CHAPTER FIVE

Chemical Signatures in Plant—Insect Interactions

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Abstract

Chemical signals are important cues throughout the life of an insect especially for mate location and for prey and host finding. The chemical signal, whether pheromone or plant volatile organic compound (VOC), remains specific because of the mixture, of the ratio of the components in mixture and of the release quantity. The plasticity of pheromone emissions is now studied in several insect species in relation to geographic variation, host plant specialization and chemical and light environment. The actual vision is that the pheromone composition is likely to be more plastic than previously assumed. The perception of the environmental odorscape produced by living plants and animals together addressed the question on the specific detection of the pheromone signal in the atmospheric blend of molecules. In agrobiocoenosis, the cultivated plants produce a specific odorscape. The insects rely on plant VOCs to locate the crop or the host plant, after which specific mixtures act as oviposition stimulants. The insect responses to host plants and their odours vary with the physiological status of both actors: the plant and the insect. Chemical signals released by plants vary with plant physiology, diel periodicity, climatic factors and pollution, and these signals can be species or even variety specific. Many of plants signalling compounds detected by insects have important roles as warning signals, which can also function in plant—plant communication.

1. INTRODUCTION

The chemical signals or semiochemicals manage the relations between organisms belonging to the animal kingdom and plant kingdom, via air, water or soil. They are classified in different categories according to their nature, the effects and which is the beneficiary of the relation (the releaser or the receiver). The chemicals involved in an intraspecific relation are pheromones by opposition to the allelochemicals that control the interspecific relation (Dicke & Sabelis, 1988; Nordlund & Lewis, 1976).

The pheromones are classified according to the action on the conspecific. When it acts immediately on the behaviour, they are releasers and when it induces a long term physiological change they are called modifiers (Wilson, 1963).

The allelochemicals are classified on the base of the beneficiary of the released chemical: when the benefit is for the releaser they are named allomones and when it is for the receiver, they are kairomones. Nowadays, the studies of the allelochemicals are expanding in the field of plant—insect interactions.

The chemical signature is a concept that defines the specificity and the originality of the semiochemicals: pheromone and allelochemical. The
The chemical signature can be considered as a specific blend more or less complex, involving often ubiquist compounds or/and original compounds. The pheromone chemical signature is rather simple made of one to at least five compounds. The concept becomes more complex for the chemical signature produced by plants and at the worst when the chemical signature of a plant acts on the pheromone production or on the perception.

The chemical signature characterized a specific mixture of chemicals that acts on another organism. The specificity relies on the composition, the ratio of the components and the dose released. The originality and the diversity of the components are poor compared to the diversity of the fauna and flora. The combinations of these compounds make the chemical signature of an individual.

In insects, the chemical signals play an important role for each steps of the life. Intraspecific recognition is well-documented for reproduction widely mediated in insects by pheromone. The receiver perceived the signal by the olfactory receptors located on the antennae. The signal, a very precise mixture of molecules produced in a particular ratio and released in very low amount \((10^{-8}; 10^{-9} \text{ gr})\), is analyzed at the level of the brain in antennal lobes and converted in a behavioural or a physiological change.

The pheromones are mostly synthetized de novo (in Lepidoptera) but can also be sequestered from the food (in Coleoptera; in Lepidoptera male pheromone). In Lepidoptera, the fatty acids synthetized de novo in the epithelial cells of the pheromone gland undergo actions of enzymes that shorten the carbohydrate chain, change the position of double bonds then reduce and esterify (Bjostad, Wolf, & Roelofs, 1987). The pheromone molecules are rather simple: carbohydrate chain of 10–18 carbons; with one, two, rarely more; double bond. The chain has a functional group, acetate, aldehyde, alcohol on the first carbon. Rare examples exist with a triple bond and with epoxide function. This molecular construction allowed many different possibilities according to the position of the double bonds along the chain. The different functional groups contribute to increase the diversity of molecules. This particularity and the complexity of the blend of two, three or more compounds insure for most of the species the specificity of the pheromone.

