryoensis*, an ambrosia beetle (closely related to the bark beetles), is notorious for its damages on oaks. With the aim to control the situation, studies were conducted to characterize its aggregation pheromone. Secretions of both sexes either of the whole corpus or just a part of it were investigated utilizing GC-FID and GC-MS. The male volatile releases included geraniol, geranial, nerol and neral. Considering the female ones, neither of those substances was found in the releases of a special body part and only very small amounts could be established for the secretions of the whole corpus. Furthermore, citronellol was found in the dust of unmated male exemplars produced when destroying the tree, while it was not present in the dust of mated or female ones, as well as the other four substances. Electroantennography studies demonstrated reaction to all of the cited components in both sexes, leading to the idea to use them as a trap in fields. As the experiments were effective, geraniol, geranial, nerol, neral and citronellol seem to work as an aggregation signal in the ambrosia beetle.[38]

Megacyllene caryae is a cerambycid beetle, which is also known as a wood destroying pest. Male secretions were investigated and showed up eight typical components: (2S,3R)-hexanediol and (2R,3S)-hexanediol in a 10:1 ratio, 2-phenylethanol, nerol, neral, geranial, (S)-(-)-limonene and α-pinene. Each substance caused attraction in males and females in Y-tube olfactometric tests. Field tests led to the result that the use of just one compound was unsuccessful and only the whole mixture was able to bait

males and females. Leaving out one of the eight components led to a strong decrease of attraction. This complexity of a mixture of terpenoids, aromatic alcohols and alkanediols was not found before in other pheromones of cerambycid beetles.[39]

In poultry industry, *Alphitobius diaperinus*, a lesser mealworm beetle, represents a dangerous pest. While other damaging beetles are combated more and more with the aid of pheromone traps, the existence or composition of the lesser mealworm beetle's pheromone could not be found for a long time. In a study, odors of both sexes of the beetle, currently occupying poultry products, were investigated. GC-MS results pointed out five components sent out by the males: (R)-(+)-limonene, (S)-(+)-linalool, (E)-β-ocimene, (R)-(+)-daucene and 2-nonanone. Female emissions could not be found. Regarding the males, release was figured out to start one to two weeks after getting an adult and lasting for minimum one year, depending on disposability and sort of food. Synthetic mixtures of the male emitted components caused reaction of male and female beetles, suggesting the odor to work as an aggregation signal. Further studies will be needed to determine the necessity of the use of all five substances and the efficacy in field investigations.[40]

2.3. Pheromones of plant lice

Plant lice can be separated into five families: aphids, coccids, psyllids, whiteflies and phylloxerans. All together they make up about 8000 species. Body size is varying from 0.5mm to 38mm. Concerning aphids, about 800 different species are known by now in Central Europe, all over the world there are a few thousand species. Occupying a plant, the reproduction follows quickly and soon a big colony lives on shoots, buds and on the underside of the leaves. With the help of their proboscis, they steal the leave's plant sap and subsequently the leaves curl and die. Frequently, aphids transfer diseases to the plants. On their backs, aphids are fitted with two siphons, which release a sticky secretion containing the alarm pheromone. Aphids use to move about freely, whereas coccids mostly remain on a chosen place, psyllids change places by jumping.[41]

2.3.1. Pheromones of aphids

(E)-β-Farnesene is the typical alarm pheromone of most aphid species sent out in dangerous situations when having recognized a predator or an enemy to warn and save the other members of the species. Using the example of the cotton aphid, *Aphis gossypii* Glover, the amount of release related to weight and life's period was recorded using GC-MS. Both aphids in nature and aphids in glasshouses showed contents about 0.1 to 1.5 ng of (E)-β-farnesene. The higher the weight, the higher was the pheromone amount, which explained the different quantities of each individual. Interestingly, the concentration of the pheromone (ng/mg weight) was higher in the low body weight aphids trying to make up for their smaller body size, but still not being able to keep up with the large ones.[42]

Another study determined the excreted quantity of (E)-β-farnesene of the pea aphid, *Acyrthosiphon pisum*, during the raid of larvae of *Chrysoperla carnae*, the lacewing. Within one hour, the quantity was measured every second minute using gas chromatography. Instantly after the attack, a very high released amount could be detected, decreasing consequently but still being traceable after half an hour. Nymphs were characterized with a higher excretion than grown ups but the reaction time was the same in both stages. The mean amount was about 16.33 ng (+/- 1.54 ng).[43]

Stating the question, whether aphids of the species *A. pisum* react to the alarm pheromone of other members by sending out the own alarm pheromone and in this way reinforce the warning, the emission of (E)-β-farnesene after the recognition of another individual's alarm pheromone was detected. The amount of only one aphid does not help to warn a satisfactory number of colony members, nevertheless the results of the study could not approve an adoption of alarm pheromone emission.[44] The same issue was substance of another study, this time using deuterated (E)-β-farnesene to distinguish the aphid's pheromone from the synthetic substance. Again individuals of *A. pisum* were in the focus of interest. The results once again showed no intensification of the alarm pheromone.[45]

Insect odorant-binding proteins (OBPs) are made responsible for cognition and distinction of different scents. Four genes including the code of the OBPs in the pea aphid were cloned and three of the proteins (1, 2 and 8) subsequently were expressed. The three OBPs in some cases showed similarity, in other cases diversity concerning

different ligands. OBP3 presented high attraction to (E)-β-farnesene and farnesol, whereas the other ones did not. According to that, OBP3 seems to be participated in the reaction to the alarm signal in aphids.[46]

Mentha x piperita (Lamiaceae) was used to extract the (E)-β-farnesene synthase gene to further clone it and finally express it in *Arabidopsis thaliana (Brassicaceae)*, a helpful plant concerning experimental purposes. Releasing the aphid's alarm pheromone, changes in behavior were found in the *Myzus persicae* species. Following the idea of avoiding aphid damages by using manipulated plants, it will need more than that, because only one exemplar of the pest can lead to destruction of the whole plant.[47]

Many tomato species release different volatiles when being colonized by damaging insects such as *Myzus persicae* Sulzer than in normal situations to allure hostile insects. Attacked by the aphid species, tomatoes show a higher attraction to *Episyrphus balteatus* De Geer, a hoverfly, which subsequently lays eggs on the affected plant. Comparing the volatiles emitted by attacked tomato species and non-attacked tomato species using solid phase microextraction GC-MS, α-phellandrene, β-phellandrene, Δ^2-carene, Δ^3-carene and α-pinene were characterized as the major compounds showing high reaction in electroantennography experiments on the hoverfly. (E)-β-Farnesene turned out to be the only sesquiterpene with attraction to *E. balteatus* De Geer and therefore seems to be responsible for the egg laying on the tomato species.[48]

The Lamiaceae species *Hemizygia petiolata* Ashby, the purple sage, shows large amounts of (E)-β-farnesene in its essential oil. Therefore, the suggestion was made to obtain the alarm pheromone out of the plant and use it as a pest monitoring. Reaction to the essential oil was studied on *M. persicae* Sulzer and *A. pisum* in laboratory as well as outside. Attraction was not as high as estimated because of the further compounds (+)-bicyclogermacrene and (-)-germacrene D, decreasing the reaction of the aphids to the alarm pheromone. Olfactometric tests, however, showed plant-shunning behavior of the oil by *A. pisum* and *Sitobion avenae* F., known as the grain aphid. The last-mentioned species also was influenced by (-)-germacrene D. *M. persicae* Sulzer could not directly be allured by the plant's oil, but it obstructed the affinity to host plants. Field studies revealed a lower quantity of pea aphids in essential oil-manipulated areas than in untreated places.[49]

Beside the alarm pheromone, even the sex pheromone controls the aphid's behavior. The main compounds (1R,4aS,7S,7aR)-nepetalactol and (4aS,7S,7aR)-nepetalactone also appear in *Nepeta cataria (Lamiaceae)*, the catmint, and thus can be obtained by these plants in a regenerative way. The easy access to the sex pheromone compounds with the aid of the catmint simplifies the realization of new pest management plans. Combined with other typical volatiles, parasitoids such as the lacewing can be attracted and help controlling the pests. So, *N. cataria* is the first plant used for obtaining an insect pheromone.[50]

Males, winged virginoparae and gynoparae of the bird cherry-oat aphid, *Rhopalosiphum padi*, were studied on their behavior when being exposed to (-)-(4aS,7S,7aR)-nepetalactol and (+)-(4aS,7S,7aR)-nepetalactone and also benzaldehyde, a host volatile. Electroantennogram responses could be pointed out for each substance, highlighting the male exemplars with the strongest response. The two sex pheromone compounds were higher attractive to gynoparae than to the virginoparae, which in turn showed higher response to the host volatile. Even though the lactone is not part of the bird cherry-oat aphid's sex pheromone, it led to equal electroantennogram reactions as the lactol. Olfactometric tests detected extremely higher attraction to nepetalactol-manipulated, nepetalactone-manipulated and also benzaldehyde-manipulated air than to normal air in males and also showed a stronger reaction to the lactol than to the lactone. However, blends of these two components had no effect at all. Virginoparae were not attracted to any of the three substances, gynoparae only reacted to nepetalactol, but not comparable to the males. With the aid of this study, differences between the winged virginoparae and the gynoparae could be pointed out as well as the importance of the sex pheromone components for mating activities and benzaldehyde for host choice in males.[51]

The pheromone release of female individuals, strictly speaking virgin female oviparae, of *Macrosiphum euphorbiae*, the potato aphid, was studied with the help of GC and GC-MS. (1R,4aS,7S,7aR)-Nepetalactol and (4aS,7S,7aR)-nepetalactone could be identified in a 4:1 or 2:1 relation, depending on the aphid's age. During wind tunnel experiments, male potato aphids were attracted to oviparae-occupied potato plants, whereas unoccupied potato plants were not attractive. Reaction was also determined to virgin aphids and mixtures of nepetalactol and nepetalactone in 3:1 up to 5:1 ratios and similar attraction could be shown. The virgins were found faster than the pheromone baits. 1:1 blends caused less attraction and one-component baits did not cause any

reaction at all. However, in the fields the attraction rate to the pheromone blends was low.[52]

Considering the fact, that all aphid species use the same pheromone compounds, leads to the quest of factors being responsible for the species specifity. Studies investigating a time-dependent release were conducted on *Dysaphis plantaginea*, the rosy apple aphid. Pheromone releases of oviparae were gathered to further study them with the help of GC, MS and microscale NMR spectroscopy. A 3.7:1 relation of nepetalactol and nepetalactone could be pointed out, leaving the question of the exact point of time. Therefore, releases of 95 oviparae, showing the same age, were collected every hour for a timeframe of 20 days. In the photophase, the amount of nepetalactol and nepetalactone was high, whereas in the scotophase an obvious decrease was detected. The initial three photophase hours could be characterized with increasing quantities, then being stable until the beginning of the scotophase. The 3.7:1 relation of the two pheromone components remained constant until day 14, changing into a reduction of nepetalactol on day 15. The biggest amount was sent out on day 8. Regarding the results, further items may be responsible for species distinction.[53]

One of these factors can be shown with the following study. Females of the rosy apple aphid raise volatile release in occupied trees. Hexylbutyrate, (E)-2-hexenylbutyrate, (Z)-3-hexenyl-3-methylbutyrate and hexyl-2-methylbutyrate enforce the response of males to the (4aS,7S,7aR)-nepetalactone and (1R,4aS,7S,7aR)-nepetalactol sent out by the females. Using pheromone traps containing a 1:1:1 blend of three of the four esters, higher attraction to the rosy apple aphid than to other aphids was caused. Traps only consisting of the esters were not successful just as the addition of only one ester to the pheromone mixture. With the aid of these results, for the first time a plant eater-effected volatile release could be attested, helping the males of the *D. plantaginea* species to find their way and not drawing males of other species, which use the same sex pheromones.[54]

A further survey was performed on *D. plantaginea* to question the existence of another sex pheromone compound beside nepetalactone and nepetalactol. Volatiles of the female rosy apple aphids, currently eating their favorite fruit, were taken and four of the components were found to cause attraction in the males: nepetalactone, nepetalactol, phenylacetonitrile and (1S,2R,3S)-dolichodial. For the first time, dolichodial could be detected as an aphid-released substance, leading to electrophysiological attraction and

change in behavior. The role of (1S,2R,3S)-dolichodial as a part of the rosy apple aphid's sex pheromone needs to be debated.[55]

2.3.2. Pheromones of coccids

Lavandulyl senecioate was detected as the sex pheromone of mealybugs, more specifically the vine mealybug, *Planococcus ficus* Signoret. The racemic substance showed the same effect in male individuals as (S)-lavandulyl senecioate, the only enantiomer released by the females. Volatiles of virgin females furthermore contained lavandulol, which was found to inhibit the male's attraction in high amounts. Baits manipulated with pheromone amounts reaching from 10µg to 1000µg showed the same response. In the fields, traps containing 100µg of the racemic substance kept the mealybugs effectively off for twelve weeks and worked in a circle of 50 meters.[56]

In the course of another study, also investigating the vine mealybug, two pheromone compounds were found in individuals nurtured on potato plants. Additionally to (S)-lavandulyl senecioate, also (S)-lavandulyl isovalerate could be detected using GC and GC-MS. Males showed response to either substances in Petri dishes and flying around in the nurturing area. Going outside into the vineyards, the males only responded to (S)-lavandulyl senecioate. According to that, the pheromone was studied again and it could be shown that the first generation of female descendants of the outside-gathered exemplars only released (S)-lavandulyl senecioate, whereas the further laboratory nurtured descendants also produced (S)-lavandulyl isovalerate with a rising quantity in each generation. Regarding these results leads to the conclusion that the sex pheromone of wild exemplars only consists of (S)-lavandulyl senecioate, whereas the sex pheromone of exemplars nurtured on potato plants in the laboratory furthermore contains (S)-lavandulyl isovalerate.[57]

Californian vineyards served as location for pest management studies on *P. ficus* Signoret. Racemic lavandulyl senecioate was distributed in a microencapsulated form with the help of a sprayer in 2003 and 2004. In June 2004 even a formulation of buprofezin was added to the pheromone bait. Results were matched with untreated areas for the whole season. In 2003, the frequency of mealybugs in the pheromone-treated areas was lower as well as the number of damages, which was lower in 2003 and 2004.

In the timeframe of April to September, the frequency of vine mealybugs was only 12.0 per cent (+/- 15.6) lower in 2003 and only 31.1 per cent (+/-11.6) lower in 2004. In 2003, there was also a decreasing number of egg laying in the pheromone manipulated vineyards. Based on the mealybug frequency, vine sorts had been characterized as low, medium or high mealybug attracting sorts in the time before the pheromone treatment. Low attracting vine showed a decrease of 86.3 per cent (+/- 6.3) in mealybug infestation after the utilization of the pheromone bait. On the other hand, no decrease was detectable on high mealybug attracting vine sorts. Moreover, the effect of the sprayer was also lowered by the brief lifetime of the microencapsulated formulation.[58]

The sex pheromone of female exemplars of *Maconellicoccus hirsutus*, the pink hibiscus mealybug, was found to contain (R)-lavandulyl (S)-2-methylbutanoate and maconelliyl-(S)-2-methylbutanoate, actually named [(R)-2,2-dimethyl-3-(1-methylethylidene)cyclobutyl]methyl (S)-2-methylbutanoate. A 1µg blend consisting of the two synthetic components in a ratio of 1:5 was effective in field tests. Maconelliol represents a cyclobutanoid monoterpene and maconelliyl-2-methylbutanoate has not been detected in nature before.[59]

A further study was conducted to reveal the importance of the two chiral centers appearing in the sex pheromone components of the pink hibiscus mealybug. Pheromone baits containing the different isomers were prepared. The natural mealybug-released substances (R)-lavandulyl (S)-2-methylbutanoate and (R)-maconelliyl-(S)-2-methylbutanoate furnished the best results, whereas (S)-lavandulyl (S)-2-methylbutanoate and (S)-maconelliyl-(S)-2-methylbutanoate caused low response in the males. Baits consisting of the two components in an R-R or S-R configuration did not achieve any effect at all. Adding these substances to the successful R-S configuration trap, an avoiding reaction could be detected. Considering the outcomes of this study, it gets obvious, that males of *M. hirsutus* precisely differ between the different configurations, whereby the (S)-configuration in the functional group of the acid turned out to be more important than the (R)-configuration in the functional group of the alcohol.[60]

Since the discovery of the components of the pink hibiscus mealybug's sex pheromone, several pheromone baits have been developed. In 2007, a study was accomplished to compare the different products in their effect against the pest. Green Delta, Jackson, Pherocon IIB, Pherocon V and Storgard Thinline are names of pheromone traps that

were tested in Homestead, Florida, a state very pestered by the mealybug. The evaluation was a consequence of the amount of caught males, the quantity of males found on each unit (cm^2) of the trap surface, the number of other trapped stuff and finally, how long it took to elicit the captured amount. Green Delta, Pherocon IIB and Pherocon V, presenting a large surface, attained a higher number of trapped mealybugs than the other two baits, showing a smaller surface area. The least amount was caught by the Storgard Thinline baits. On the other hand, the number of mealybugs trapped per cm^2 was similar in the Jackson bait as in the three large-surface baits. Furthermore, it took the least time to count the number of pests in the Jackson baits. The amount of other material captured by the five traps was negligible. Considering these results, the Jackson pheromone bait seems to be the most effective product.[61]

The Madeira mealybug, *Phenacoccus madeirensis*, was investigated to characterize its sex pheromone. Volatile releases of virgin females were collected and (1R,3R)-chrysanthemyl (R)-2-methylbutanoate and (R)-lavandulyl (R)-methylbutanoate were detected in a relationship of 3:1 with the aid of GC, MS and microchemical examinations. Synthetic components were utilized to determine the chirality. Male mealybugs showed high response to the synthetic trans-(1R,3R)-chrysanthemyl (R)-2-methylbutanoate in the laboratory, whereas only low reaction to synthetic (R)-lavandulyl (R)-2-methylbutanoate could be assessed. A blend of the two components did not show amplification of attraction.[62]