The chemical structure, especially the chain length, has followed evolution and can be used as an added criterion in systematic (Descoins & Frérot, 1979; Roelofs & Brown, 1982). In Coleoptera, the gut bacteria used the terpenoids ingested to biosynthesize the pheromone. As a consequence,
the same molecules are shared by several species of bark beetles. In this group, the attraction of conspecifics is achieved by the combination of the pheromone and tree odours. The signal became an original chemical signature because of this association (Blum, 1987).

The universality of the pheromone components in the animal kingdom is documented by the examples of bumblebees and the elephant. Males of some bumblebee species mark a territory by depositing on plant products secreted by the labial glands. The molecules involved in this behaviour are the same as those identified as sex pheromone in some noctuid moths (Berström et al., 1985; Descoins et al., 1984). In mammals certain molecules found in urine inform conspecific on the physiological and social status. One of the compounds, \((Z)-7\)-dodecen-1-yl acetate (Z7-12:Ac), identified in urine of elephant females is a component of the mating pheromones for numerous species of Lepidoptera (Rasmussen, Lee, Roelofs, Zhang, & Daves, 1996).

The interspecific communication and especially the interaction between plants and insects followed a schema closely related to the intraspecific communication. The insects are tuned to a specific signal released by the plants. The signal could be more complex than the pheromone blend in number of components. The chemicals originate from primary and secondary metabolites and are bio-synthetized by the plants. The diversity of the plant signals depends of the botanical family but is also achieved by combining molecules more or less common to the plant kingdom.

2. PLASTICITY AND SPECIFICITY OF THE CHEMICAL INFORMATION

2.1 Plasticity and Specificity in Pheromone Communication

In most nocturnal moths, females are the pheromone signallers and males are the responders. The female signal is exclusively chemical and produced in a pheromone gland de novo every night. Females of each moth species produce a species-specific sex pheromone blend, which is determined by the combination of, in general, two—five pheromone compounds that are emitted in species-specific ratios.

The predominant role of moth sexual communication is species recognition (i.e., to not attract or be attracted by other closely related species with a similar pheromone blend) (Butlin, Hewitt, & Webb, 1985; Droney, Musto, Mancuso, Roelofs, & Linn, 2012; Löfstedt, 1993; Paterson, 1985).
As these communications are generally thought to be mostly important to distinguish between species and not in intraspecific sexual selection, they are generally hypothesized to be under stabilizing selection and to exhibit low intraspecific variation. As males are behaviourally fine-tuned to their species-specific pheromone blend (Cossé et al., 1995; Linn, Young, Gendle, Glover, & Roelofs, 1997), a mutation that alters the female pheromone blend is likely to lower her reproductive fitness (Butlin & Trickett, 1997; Zhu, Chastain, Spohn, & Haynes, 1997). Therefore, the means by which novel signals in sexual communication can evolve, in the face of selection against such change, is still an evolutionary mystery, especially as moths are one of the most diverse group of animals on earth, with \( \sim 120,000 \) of 160,000 Lepidoptera species (Bazinet, Cummings, Mitter, & Mitter, 2013).

To resolve the dilemma of how the female signal and male preference can concomitantly change, the genes that are responsible for intraspecific variation in and interspecific divergence of the pheromone systems should be more extensively identified (Groot, Dekker, & Heckel, 2016). So far, only five such genes have been identified (Albre, Steinwender, & Newcomb, 2013; Fujii et al., 2011; Gould et al., 2010; Lassance et al., 2010; Leary et al., 2012). In the moth species studied until now, the genomic regions involved in female pheromone production and male response do not appear to overlap (i.e., they are located on different chromosomes). Without a genetic association between signal and response, genetic changes seem to have evolved independently, which makes Fisherian runaway selection (a hypothesized genetic sexual selection mechanism for the evolution of exaggerated male ornamentation) unlikely. Therefore, other evolutionary scenarios have been proposed for moth sexual communication, including stabilizing and directional selection, the asymmetric tracking hypothesis (Phelan, 1992) and the rare male hypothesis (Roelofs & Rooney, 2003) (see Groot et al., 2016 for a more detailed description). Once the genes underlying variation in moth sex pheromone signals and responses are eventually known, more powerful tests based on comparisons of allele frequencies among populations and over time will be possible.