2.4. Pheromones of moths and butterflies

2.4.1. Pheromones of moths

The codling moth represents a big problem for apple trees all over the world. Appearing to be an innocent butterfly, it causes huge damages that can be seen by early fall of the fruits. Eggs are laid on leaves and fruits and one or two weeks later, the caterpillar hatches out. Cutting an affected apple in two pieces, a faeces-containing burrow leading to the core, which is occupied by the caterpillar, can be seen. Female codling moths are able to lay 30 to 60 eggs.[63]

Larvae of the species *Cydia pomonella* L., also known as the codling moth, communicate with the aid of an aggregation pheromone to draw other larvae or in other cases warn them when looking for save pupation spots. The hostile parasitoid *Mastrus ridibundus* Gravenhorst, an ichneumon fly, is also able to recognize these chemical volatiles, but as shown in experimental tests, only prepupae or larvae with the maximum age of three days awaken the female host's interest. Ten chemical components were found to be responsible for the attraction: Myrcene, Δ^3-carene, geranylacetone, heptanal, octanal, (E)-2-octenal, nonanal, (E)-2-nonenal, decanal and sulcatone. A mixture consisting of these ten substances in addition with (+)-limonene, known as a common compound of cocoon releases, presented the same attraction to the female host as well as to other larvae. Just three of the eleven compounds did not decrease the reaction of the ichneumon fly when being left out, demonstrating the importance of the multiplex blend.[64] In a further study, cartons were fixed on trees representing pupation locations, partially colonized with other larvae or treated with the aggregation pheromone. The number of larvae spinning their cocoons in the manipulated areas of the cartons was higher than in the untreated areas. These results support the idea of using the aggregation pheromone for controlling larvae of *C. pomonella* in fruit gardens.[65]

The results of studies with male *C. pomonella* exemplars subjected to the sex pheromone (E,E)-8,10-dodecadien-1-ol, the codlemone, were analyzed with the assistance of electroantennogram and flight tunnel tests. Therefore, pheromone volatiles out of an Isomate-C Plus trap (a sex pheromone dispenser used in fields) were emitted for seconds or maximum a few minutes and after that the flight behavior of the males was studied. Baits were laid containing either 0.1 mg of (E,E)-8,10-dodecadien-1-ol or a 0.1 mg mixture of (E,E)-8,10-dodecadien-1-ol, dodecanol and tetradecanol. After the cognition of the Isomate-C Plus releases, completely disorientated behaviors were detected, although no differences between the manipulated males and untreated males regarding their electroantennogram responses could be pointed out. Even the one-component bait and the three-component bait were tested on their effect when presented before flying, showing a similar but weaker success than the Isomate-C Plus trap. Outside, Isomate-C Plus releasers were fixed on trees, each time ten samples. Baits containing 1 mg or 0.1 mg of codlemone were not located by 88.3 per cent or 95.9 per cent of the males, meaning that a certain amount still found the right way and could not be influenced by the Isomate-C Plus traps.[66]

For three seasons, apple gardens in Michigan served as the location for the development of five dual pheromone traps against *C. pomonella* and *Grapholita molesta*, the oriental fruit moth. The blend consisting of Isomate CM (against the codling moth) and OFM TT (against the fruit moth) was as effective as the single chemical formulations in manipulating the male individuals, although reaching the season's end, the attraction to the fruit moth decreased, ending up in a higher number of damages. This decrease was traced to the degradation of the chemicals. Another formulation consisting of Isomate CM, OFM TT and LR (against the leafroller) showed the same effectiveness. Especially orchards attacked by several pests could benefit from the multiple traps, nonetheless further experiments will be needed to improve and particularly prolong the attraction.[67]

The codlemone antagonist (E,E)-8,10-dodecadienyl-trifluoromethyl-ketone might submit a new way in combating *C. pomonella*. Electrophysiological tests showed less reaction to the sex pheromone in individuals previously being exposed to the antagonist compared to untreated individuals. 1:1, 1:5 and 1:10 mixtures of the codlemone and its antagonist all showed modification and interrupt of behavior in the male moths when nearing the trap in a wind tunnel, resulting in alternating flights. As opposed to this, individuals only exposed to the pheromone presented normal behavior. Going out to the fields, the 1:10 blend also led to less reaction. Nevertheless, (E,E)-8-10-dodecadienyl-trifluoromethyl-ketone had no influence on the degradation enzymes of the codlemone, being located in the male moth's antennae, leading to the conclusion that its electrophilic potential is not high enough to bind on the enzyme's cysteine.[68]

Apple and pear orchards served as location for studies of a sex pheromone spray consisting of microcapsules against the codling moth. Little amount releases of the highly concentrated product were most effective using twelve to 23 liters per ha with pressures of 172 to 207 kilopascals. Studies conducted in the year 2004 had matched the activity of two sprayers releasing little quantities to a spray with higher output. Here the amount of codling moths trapped by the spray was not comparable to the amount caught by the high-volume releaser. Regarding these findings, the concentration of the spray seems to determine its activity. Further studies in apple and pear gardens were made in 2005 and 2006. Little amount releasing sprays were compared to pheromone releasers used by hand. The number of damages did not show mentionable distinctions between the different applications. However, the microencapsulated product showed a higher quantity of moths being caught in the apple orchards in 2005. Further results will be

needed to judge about the usage of microencapsulated sex pheromone sprayers in fruit gardens.[69]

2.4.2. Pheromones of butterflies

Although not counted to the pests, the butterfly's pheromones containing essential oil components will be further discussed because of its prominence.

Regarding female butterflies, finding a partner for mating depends on both visual factors and odors. Volatiles especially play a role in short distances. *Pieris napi* is the green-veined butterfly of which its male individuals spray citral. A study dealt with the question after the reasons and the requirements for the citral output. It could be demonstrated that the butterfly species is sending out the component when acting on males or females of the same species or males counting to another species and even when being alone. Flight behavior was found to have strong influence on the emitted amount and being responsible for the different recorded quantities in each butterfly. Therefore, it seems likely that citral is not emitted consciously, but automatically within flight activities. Females revealed a much higher attraction to citral compared to males during electroantennogram studies, resulting in a relationship of 10:1. Presenting wings, freshly cut out of male butterflies, or citral-manipulated wings to female butterflies caused higher response than the presentation of normal wings. Due to the results of this study, citral seems to act as a sex pheromone, released by the male butterflies of *P. napi* during mating activities.[70]

The presumption of an existence of a monogamy promoting substance released by already mated females of the butterfly species *Heliconius erato* was spoken out by Gilbert in 1976. During a study, conducted in 2008, the pheromone components of the related species *H. melpone* were analyzed. Furthermore, the butterfly's behavior was investigated and precursors of the pheromones were presented to the species. Odors found in the secretions of the male abdominal glands presented (E)-β-ocimene as the major component beside a few detected tracing substances and several fatty acid esters containing ethanol, isopropanol, 1-butanol, isobutanol, 1-hexanol or (Z)-3-hexenol as alcohol components. These substances are produced within the initial days of an adult butterfly. During mating, the blend, which was not found in virgins, is transmitted to the

female individuals. This fact could be proved by studying male and female exemplars of the butterfly species previous to mating activities as well as afterwards. Furthermore, male butterflies were nurtured on D-$^{13}C_6$-glucose and consequently presented and transmitted ^{13}C-ocimene within the mating activities. Virgins manipulated with ocimene elicited avoiding behavior in their male conspecifics, which was not caused when manipulated with the fatty acid esters. Nevertheless, the fatty acids were responsible for a decreased frequency of volatile output. According to the results of this study, β-ocimene functions as a so called antiaphrodisiac pheromone in *H. melpone*.[71]

2.5. Pheromones of termites

Termites are represented by 2000 species, mostly tropical ones. Similar to ants, termites live in colonies and have a strict caste system. They exclusively feed on plants. Thus, they are pests for every house, eating and burrowing through the wooden parts. 1930 *Reticulitermes flavipes* was imported to Europe by the USA.[72]

Reticulitermes species in America were investigated to figure out the components of soldier volatile releases. 45 different substances could be pointed out, representing monoterpenes, diterpenes and sesquiterpenes. (-)-α-Pinene, (-)-β-pinene, (-)-camphene, myrcene, (-)-limonene and both (Z)- and (E-)-ocimene, (+)-γ-cadinene, (+)-γ-cadinenal, (-)-germacrene A and B, γ-himachalene and finally β-bisabolene were identified.[73] In Europe, seven *Reticulitermes* species were studied, finding 16 different components: α-pinene, β-pinene, limonene, germacrene A, B and C, β-, γ- and Δ-selinene, (E)-β-farnesene, nerolidol, γ-cadinene, geranyl linalool, geranyl geranial, geranyl geraniol and geranyl farnesol.[74]

Mastotermes darwiniensis, *Porotermes adamsoni* and *Stolotermes victoriensis* are three widely spread species of termites which were analyzed to research their trail pheromone. *M. darwiniensis* workers release a trail pheromone with the help of their sternal gland, despite not investigating new surroundings in groups but on their own and not being able to succeed experimental tracks in field studies. During the studies, (E)-2,6,10-trimethyl-5,9-undecadien-1-ol, a norsesquiterpene alcohol, turned out to be the main pheromone compound in each of the three species, but showing different release

amounts with 20 pg in *M. darwiniensis*, only 4 pg in *S. victoriensis* and 700 pg in *P. adamsoni*.[75]

The soldiers of the three termite species *Prorhinotermes canalifrons*, *P. simplex* and *P. inopinatus* were focus of a study concerning the components of frontal gland volatiles. Beside the presence of nitroalkenes, also the appearance of sesquiterpene hydrocarbons could be demonstrated. (E)-1-Nitropentadec-1-ene was discovered as the main compound, occurring in amounts of 152 µg, 293 µg and 207 µg per soldier, besides two nitrodienes and further four 1-nitroalkenes could be identified. Proceeding to the sesquiterpenes, differences in the three species became visible. Regarding *P. simplex*, just the (3Z,6E) isomer of α-farnesene could be found, whereas the other two species also released the (3E,6E) isomer. Even the amounts of (3Z,6E)-α-farnesene differed, coming to 39 µg per soldier in *P. simplex*, but only 0.5 µg in *P. canalifrons* and 1.5 µ in *P. inopinatus*. Additionally, also small amounts of trans-β-bergamotene and (Z)-γ-bisabolene could be attested in the releases of *P. canalifrons*.[76]

Individuals of *P. canalifrons*, were investigated to explore behavior and electroantennographic reaction to releases of the frontal gland, containing the main compounds (E,E)-α-farnesene and (E)-1-nitropentadec-1-ene. (E)-1-Nitropentadec-1-ene given alone had no effect at all. On the other side, (E,E)-α-farnesene induced the same alarm response as the whole volatile releasing, and therefore was accounted for the alarm pheromone in termites. Being exposed to (E,E)-α-farnesene, the walking speed of the termites increased and other colony individuals were advised. Beside the soldiers, also pseudergates[2] were attracted by the chemical component, but did not show a similar strong reaction as the soldiers. Both groups showed an increase in reaction when raising the dose of (E,E)-α-farnesene, leading to the conclusion that the appropriate receptor is the same.[77]

With the aid of GC-MS, neocembrene (cembrene A) was figured out to make up the main compound of a trail signal released by three *Prorhinotermes* species: *P. canalifrons*, *P. inopinatus* and *P. simplex*. Dodecatrienol quite likely represents a further compound of this pheromone, appearing in lower amounts. These conclusions were drawn due to results of electroantennogram and GC combined electroantennogram studies conducted with *P. simplex*. Further approval was given to the presumption of

[2] Members of the workers in lower termites, being able to turn into a reproductive

neocembrene and dodecatrienol representing the trail pheromone during trail-following experiments.[78]

The termite species *R. flavipes* was observed to figure out whether the soldiers produce a primer pheromone with the function to control the several tasks of each colony member and either elicit or avoid caste specialization. Head essences of soldiers combined with the juvenile hormone were found to be responsible for the specialization of workers. The single utilization of the head extraction did not influence the differentiation or other issues in workers. According to that, soldier volatiles seem to work as primer pheromones, enforcing the potential of the juvenile hormone. Former studies had recognized γ-cadinene and γ-cadinenal as the major compounds of the soldier head extracts using GC-MS and NMR. Tests applying synthetic cadinene showed positive outcomes. Recently found further terpenes indicated similar results, drawing the conclusion that terpenes operate as primer pheromones in *R. flavipes*, maintaining the function of the juvenile pheromone regarding caste specialization.[79]

2.6. Pheromones of bugs

Bugs are represented by a number of 40000 species worldwide and about 8000 species in Europe. They are plant sap sucking pests, predators or parasitoids like the blood sucking bed bug. Two antennae and a proboscis are placed on their heads. Predatory bugs are able to transfer various diseases, whereas plant sapping bugs are troublesome pests in the nature. The plant damaging stink bugs got their name because of their disgustingly smelling secretion released in cases of danger. Bed bugs are night active insects and exhibit a size of about four to six millimeters. They prefer warm places like human living areas or henhouses. Females lay about 250 to 300 eggs during their life. After blood sucking, they leave red itching dots on the human victims.[80]

2.6.1. Pheromones of stink bugs

Y-tube studies on adults of *Acrosternum hilare*, the green stink bug, showed that virgin male exemplars caused interest in virgin female ones. Other males did not show reaction to their conspecifics, leading to the presumption, that a sex pheromone is

released by the male bugs. Secretions of both sexes were taken to identify the components and the male ones were found to comprise (4S)-cis-(Z)-bisabolene epoxide as the main compound. The sex pheromone was not found in premature males. The effect of the whole secretion was studied on the female exemplars, showing reaction to it, but divided in four parts, the three parts without (4S)-cis-(Z)-bisabolene epoxide and the less important (4S)-trans-(Z)-bisabolene epoxide did not show any effect. The bisabolene epoxide-containing fraction caused the same attraction as the whole secretion. Single presentation of synthetic (4S)-cis-(Z)-bisabolene epoxide or (4S)-trans-(Z)-bisabolene epoxide was ineffective, on the other hand a mixture with a 95:5 ratio of cis and trans - representing the genuine secretion of the male bugs - showed positive results in Y-tube tests. In the fields, baits were fixed in alfalfas, containing either 1 mg of the 95:5 cis:trans mixture or pentane as a comparison. The female bugs showed much higher attraction to the pheromone trap contrary to the pentane trap and the highest effect was attained in the time of late afternoon and evening.[81]

Studies on *Chlorochroa sayi*, the large green stink bug, were performed to investigate its sexual attitudes. Previous to the first mating process, a ten day lasting sexual maturing stage was passed by the males. Competing for the females, the males used their antennae and started head-butting until the females showed interest and the mating could start. The production of the sink bug's sex pheromone is the highest in the hours of late afternoon and evening, resulting in a large number of matings accomplished during this time. Secretions of the adult male stink bugs contained three typical compounds: methyl geranate, methyl (E)-6-2,3-dihydrofarnesoate and methyl citronellate in a relationship of 100:1.6:0.45. Females showed response to the male volatiles in a y-tube olfactometer as well as to a mixture of the three pheromone compounds. In the fields, the quantity of females allured by the pheromone trap was low, maybe because of other factors playing a part in attracting them, e.g. vibrations.[82]

Regarding the sex pheromone of another species of stink bugs, *Thyanta pallidovirens*, the red-shouldered stink bug, (+)-α-curcumene, (-)-zingiberene, (-)-β-sesquiphellandrene and (E2,Z4,Z6)-decatrienoate were determined as main components. Studies showed that adult males could engage the adult female's attention, whereas other males could not be influenced as well as females were not able to cause attraction in conspecifics of the same sex. Secretions of both sexes were explored, revealing that certain components appearing in adult males were not present in female adults or

premature males. Furthermore, the male secretions were shown to cause attraction in the female bugs. Dividing the extracts into fractions, the whole effect was gone, leading to the conclusion that minimum two chemically different substances are responsible for the pheromone's activity. Adding one of the three sesquiterpenes to (E2,Z4,Z6)-decatrienoate, the reaction of the females was recovered. Adding two or all three of them, no rise of response could be demonstrated. A mixture of synthetic substances was as successful as the natural male secretion. In a certain field section, traps containing the synthetic components showed success in catching the females, whereas tests in larger field areas could not attain the desired results. Secretions of the closely related species *T. accerra custator* McAtee showed high similarity to the releases of *T. pallidovirens*, differing from each other just by the addition of (E)-2-decenal.[83]

Olfactometric tests were also conducted in *Tibraca limbativentris*, the Brazilian rice stalk stink bug. Adults of both sexes were investigated for their attraction to secretions of their conspecifics. Results showed that female bugs were interested in the releases of their male congeners, whereas males did not demonstrate reaction, just like males and females were not interested in other female emissions, submitting that a male produced sex pheromone is in the case. The best effect of the pheromone was achieved in the night hours. Studies using GC and GC-MS were accomplished to characterize the emissions of both sexes. Either volatiles contained defensive components, but in the male releases even two isomers of 1'S-zingiberenol could be pointed out. There are three chiral centers in zingiberenol. An unspecific syntheses of the substance generates two isomers: zingiberenol I (1RS, 4RS, 1'R)-4-(1'5'-dimethylhex-4'enyl)-1-methylcyclohex-2-en-1-ol and zingiberenol II (1RS, 4RS, 1'S)-4-(1'5'-dimethylhex-4'-enyl)-1-methylcyclohex-2-en-1-ol. The attraction to the females of the two of them was higher than the attraction of a hexane trap serving as a check. There is a need to clarify the whole configuration of the sex pheromone compounds.[84]