Although moth sex pheromones are generally assumed to be under stabilizing selection, there are numerous descriptive studies of geographic variation in female sex pheromone blends, male responses and attraction to artificial lures formulated to mimic females (Cossé et al., 1995; Gries, Schaefer, Gries, Liska, & Gotoh, 2001; Groot et al., 2009; Linn et al., 1997; McElfresh & Millar, 1999, 2001; Unbehend et al., 2014). Geographic variation in moth
sexual communication systems could result in reproductive isolation and subsequently may lead to speciation (Coyne & Orr, 2004; Löfstedt, 1993; Smadja & Butlin, 2009; Symonds & Elgar, 2008). The causes of geographic variation may be different selection forces in different regions, stochastic events or habitat variation, which includes abiotic factors, such as temperature, relative humidity and day length. In moths, pheromone production has been shown to be affected by host plant volatiles (McNeil & Delisle, 1989a; Raina, Jackson, & Severson, 1997) (see Section 2.1), sucrose nutrition (Foster, 2009) and temperature (Raina, 2003). However, these factors affect the quantity rather than the quality of the pheromone blend. Biotic factors in the habitat that likely affect chemical communication are the presence and abundance of species with similar chemical cues because they may either affect the signal-to-noise ratio (e.g., Eizaguirre, Albajes, Lopez, Sans, & Gemeno, 2007; Gemeno, Sans, Lopez, Albajes, & Eizaguirre, 2006; Haynes, Gemeno, Yeargan, Millar, & Johnson, 2002; Sole et al., 2008) or generate communication interference (e.g., Butlin, 1995; Cardé, Cardé, Hill, & Roelofs, 1977; Groot et al., 2006; McElfresh & Millar, 1999, 2001). Both signal-to-noise ratio and communication interference would result in selection for females with the most distinct, optimized pheromone blend (i.e., negative frequency-dependent selection). Such local natural selection forces may alternate directions or be unidirectional, similar to what has been found for beak sizes in the Galapagos finches (Grant & Grant, 2002). Only when specific local environmental conditions persist, selection forces from the environment may result in directional or divergent selection. Variation related to selection across a persistent environmental gradient has been referred to as the “selection hypothesis” (Groot et al., 2009).

Even though the finding of variation often leads to the conclusion that differences are due to selection that may lead to speciation, as in the Galapagos finches, not all variation may be subject to selection. An alternative, more speculative hypothesis to explain variation in chemical communication signals, is that females and males exhibit phenotypic plasticity in sexual signalling, and experience — either by immature stages or by early adults — shapes the expressed phenotype. Early adult (postimaginal) experience has been shown to be an important factor in female oviposition preference (Barron, 2001; Jaenike, 1983, 1990; Van Emden et al., 1996), and preexposure of male moths to sex pheromone blends has been shown to affect their subsequent responses to sex pheromone (Anderson et al., 2007; Anderson, Sadek, & Hansson, 2003; Anderson, Sadek, Larsson, Hansson, Thoming, 2013). Whether preexposure to pheromone blends and other semiochemicals
could also cause females to alter the blend that they produce has only been investigated in one species so far (Groot, Classen, Staudacher, Schal, & Heckel, 2010).

Plasticity in female sex pheromone composition in moths can be expected because of a number of characteristics. First of all, females produce their pheromone de novo every night (e.g., Jurenka, 2004; Rafaeli, 2005), which may allow adjustment of time and temporal patterning of pheromone release (calling) in relation to environmental conditions (Lim & Greenfield, 2007; Schal & Carde, 1986). Also, in many moth species females can perceive their own species’ female pheromone compounds (Den Otter, Schuil, & Sandervanoosten, 1978; Groot, Gemeno, Brownie, Gould, & Schal, 2005; Hillier, Kleineidam, & Vickers, 2006; Schneider, Schulz, Priesner, Ziesmann, & Francke, 1998), so that they are likely to perceive conspecific as well as heterospecific sex pheromone, at least if their own pheromone blend overlaps with that of other species. In addition, pheromone receptors have been found in sensilla on the ovipositor of a female moth (Widmayer, Heifetz, & Breer, 2009), suggesting that these receptors might allow a feedback mechanism onto the gland that might affect pheromone emission. Hence, it may be possible that females can vary their biosynthesized as well as emitted pheromone blend to some extent, depending on the prevailing olfactory cues in their habitat. This hypothesis has been referred to as the “experience hypothesis” (Groot, Classen, et al., 2010; Groot et al., 2009).

In many species, the attractiveness of a potential mate is determined by the quality of a sexual signal (Domb & Pagel, 2001; Groot et al., 2014; Scheuber, Jacot, & Brinkhof, 2003), which may be affected by immune defence responses, as generally hypothesized by the Zahavi handicap principle (i.e., hypothesis proposed to explain how evolution may lead to “honest” or reliable signalling between animals which have an obvious motivation to bluff or deceive each other). The level of sexual attraction may signal the level and extent of health. However, if sexual attractiveness and immunity compete for the same resource pool, they may negatively affect each other. Very little data exist on whether and how immune responses affect sexual communication in moths. In the tobacco budworm, Heliothis virescens (Fabricius, 1777) (Lepidoptera: Noctuidae), the sex pheromone blend shifted towards an unattractive blend when females were infected with the entomopathogenic bacterium Serratia entomophila (Barthel, Staudacher, Schmaltz, Heckel, & Groot, 2015), suggesting that at least in this species sex pheromone production is condition dependent.
2. Relevant Examples of Intraspecific Variation in Moth Sexual Communication

2.1.1 Pheromone Races of Ostrinia nubilalis (Fig. 1A)

The existence of pheromonal races within the same species is known since 1975 (Klun, 1975), who identified two pheromone races in the European corn borer Ostrinia nubilalis (Hübner, 1796) (Lepidoptera: Crambidae), the E- and the Z-strain. The sex pheromone of the two O. nubilalis strains consists of two pheromone components, i.e., (Z)-11-tetradecenyl acetate (Z11-14:Ac) and (E)-11-tetradecenyl acetate (E11-14:Ac), which are produced in a 97:3 Z:E ratio in the Z-strain, while E-strain females emit a 1:99 Z:E mixture (Klun, 1975; Kochansky, Cardé, Liebherr, & Roelofs, 1975; Roelofs et al., 1987). Males of each strain are highly attracted to females of their own strain (Linn, 1997; Roelofs et al., 1987), which leads to assortative mating in the field (i.e., a form of nonrandom mating in which pair bonds are established on the basis of phenotype) (Dopman, Robbins, & Seaman, 2010; Klun & Huettel, 1988; Malausa et al., 2005). Following the first evidence for pherotypes in US population of O. nubilalis, a more recent

![Figure 1](image-url)