Soybean fields in the centre and south of America are often seriously damaged by *Piezodorus guildinii*, the neotropical redbanded stink bug. With the aid of GC-MS and following tests, its sex pheromone could be characterized as (7R)-(+)-β-sesquiphellandrene. Only the volatiles of male adults contained the substance, spreading about 40 ng of it in one day. Olfactometric experiments with 200 ng of the synthetic component, put on a piece of filter paper, showed the same positive effect as the

releases of males with the age of 15 days as well as the collected volatiles and also the extracted part of the volatiles including the sesquiterpene hydrocarbon.[85]

The attraction of stink bugs to pheromones of another stink bug species was studied on farms for two years. The effect of the pheromones of *Acrosternum hilare* (the green stink bug), *Nezara viridula* (an exotic species, the southern green stink bug), *Euschistus servus* (the brown stink bug) and *Plautia stali* Scott (the brown-winged green bug) was investigated on the first three mentioned species. Studies on fields realized first that *N. viridula* could be manipulated with traps treated with its pheromone, a 1:3 ratio of cis-(Z)-α-bisabolene epoxide and trans-(Z)-α-bisabolene epoxide. Higher pheromone amounts led to a stronger response. The 95:5 mixture of cis-(Z)-α-bisabolene epoxide, the sex pheromone of *A. hilare*, did not show any effect to the exotic stink bug. On the other hand, the members of *A. hilare* reacted to the pheromone of *P. stali*, known as methyl (E,E,Z)-2,4,6-decatrienoate. Methyl (E,Z)-2,4-decadienoate, the reported pheromone of *E. servus*, showed the highest attraction to *E. servus*, compared to the other three species – with or without the addition of methyl (E,E,Z)-2,4,6-decatrienoate. Further results demonstrated that stink bug parasitoids were also attracted by the secretions of the male exemplars, e.g. the response of *Trichopoade pennipes* to pheromone baits of *A. hilare* and *N. viridula*, or the reaction of *Cylindromyia* species to pheromone traps of *E. servus*. Finally, it seems that the highest effect to catch or prove the involvement of the named stink bugs in the fields can be achieved by using a combination of the pheromone mixture of *N. viridula*, the pheromone of *E. servus* and the pheromone of *P. stali*.[86]

2.6.2. Pheromones of bed bugs

Regarding *Cimex lectularius*, the common bed bug, it is known that after frequenting their victims, they turn back to an aggregation point. Therefore, the search for an aggregation pheromone started. Odors of their meeting points were collected and analyzed with the help of LC and olfactometric studies. 14 different components with a presence over 100 pg were detected using GC-MS. Ten of them could be established as crucial ingredients: (+) and (-)-limonene, sulcatone, benzyl alcohol, nonanal, decanal, (E)-2-hexenal, (E)-2-octenal, (2E,4E)-octadienal and benzaldehyde.[87]

2.7. Pheromones of flies

2.7.1. Pheromones of fruit flies

All over the world, about 4500 species of fruit flies are known, only 290 of them appearing in Central Europe. The life expectancy comes up to only a few days. Females are equipped with a kind of sting on their backs, used for putting their eggs directly into fruits or other parts of plants. The larvae are able to survive in any plant part. Many species cause huge damages in the fields and orchards.[88]

Pheromone compounds of *Anastrepha suspensa*, the Caribbean fruit fly, were identified as α-farnesene, β-bisabolene, anastrephin, epianastrephin and suspensolide. Epianastrephin was found to make up the major part of the pheromone, whereas α-farnesene represented the minor compound in the male oral secretions. The components were also found in the affected crop. Releases of female fruit flies did not contain any of the mentioned substances. Studying the effect of the male releases to the female individuals, attraction could be shown, whereas females did not react to the releases of other females. The quantity of the released anastrephin and epianastrephin was varying during the day and during the life of a male fruit fly. α-Farnesene, β-bisabolene and suspensolide showed higher presence in the tissue of the crop than in the sap. On the other hand, anastrephin and epianastrephin again showed different quantities in crop and liquor within 24 hours, but in total, a rise of these substances from morning to evening and a decline of β-bisabolene and suspensolide during this time could be attested. Additionally, the enzymatic conversion of suspensolide to anastrephin and epianastrephin in the crop was attested.[89]

Methyl eugenol is the reactant of 2-allyl-4,5-dimethoxyphenol and (E)-coniferyl alcohol, the two compounds of the sex pheromone of *Bactrocera dorsalis*, a tephritid fruit fly. Methyl eugenol is known for its strong response and reaction in male flies. The two biosynthesized substances are then accumulated in the rectal gland and further sent out within the mating in the evening hours. 2-Allyl-4,5-dimethoxyphenol and (E)-coniferyl alcohol could be found in the crop organ as well as in the haemolymph. The latter was investigated consequently in fruit flies baited with methyl eugenol and fractions of extracts were collected for further studies. Two of the gathered fractions caused strong interest in the male exemplars. The protein absorbance of the haemolymph elution was significantly higher in the two attracting fractions of the male

fruit flies baited with methyl eugenol, showing a molecular weight of about 3.3 kDa to 5.5 kDa, in contrast to the elution of untreated males. Finally, the occurrence of the two methyl eugenol derived substances could be demonstrated by using GC-MS.[90]

Tephritid fruit flies are attracted by the flowers of *Bulbophyllum vinaceum (Orchidaceae)* which contain high amounts of phenylpropanoids. Substances like methyl eugenol, 2-allyl-4,5-dimethoxyphenol, trans-coniferyl alcohol and trans 3,4-dimethoxycinnamyl acetate are the main compounds incorporated by the fruit flies and then used for the production of the sex pheromone. Besides, also eugenol, trans-3,4-dimethoxy cinnamyl alcohol, cis-coniferyl alcohol and eusarone are gathered. The largest amount of the useful substances is located in the flower's lip, which is soon headed for by the flies after their landing on the plant's petals. The lip must be opened by the insects to get to the right position and gain the attracting substances. After leaving the lip, it turns back into a closed status. The whole process represents a big but precious effort for the fruit flies, gathering a high number of phenylpropanoids used for the production of the sex pheromone compounds and therefore carrying the orchid's pollinarium, which is now sticking on their backs, and thus enabling the pollination.[91]

B. dorsalis, the oriental fruit fly and *B. curcubitae*, the melon fly, are two serious pests in Hawaii. Therefore, three different formulations were tested in the fields to control the situation: the dispensers SPLAT ("specialized pheromone and lure application technology" with methyl eugenol or cue lure), Acti-Gel and Min-U-Gel were combined with either Spinosad or Naled, both insecticides. Min-U-Gel methyl eugenol combined with Naled served as control during the 12 week long lasting study. SPLAT methyl eugenol combined with Spinosad showed the same effect as the control formulation. On the other hand, SPLAT cue lure in combination with Spinosad was only similar attractive in the last six weeks and not in the first six weeks. According to these results, SPLAT methyl eugenol and Spinosad was scrutinized closely and matched with Min-U-Gel methyl eugenol plus Naled. During the twelve weeks, the first six weeks showed assimilable outcomes for both formulations, but from that point on, SPLAT methyl eugenol plus Spinosad was more successful in a papaya as well as in a guava field. Under different weather conditions, the cue lure releasers SPLAT and Naled, SPLAT and Spinosad and Min-U-gel plus Naled all worked for about 70 days. SPLAT methyl eugenol combined with Spinosad was similar effective as new releasers for up to four weeks under bad weather conditions. These outcomes lead to the conclusion, that both

SPLAT methyl eugenol and SPLAT cue lure sprayers combined with Spinosad are an alternative for insecticides containing organophosphates in the fight against fruit flies in Hawaiian orchards.[92]

2.7.2. Pheromones of sand flies

Lutzomyia longipalpis (Lutz & Neiva), the phlebotomine sand fly, is known as the major vector of *Leishmania chagasi* (Chagas and Cunha) and therefore responsible for causing the American visceral leishmaniasis (Kala Azar), which afflicts the inner organs. Intermittent fever attacks, enlargement of the spleen and the liver, reduction of weight or even anaemia are noticed. Only female sand flies exert this vectorial function by sucking blood out of their hosts, either animals or humans, when attracted to groups of males flying around the potential victim and releasing their characteristic sex pheromone. Thus, by noticing the pheromone, the fly on one hand finds a host and on the other hand can practice mating with a male conspecific. *L. longipalpis* is located in temperate and tropical regions. A vaccination does not exist, but the disease can be treated.[93]

L. longipalpis, the sand fly, is composed of four strains that have developed due to allopatric speciation[3] in South and Central America. These presumptions are also confirmed by isozyme investigations. One of the strains was found to be *L. pseudolongipalpis*, the identification of the other ones is not concluded by now. However, investigations concerning the sexual manners of sympatric[4] and allopatric sand flies, led to the presumption of the existence of not less than four further strains. Gene studies and cross-mating investigations support this idea. Sympatric exemplars of Brazilian *L. longipalpis* were analyzed within a study to clarify these contradictions. Three of the presumed species existing in Brazil, are known to show a cembrene component together with (S)-9-methylgermacrene-B and 3-methyl-α-himachalene as constituents of their sex pheromone, released by the male sand flies. During investigation using GC-MS, two different types of the sex pheromone were detected among the sympatric flies. On one side, a cembrene type already known due to prior studies in Brazilian sand flies was found and on the other side, a new type of cembrene