**Figure 1** Intraspecific variation in the pheromone composition of female moths. (A) Pheromone polymorphism of Ostrinia nubilalis Z- and E-strain females. Values on the y-axes show the relative percentages of (Z)-11-tetradecenyl acetate (Z11-14:Ac)/(E)-11-tetradecenyl acetate (E11-14:Ac). (B) Geographic variation in the relative amount of (Z)-9-tetradecenyl acetate (Z9-14:Ac)/(Z)-7-dodecenyl acetate (Z7-12:Ac) in Spodoptera frugiperda corn-strain females from Mississippi and Florida. (C) Within-population variation in the relative amount of (Z)-11-hexadecenyl acetate (Z11-16:Ac)/(Z)-7-dodecenyl acetate (Z7-12:Ac) in common and rare Heliothis virescens females. (D) Phenotypic plasticity in the pheromone composition (relative amount of Z11-16:Ald/(Z)-11-hexadecenyl acetate (Z11-16:Ac)) of Heliothis subflexa (Hs) females that were reared in the presence of Hs odour or H. virescens (Hv) odour. (E) Light-dependent phenotypic plasticity of the pheromone composition (relative amount of Z11-16:Ac/(Z)-9-hexadecenyl acetate (Z9-16:Ac) + (Z)-11-hexadecenol (Z11-16:OH)) of Mamestra brassicae females that were either extracted under normal night conditions (Dark) or exposed to artificial green, red, and white light (Light).
study demonstrates that in France the two pheromone populations were also present but segregated by the host plant. The Z population was specialized on maize whereas the E population was on hop and mugwort, whatever was the geographic location (Pelozuelo, Malosse, Genestier, Guenego, & Frérot, 2004). Genetic approach evidenced a very low gene flow between the two populations (Bethenod et al., 2005). Thus, although hybridization is still possible (Liebherr & Roelofs, 1975), the Z- and E-strains seem to be in process of speciation and are isolated enough to be considered sibling species (Cardé et al., 1978; Malausa et al., 2007).

2.1.2 Geographic Variation in *Spodoptera frugiperda* (Fig. 1B)
The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), consists of two genetically and behaviourally distinct strains, the corn- and rice-strain, that appear to be undergoing ecological speciation in sympatry (Dumas et al., 2015; Kergoat et al., 2012). One of the prezygotic isolation barriers is differentiation in sexual communication (Groot, Marr, Heckel, & Schoefl, 2010). Strain-specific as well as geographic variation in the sex pheromone composition has been found in this species (Batista-Pereira et al., 2006; Groot et al., 2009; Lima & McNeil, 2009). Genetic analysis showed that only 9% of the strain-specific pheromone variation was explained by genetic differences (Unbehend, 2013), which suggest that environmental conditions influence the female pheromone phenotype to a large degree. In addition, males of the two strains were similarly attracted to the blends of both strains, and there was more geographic than strain-specific variation in male response (Unbehend et al., 2014). Thus, this prezygotic isolation barrier in itself does not seem strong enough to keep the two strains separated. However, if environmental conditions affect the female signal and male response in the same direction, geographic differentiation in sexual communication may ensue.

2.1.3 Within-Population Variation in *Heliothis virescens* (Fig. 1C)
In the moth *H. virescens*, consistent high phenotypic variability in the female sex pheromone blend was found within each of four geographically distant populations (Groot et al., 2014). The within-population variation found in *H. virescens* is in ratio of unsaturated to saturated pheromone compounds; common females produce mostly unsaturated compounds while rare females produce higher relative amounts of saturated pheromone compounds. Selection experiments in the laboratory showed a genetic basis of this
within-population variation, while field experiments showed that females producing a higher relative proportion of the saturated compounds are less attractive to males (Groot et al., 2014). Interestingly, *H. virescens* females expressing the unusual phenotype (high relative amounts of saturated compounds) were found in all populations, across regions and years, albeit at low frequency (Groot et al., 2014). Such a polymorphism may be maintained through balancing selection, in this case specifically through heterozygote advantage, as heterozygote females produce significantly more of the critical sex pheromone component through which males are attracted. Thus, selection need not act solely in a purifying role, eliminating genetic variation in signal—response systems as is generally assumed but can also act to maintain genetic variation in them.

### 2.1.4 Phenotypic Plasticity in *Heliothis subflexa* Due To Varying Chemical Environments (Fig. 1D)

In the moth, *Heliothis subflexa* (Guenée, 1852) (Lepidoptera: Noctuidae), one compound in the sex pheromone blend was found to have a dual function: the acetate (Z)-11-hexadecenyl acetate (Z11-16: Ac) not only enhances the attraction of conspecific males but also inhibits the attraction of the closely related heterospecific *H. virescens* males (Groot et al., 2006; Vickers & Baker, 1997). In these two closely related species, not only geographical but also temporal variation in the pheromonal signals was found (Groot et al., 2007, 2009). The geographic and temporal variation in the sex pheromone of *H. subflexa* was correlated with *H. virescens*; when *H. virescens* was highly abundant, *H. subflexa* females contained significantly more of this acetate than when *H. virescens* was much less abundant (Groot et al., 2009). In the laboratory, when *H. subflexa* females emerged and remained in the odour of *H. virescens* for three days, these females contained significantly more acetate compared to *H. subflexa* females that emerged in control odour or in the sex pheromone odour of conspecific females (Groot, Classen, et al., 2010). Thus, early-adult experience of different chemical environments affects the sex pheromone composition in *H. subflexa* females. Because a higher acetate level increases the attraction of conspecific males (Groot et al., 2006), this behavioural adjustment may lead to assortative mating.

### 2.1.5 Phenotypic Plasticity in *Mamestra brassicae* Due To Varying Light Regimes (Fig. 1E)

The cabbage moth, *Mamestra brassicae* (Linnaeus, 1758) (Lepidoptera: Noctuidae), is a common species in (sub)urban areas of western Europe,
and adult moths are frequently exposed to artificial light at night, as they are nocturnal and attracted to sources of artificial light. Van Geffen et al. (2015) tested the effect of low levels of artificial night lighting with different spectral compositions on the amount and composition of the sex pheromone produced by *M. brassicae* females. This experiment showed that artificial light at night not only strongly reduced the total amount of sex pheromone, but also changed the chemical composition of the pheromone blend. The range of variation that was found in the pheromone composition of *M. brassicae* under different light conditions is similar to or even higher than that found in the geographic variation of, for example, *H. virescens*, *H. subflexa* (Groot et al., 2009), *Agrotis ipsilon* (Hufnagel, 1766)(Lepidoptera: Noctuidae) (Gemeno, Lutfallah, & Haynes, 2000), *Agrotis segetum* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae) (Wu, Cottrell, Hansson, & Löfstedt, 1999), and *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae)(Dumenil et al., 2014). Hence, varying only one environmental factor, in this case light, can already change the sex pheromone blend significantly.

2.3 Plasticity and Specificity in Plant Volatile Organic Compounds

2.3.1 Major Groups of Plant Volatiles

Plants are capable of emitting wide variety volatile organic compounds (VOCs). Because of their biological origin these compounds are often called biogenic VOCs (BVOCs) to separate them from volatile compounds of anthropogenic origin. Volatiles can be emitted from different plant organs including leaves, flowers, stem and root system and they may have different ecological functions such as activating defences in neighbouring plants, attraction of pollinators by flower volatiles or attraction of parasitoids and predators of defoliating herbivores by leaf volatiles (Holopainen & Gershenzon, 2010).

Majority of VOC emitted by plant originate from three major biosynthesis pathways: (1) terpenes form most diverse group of VOCs, (2) oxylipins are fatty acid—derived six-carbon C6 compounds and (3) shikimate and benzoic acid pathways produce benzenoids that are aromatic compounds such as methyl salicylate and indole (Maffei, 2010). In addition, there are several low molecular weights, C1 and C2, compounds, such as methanol, ethanol, formaldehyde, and acetaldehyde methane and ethylene synthesized via other biosynthetic routes (Loreto & Schnitzler, 2010). Most of common plant VOCs can be classified into alkanes, alkenes, alcohols, esters, aldehydes, and ketones (Maffei, 2010). Furthermore, the breakdown products of glucosinolates are very important group of VOC in the family
Brassicaceae (e.g., Blande, Holopainen, & Niinemets, 2014; Pinto, Blande, et al., 2007).

Terpenes represent highly diverse group of volatile chemicals found from plants and they are also most intensively studied. Isoprene, synthesized in plant chloroplast in light and temperature-dependent way (Loreto & Schnitzler, 2010), is the single compound emitted from the vegetation in the atmosphere in the highest rate, but not all plant species emit this compound (Laonthawornkitkul, Taylor, Paul, & Hewitt, 2009). Isoprene unit (C5) is the basic structure of terpenes. Other major volatile terpene (isoprenoid) groups are monoterpenes (C10) and sesquiterpenes (C15) (Holopainen & Gershenzon, 2010). Recently even some volatile diterpenes (C20) have been detected from plant headspace of tobacco (Jud et al., 2016), although diterpenes are mostly nonvolatile compounds. Herbivore-inducible homoterpenes (E)-4,8, dimethyl-1,3,7-nonatriene (C11, DMNT) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (C16, TMTT) (Dicke, 2009) are important signalling compounds in plant—carnivore communication as their emissions are induced by herbivore feeding.