[3] Development of a new species due to geographic separation
[4] A new species that developed out of an existing species, without an influence of geographic isolation

could be demonstrated. Therefore, the latter presumption seems to be confirmed and *L. longipalpis* seems to consist of four further strains in Brazil.[94]

As already mentioned, males of *Lutzomyia longipalpis* aggregate in the near of hosts. Thereby, they emit (1S,3S,7R)-3-methyl-α-himachalene as their sex pheromone out of their secretion glands, located in the tergites with the intent to attract female conspecifics. Thus, a study was performed searching for a function of (1S,3S,7R)-3-methyl-α-himachalene not only as a sex pheromone, but also as an aggregation pheromone among the male sand flies. Chiral GC studies of the male releases as well as of the synthetic version of the pheromone and a synthetic blend consisting of the eight 3-methyl-α-himachalene isomers were combined with electroantennogram analysis of both sexes. Regarding all compounds of the male produced gland extract, attraction could only be shown to (1S,3S,7R)-3-methyl-α-himachalene by males and females. The synthetic blend only caused reaction in the (1S,3S,7R)-3-methyl-α-himachalene containing part. Wind tunnel experiments revealed an attraction to all three traps (male gland extract, (1S,3S,7R)-3-methyl-α-himachalene and the synthetic isomer blend) by both sexes. Therefore, the sex pheromone of *L. longipalpis* seems to cause a female attraction on one side and act as an aggregation substance for male conspecifics on the other side.[95]

As there may be differences in the vectorial function within the various strains in the sand fly, it is necessary to be aware of their distinctions. The male produced sex pheromone, as already mentioned, varies among the four Brazilian species. Eleven Brazilian and Venezuelan populations were studied with the aim to confront their sex pheromone with their different phylogeographic profiles. Same geographic locations did not show mentionable distinctions in the genes of the regarded populations. Geographic separation, by contrast, showed significantly high differences. A correlation between the dimension of distinction and an increasing geographic displacement could only be shown in a very small extent. Finally, five different clusters could be demonstrated: *L. cruzi* in Brazil, *L. pseudolongipalpis* in Venezuela, two further species with 9-methyl-germacrene-B as the major pheromone in both countries and a complex group of cembrene-releasing sand flies in Brazil.[96]

A further study was concerned with the comparison of the sex pheromones released by two *L. longipalpis* populations occurring in two different cities in São Paolo (Arcatuba and Espírito Santo do Pinhal) by the use of GC-MS. The pheromones were figured out

to disagree among the two populations. In addition to the fact that the two cities present a significantly diverse epidemiological state, these results reinforce the conclusion of (S)-9-methylgermacrene-B releasing and cembrene-1 releasing individuals possessing a varying vectorial function.[97]

Up to now, the total amount of different species belonging to the *L. longipalpis*-complex is still not clarified. Exemplars of the sand fly, collected from all over Brazil, were investigated regarding a certain gene that is responsible for regulating the circadian rhythm and triggering the constant frequency of mating songs used by males of *Drosophila melanogaster* and related species. Furthermore, the songs as well as the pheromones were scrutinized. Two big clusters of *L. longipalpis* were detected. On one side, a sole species, characterized by emitting cembrene-1 as a pheromone and presenting burst-type mating songs, on the other side a much more involved cluster, consisting of a number of species and differing from each other in their pheromone output as well as in the production of diverse pulse-type mating songs. Concerning these results, Brazilian sand fly populations show remarkable genetic differences and once again the considerations of epidemiological effects caused by the specification occurrence come to the fore.[98]

Asunción in Paraguay is a region with a very high population number of *L. longipalpis* and therefore, investigations searching for the sex pheromone of this certain species were conducted. Using GC-MS, (S)-9-methylgermacrene-B was detected as the male released pheromone of *L. longipalpis* in the Paraguayan area, which is also already known as the major component of several regions in Central America, Brazil and Colombia.[99]

New management plans against the sand fly were put to the test by using the male produced sex pheromones in 2007. Furthermore, odors of sand fly hosts were added to some traps to notice an eventual rise of attraction to the bait. Traps only consisting of the sex pheromone were shown to cause reaction in the females. A ten time higher concentrated pheromone blend could neither attract a larger quantum of insects, nor decrease the not attracted amount. Scents out of *Mesocricetus auratus*, the Syrian hamster, were used to represent the host odor and elicited higher response in the females than clean air, when used without the addition of the pheromone, but this reaction was referred to the presence of hexane in the scent. Thus, the single presence of the hamster volatiles acting as an attracting odor did not seem to be successful. A blend of host scent

and pheromones led to higher numbers of virgin sand flies showing interest in the bait and also decreased the amount of not reacting females. Therefore, the presence of the hamster odor seems to amplify the pheromone elicited effect. No bait, no matter if containing only the pheromone, only the host odor, or both components, was effective in male exemplars. However, adding the sex pheromone to the host odor was shown to cause shunning behavior in the males. Regarding these outcomes, the male released pheromones of *L. longipalpis* obviously mainly act as female attracting volatiles and an amplification of a trap's effect can be achieved by using a blend of pheromones and host odor or placing the pheromone baits near to host animals.[100]

A further study was conducted with the aim to find an economic way to combat the sand fly *L. longipalpis*. The synthetic sex pheromone (+/-)-9-methylgermacrene-B, obtained from an intermediate of plants, could be found to elicit female response during laboratory investigations. Field tests were accomplished with releasers containing the pheromone and emitting it in a frequency like being noticed in the male sand flies. Both females and males showed reaction to the baits outside. The releasers were same effective when equipped with cheap sticky traps. Hence, traps containing (+/-)-9-methylgermacrene-B could possibly reduce the vectorial activities of *L. longipalpis* when placed in appropriate locations. Additionally, this management demonstrated a low-cost affair.[101]

So far, existing management plans against *L. longipalpis* have not brought the desired success and the number of diseases in Brazil is proceeding. Vaccines have still not been found and dogs being infected get killed. In the course of the following study, conducted in 2010, it was tried to find a contribution for controlling the insect with the help of the known pheromone (+/-)-9-methylgermacrene-B. Two types of strategies were tested: in the first one, sticky insecticide traps were placed on animal hutches, the second plan consisted of pheromone traps being located on these insecticide treated hutches. The combination of the sex pheromone to chicken houses, manipulated with the insecticide, attracted and killed higher amounts of both sexes in comparison to traps without the addition of the pheromone. The emission of a ten times higher quantum of pheromone raised the number of captured females. The single usage of the insecticide significantly reduced the caught male amount and almost significantly reduced the female amount. Then again, the same effect was noticed when adding the synthetic (+/-)-9-methylgermacrene-B during insecticide release. Regarding the insecticide traps,

different colors are disposable. A larger amount of male sand flies was found to be captured by the yellow ones in contrast to blue colored ones. Further, the combination of the yellow traps with the pheromone baits led to significantly higher numbers of attracted male individuals compared to traps without pheromones. Despite the fact, that females demonstrated an equal interest in blue and yellow pheromone containing sticky traps, higher than in white traps, no remarkable amount of female sand flies could be captured by any of the traps in the chicken houses. Regarding the results of this study, the usage of the synthetic sex pheromone seems to be most successful in addition to insecticides. Excluding the insecticide, the further release of host scent might be needed to be most effective.[102]

2.8. Pheromones of bumblebees, bees and wasps

2.8.1. Pheromones of bumblebees

Although not counted as a pest, the bumblebee and the bee will be further discussed because of its interesting pheromones and its big role as a pollinator in nature. Bumblebees prefer northern temperate areas. Among the several species, *Bombus terrestris,* the buff-tailed bumble bee, seems to be the most pervasive one. An exceptional position is represented by the cuckoo bumblebee, which is not able to gather pollen anymore and depends on other bumblebee species. Its name points out the similar behavior to the cuckoo – they both place their eggs in other nests. Generally, bumble bees exhibit hairy legs, a red, white, brown, or yellow-brown tail and two yellow stripes – one in the front of the abdomen and the other one more behind. Their nests are located on the ground and can comprise up to 350 workers. Initially, the queen of the *B. terrestris* species has to collect the nectar of 6000 flowers to reach the required temperate for brooding the eggs. With the help of a pheromone, the queen makes workers out of the larvae.[103]

Marking pheromones of male exemplars of bumblebee and cuckoo bumblebee species are known as 2,3-dihydrofarnesol, 2,3-dihydrofarnesal and citronellol. Enantioselective GC was used to elicit the exact configuration of the three components. *B. terrestris*, *B. pratorum*, *B. lucorum*, *B. pyrenaeus*, *B. impatiens*, *B. jonellus* and finally *B. bohemicus*, the cuckoo bumblebee, all exhibited (-)-(S)-2,3-dihydrofarnesol, (-)-(S)-2,3-

dihydrofarnesal and (-)-(S)-citronellol in their labial glands. For several years, samples were taken out of the seven species being located in different places. All absolute configurations could be proved with the exception of the configuration of 2,3-dihydrofarnesol in *B. terrestris*, which could not be clarified.[104]

Bumblebees perform characteristic dances to signal the need for food. Former interpretations of their behavior had stated that this dance could be an analog to the dances of honeybees. Now it is known that the dances are conducted with the objective to spread a recruitment pheromone. Eucalyptol, ocimene and farnesol were detected in the tergal glands and in the volatiles of bumblebee nests. The amount of the three released substances rose during forage activities. During tests, in which nests were subjected to the terpenes, eucalyptol showed the highest recruitment behavior. Terpenes are also released by honeybees for indicating food spots.[105] So, the bumblebee's recruitment pheromone is released with the purpose to request the colony members to abandon the nest on the one hand and to inform the others about a good food place on the other hand. A study was conducted in 2009 to figure out, if this pheromone leads to a higher memorization of floral scents. The pheromone was presented to bumblebee colonies in combination with the odor of anise. No enhancement in learning the flower's odor could be approved. Spreading the floral odor in the surrounding air of the colony led to an increase of learning as well as the odor's presence in a honeypot's nectar did. The latter result was probably achieved because of the connection between odor and consumption. The best effect in learning a new odor was reached when successful foraging bumblebees came back to the nest with anise-containing nectar. Therefore, a so far unknown cue, released by the fruitful foragers, seems to improve the learning of new floral scents in the colony members.[106]

B. terrestris, a eusocial bumblebee, was center of a study searching for the compounds of its queen sex pheromone. GC-EAD, GC-MS and studies of behavior were conducted. Male bubmblebees showed strong response to virgin queens that were frozen at a temperature of -20° C before. Subsequently, fake bees were manipulated with secretions collected out of the cephalic part of the labial gland and the top of frozen (-50° C) virgin queens and compared to untreated fake bodies, resulting in a higher reaction to the manipulated fake bumblebees by the males. Furthermore, extracts out of the cephalic region were applied onto the bodies of queens and caused attraction in the males. Altogether, eletroantennographic recordings showed reaction to 21 components found

in the cephalic secretions and in the surface of the queens. Beside several fatty acids, either saturated or unsaturated, and some of their methyl esters or ethyl esters, also geranyl geraniol, heptacosane and 2-nonanone were identified. Synthetic mixtures as well caused attraction in males. The fake bumblebees did allure the males but did not induce mating behavior. Therefore, apparent signs could be useful. Further investigations regarding the response to virgin queens showed that the allurement declined with the rising time spent in the freezer at -20° C. Due to these results, the compounds of the bumblebee's queen sex pheromone seem to change and decompose while being frozen.[107]

In the course of another study, the four *B. terrestris* subspecies *B. terrestris terrestris* (1), *B. terrestris sassaricus* (2), *B. terrestris lusitanicus* (3) and *B. terrestris dalmatinus* (4) were investigated to identify the components located in the cephalic proportion of their labial glands. Altogether, 95 components were found, each time 54 in subspecies 1 and 3, 49 in subspecies 2 and 44 in subspecies 4. The major part was represented by (E)-2,3-dihydrofarnesol in subspecies 2 and 4 and by dihydrofarnesyl dodecanoate in subspecies 1 and 3.[108]

The attracting labial gland secretion compounds of the male exemplars belonging to the *B. terrestris* and the *B. lucorum* species were analyzed to detect eventual changes in the released secretion depending on the bumblebee's age. Electroantennographic experiments showed attraction to ethyl dodecanoate, 2,3-dihydrofarnesol, 2,3-dihydrofarnesal, geranyl citronellol, hexadecane-1-ol and octadeca-9,12,15-trien-1-ol by *B. terrestris* virgin queens and attraction to hexadecal-1-ol, hexadec-7-enal, ethyl dodecanoate, ethyletradec-7-enoate, ethyltetradec-9-enoate, octadecan-1-ol, octadeca-9,12-dien-1-ol and octadeca-9,12,15-trien-1-ol by the virgin queens of *B. lucorum*. During the life of a male bumblebee, the amounts of these secretion substances varied. *B. terrestris* as well as *B. lucorum* presented the highest quantity of components one week after becoming an adult. Thereupon, a fast decline of the number was detected in *B. terrestris*. On the other hand, *B. lucorum* showed a steady or an only briefly declining component quantity. In *B. terrestris*, the programmed cell death of labial gland cells during day five and day ten of the bumblebee's existence was visualized in the microscope. However, the cells of *B. lucorum* survived for the whole life time.[109]

2.8.2. Pheromones of bees

Most people associate the word bee with the honeybee, forgetting that about 20000 species are widespread all over the world and 700 of them are being found in Europe. The abdomen makes up the biggest body part of a bee, presenting the poisonous sting. As vegetarians, they feed on nectars and pollen. Some species live as social bees, other as solitary ones. Pheromones are released due to various reasons.[110]

The female released sex pheromone of the non-social sand bee species *Andrena nigroaenea* was contrasted with the odors of orchid flowers belonging to the species *Ophrys sphegodes (Orchidaceae)*, the pollination of which is enabled by the certain bee. Analyzes were conducted using GC, electroantennogram experiments, GC-MS and behavior studies in the fields. Fake bees were either covered with extracts out of the cuticula of virgins or extracts of the orchid's lip. The male response to the manipulated bee bodies was significantly higher than to unscented fakes. Hence, chemical signals for inducing mating activities were shown to exist in the orchid flowers and the cuticula of female bees. The male attracting substances both in bee and orchid volatiles were identified as all-trans-farnesol, all-trans farnesyl hexanoate, n-alkanes, n-alkenes, esters and aldehydes. The profile of alkanes and alkenes was assimilable among bees and plant. The components were also detected in the orchid's leaves, but showing varying amounts. Experiments of behavior, using the synthetic versions, demonstrated a strong male interest in alkene mixtures, but no effect of alkanes. Blends consisting both of alkenes and alkanes were most successful and therefore are being regarded as the sex pheromone components of *A. nigroaenea* and the pollination enabling scent released by *O. sphegodes*.[111]

In the releases out of the cephalic part of females, virgin ones as well as mated ones, and very young or patrolling males of the bee species *Colletes cunicularius*, (S)-(+)-linalool was found to be the major component. The amount of this compound highly decreased after mating activity. Both (S)-(+)-linalool and (R)-(-)-linalool were attractive to males in electrophysiological tests. Experiments in the fields, conducted with the two enantiomers and the racemic version, demonstrated a strongest reaction to (S)-(+)-linalool. The exposure of either (S)-(+)-linalool or (R)-(-)-linalool in the mating zone pointed out a much higher allurement of males under the presence of (S)-(+)-linalool.

Due to these results, (S)-(+)-linalool seems to work as an attracting pheromone, releasing mating behavior.[112]

The presence of the sex pheromone released by female bees of the *C. cunicularius* species leads to the pollination of *Ophrys exaltata*, another orchid species, by the males because of its orchid scent imitation. GC-EAD studies combined with contemporaneous FID represented the biological activity of the odors, the components of which later were recognized by GC-MS. Altogether, 22 active components were found in the volatiles out of the female cuticula. Alkenes were found to make up the major part of the releases, especially the (Z)-7 unsaturated ones. All of the attracting components could also be pointed out in the odors of *O. exaltata* subspecies *archipelagi*. Referring to the preceding study, the attraction of synthetic linalool was reviewed, resulting in an approval of its attraction to male bees but also showing a very low effected mating behavior. A mixture of linalool with the active alkenes caused reaction as well as mating behavior in the male patrollers. Regarding these outcomes, linalool seems to act as an attracting substance for longer distances in contrast to the cuticular alkenes, which are responsible for enabling mating activities in nearby males.[113]

Stingless bees were investigated in the course of a study dealing with electroantennographic analysis. The two Brazilian *Frieseomelitta* species *F. silvestrii* and *F. varia* both demonstrated that the bees reacted to head and abdominal extracts, to their own extracts as well as to the extracts of the other species, and to the posterior tibia extracts of *F. silvestrii*. Further, response could be figured out to 2-heptanol plus 2-nonanol, existing in the mandibular glands of both bees and to humulene, β-caryophyllene and α-cubebene, emitted by the tibia and being present in their cerumen.[114]

A blend of terpenoids and oxygenated components as well as hydrocarbons was found in the secretions of the bee species *Melipona beecheeii*. In contrast to the eleven Brazilian species being investigated at that time, 2-heptanol could not be proved in the mandibular gland releases, but on the other hand, rose oxides were detected. Geranyl hexanoate was figured out to be the main component in the secretion. However, geraniol and both cis-rose oxide and trans-rose oxide made up the majority regarding the airborne part of it. Electroantennographic studies were conducted with five of the detected compounds and the whole mixture. The highest effect was attained by geraniol and the whole mixture. Geraniol, farnesyl acetate and the whole blend caused the

highest attacking behavior in worker bees and obviously work as an alarm pheromone, in which the rose oxides do not seem to enact a part.[115]

Caste differentiation into working bees or queens is based on the nutrition of the larvae in almost exclusively all bee species. Regarding the several *Melipona* species, there had been no explanation about the factors leading to the development of a queen. On one side, there had been presumptions about genetic factors, on the other side it had been proposed, that larvae choose about their caste on their own. In a study conducted in 2010, the nurture of the larvae of *M. beecheii* was laced with geraniol, which is known as the secreted major component of the nurse worker's labial gland. The amount of larvae turning into queens was detected to rise under these conditions and attained the same number that was assumed in the context of the theory concerning genetic factors of influence. Therefore, secretions out of the bee's labial gland combined with the larvae's nutriment enable the development into queens in larvae that are already designated for this caste in their genes. Thus, the *M. beecheii* species obviously uses geraniol as a primer pheromone determining about the larvae's caste.[116]

2.8.3. Pheromones of wasps

α-Trans-bergamotene and β-trans-bergamotene were recognized as the male released sex pheromone components of the idiobiont parasite *Melittobia digitata*. The tiny wasp uses pupae or the final larval instar of unsocial bees and wasps as a host. Mating activities consist of complex motions of antennae, legs and wings and take place after the response of the female wasps to the sex pheromone spread by the males, which are unable to fly. Distinctions regarding the emitted pheromone amount correlate with the age.[117]

Antistrophus rufus Gillette, a gall wasp species, was in the focus of the following study. Their larvae are known for eating the galls out of flower-carrying stalks of the two *Asteraceae Silphium terebinthinaceum* Jacquin, the prairie rosinweed and *Silphium laciniatum* L., the compass flower. Male gall wasps show a faster development than female ones and deal with the function of finding conspecifics being captured in dead plant stalks. During bioassays presenting the stalk surface it was figured out that the male wasps only reacted to the *Silphium* species in which they had been nurtured.

Males, the larvae of which had been located in *S. laciniatum*, additionally were attracted by hexane extracts out of these stalks, which consisted of distinctly higher amounts of α-pinene, β-pinene and camphene compared to *S. terebinthinaceum*. Stalks of the former *Silphium* species were found to contain both enantiomers of α-pinene and β-pinene, occurring in a relationship of 1:1 in plants not being occupied by the wasps. On the other hand, stalks containing the larvae revealed highly deviating ratios. Synthetic mixtures of the two pinenes, representing the enantiomeric ratios in the galled stalks, were found to cause attraction in the male exemplars. Furthermore, it could be shown that males find their female partners in the stalks by interpreting the characteristic odors of galled plants, which are obviously effected by the occupying gall wasps that force the stalks to vary the relationship of the two enantiomers. Thus, the two *Silphium* species release a representative sex pheromone guiding the males the way to the females.[118]

3. Pheromones of arachnids

3.1. Pheromones of spiders

So far, the existence of sex pheromones in spiders has been detected in several species during studies of behavior. Like in a few other animals, sex pheromone release is mainly a female task in spiders and males serve as receivers. Pheromones are released out of the cuticula and show either volatile character or are transmitted during contact activities and further receipted with the help of chemoreceptors placed on the pedipalps[5] of males. Volatile emissions serve as attraction-releasing chemicals or induce searching activities in the male spiders. Pheromones transmitted by contact trigger mating reactions and make details about the emitter available to its receiver. The pheromones seem to be mostly lipids and probably are related to major metabolites. Species specificity does not seem to be important. Nevertheless, pheromones are even used for species identification in spiders. Male individuals are known for being very picky in mate choice and common prefer virgins or juveniles. Both females and males try to regulate the pheromone output of the female spiders with the purpose to determine about the moment and also the quantity of mating activities. Spider sex pheromone

[5] A transformed pair of extremities located on the spider's head

research is still in its infancy, but due to new methods regarding electrophysiology and chemical investigations, studying has become easier.[119]

Yet, only a small number of studies about spider pheromones have been conducted and thus, there is not much information about. However, as already mentioned, it is known that the sexual behavior of spiders is highly influenced by chemical volatiles. Studies about the species *Pholcus beijingensis* showed a higher interest on blank webs of fertile females by the males compared to blank webs of infertile females or other adult males. (E,E)-farnesyl acetate and hexadecyl acetate were found in higher amounts in the webs of the receptive females than in the other webs. A mixture of (E,E)-farnesyl acetate and hexadecyl acetate in a 2:1 ratio was similar attractive to the male spiders as the same quantity of these substances being located in the web. The presence of a single component did not cause any effect at all. Due to these results, (E,E)-farnesyl acetate and hexadecyl acetate probably act as a sex pheromone in female individuals of *P. beijingensis*.[120]

3.2. Pheromones of mites

Mites are represented by about 50000 species. Their size varies from 0.1 millimeters to 3 millimeters. They prefer moist places. The majority of the species lives on the ground, some live in the pelage, skin or stoma of animals or in textiles of humans. They can transfer diseases, cause allergies or infest food. Mostly mites are parasites – the oribatid mite is not. *Oribatida* feed on bacteria, dead plant material, rotting carcass or funguses.[121]

Trhypochthonius japonicus and *Trhypochthoniellus* sp. are two *oribatid mites*, which were investigated to find the compounds of their oil gland releases. Seven different substances were determined in *Trhypochthoniellus* sp.: geranial, γ-acaridial, neral, neryl formate, geranyl formate, (Z,Z)-6,9-heptadecadiene and (Z)-8-heptadecene. Geranyl formate was pointed out to be the major component. *T. japonicus* showed up two different types of volatiles, dependently on the position used for taking the sample. One time (Z,E)-farnesal, γ-acaridial, (E,E)-farnesal, geranial, (Z,Z)-6,9-heptadecadiene, (Z)-8-heptadecene and two obscure components were found, representing geranial as the

main compound. The other time, the same eight substances in addition to 2-hydroxy-6-methylbenzaldehyde, simultaneously making up the major part, were detected.[122]

Studying the components of the oil gland releases of *Hermannia convexa*, neral, geranial, γ-acaridial and the two hydrocarbons 8-heptadecene and 6,9-heptadecadiene were detected in young exemplars with the help of GC-MS. Eucalyptol (1,8-cineole), normally not known as a component of oil gland productions, could also be shown, if only in small amounts. 1,8-cineole was the only substance found in adult exemplars, revealing the big distinction between juveniles and adults. Histological studies issued the statement that the oil glands lose their function with advancing age, which is not known in early-derivatives or middle-derivatives of *oribatida*.[123]

Neral, neryl formate, geranial and 2-hydroxy-6-methyl-benzaldehyde are part of the oil gland releases of *Collohmannia gigantea*, another oribatid mite, with the purpose to act as an alarm pheromone. Neral, which is the major compound with a presence of 50 percent and 2-hydroxy-6-methyl-benzaldehyde (5 percent), known for its strong attraction, were made responsible for the mite's reaction during a study. Hydrocarbons like tridecane and pentadecane did not cause any reaction and probably function as dissolvers for the alarm substances. Taking notice of the alarm pheromone, the mites needed a short time for perceiving the danger, then cringed and subsequently left the place. The whole secretion of the gland, meaning the pheromone components and the hydrocarbons, was found out to act as an allomone[6] to the beetle *Euconnus oblungus*, a host of oribatid mites.[124]

Volatiles of *Chortoglyphus arcuatus*, the storage mite, were investigated using GC-MS with the intention to detect new mite pheromones. (4R,6R,8R)-4,6,8-trimethyldecan-2-one was presented as the main released component. MS evaluation, synthesis and chiral GC were used to prove its structure. From now on, the substance was called chortolure and seemed to be released with the purpose to act as an aggregation pheromone for male and female mites. Other components of the pheromone were only presented by lower amounts. Neral and geranial, being considered as an alarm pheromone in the storage mite, could only be detected in the total extracts.[125]

[6] A substance that allows communication between different individuals - in contrast to pheromones, which serve as a signal in same individuals. Different to kairomones, the transmitter profits by the emission

4. Conclusion

Regarding the outcomes of all the quoted studies, pheromone investigation has strongly developed during the last ten years, giving us an insight into the fascinating communication among insects and therefore enabling a better understanding of an insect's behavior. It is also amazing that so many substances, that are commonly known as essential oil components, besides make up compounds of various pheromones in animals. Furthermore, new plans against pest colonization have emerged by using pheromone traps, which, however, obviously still need to be improved because of partially unsatisfactory results. Nevertheless, pheromone pest management strategies seem to be on the right way, representing an alternative and often cheaper way compared to insecticides. Some examples of plants, serving as a natural source of pheromone components being used for traps, have already been mentioned and certainly there will be more of them in a few years. As the arachnid pheromone investigation is still in its early stages, it seems likely that there will be great progressions in the future.

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