Fatty acid—derived C6 oxylipins are often called green leaf volatiles (GLVs) as typically they are smelled by humans after cutting of grass. They are rapidly emitted from stressed plants, particularly from those suffering mechanical damage (Brilli et al., 2011) or insect feeding, which are causing membrane damage in plant cells. The emission rate of some GLV compounds, such as leaf aldehydes (E)-3-hexenal and (E)-2-hexenal, are following strictly the feeding activity of Lepidopteran larvae having the emission peak within 2–3 min from the start of feeding activity. Leaf alcohol (Z)-3-hexenol and (Z)-3-hexenyl acetate have the emission peaks nearly 10 min later (Maja et al., 2014; Simpraga, Takabayashi, & Holopainen, 2016). This succession of GLV compounds in plant VOC emissions has chemical information for herbivorous insects to detect the sites in plant that are already occupied by other herbivores. Value for predatory and parasitoid insects is also high as their can use typical GLV profiles of current feeding and older damage in their orientation behaviour to separate the older damage from fresh damage. This maximizes their probability to find host larvae. Furthermore, the proportion of GLV compounds in volatile bouquet is an important tool in detection of pest attack and its extension in crops when using VOC-based profiling (Jansen et al., 2011).

Quite a few benzenoid compounds are emitted by plants (Misztal et al., 2015), although some compounds such as methyl salicylate are induced in higher rate (e.g., by mites - Blande, Holopainen, & Li, 2010, by
2.3.2 Plant Physiological Functions of VOCs and Diel Periodicity

Secondary metabolites are generally considered to lack primary functions for plant physiology and act more for plant defence and communication. However, many of the terpenes (isoprene, monoterpenes) are synthesised in chloroplasts and they have crucial role to maintain plant photosynthesis under variable environmental conditions [e.g., by stabilizing chloroplast membranes under heat and pollution stress (Loreto & Schnitzler, 2010)]. This suggests that chloroplast-originating terpenes have very primary functions for plant physiology. Turnover of freshly fixed carbon to isoprenoid emissions from the chloroplasts could be unexpectedly fast in sufficient light conditions. Isoprene is the volatile compound studied most intensively in the association with plant photosynthesis. Delwiche and Sharkey (1993) found that freshly photosynthetically fixed labelled $^{13}$C carbon can be detected from isoprene emitted from oak leaves just 9 min later. Under high temperature and solar radiation conditions photosynthesis is activated and will better provide fresh carbon for isoprene production. However, drought stress causes some limitation for photosynthesis as closure of stomata does not allow carbon uptake. Recent studies have shown that isoprene synthesis and emission still continues under drought stress, but carbon source can earlier fix carbon possibly from respiration or starch breakdown (Loreto & Schnitzler, 2010). While stomata are closed, plant volatiles can be emitted by passive diffusion from cells to inter cellular gas space and from there by diffusion through leaf epidermis. Recently it has been suggested that specific transporter molecules might transfer VOC molecules in plant tissues (Widhalm, Jaini, Morgan, & Dudareva, 2015). In general, emission rates of isoprene can be substantially higher in photosynthetically active mature leaves than in young and old leaves (Niinemets, Sun, & Talts, 2015).

Diel periodicity of leaf volatiles, showing highest emission rates of VOC during day time and low emission rates at night can be directly linked to their very strong coupling of photosynthetic activity of foliage during day time. However, under 24h sunshine subarctic conditions some plant species can keep their VOC emission rates at midnight on the same level as during daytime, while in colder high arctic condition plants seem to show diel periodicity under sunny nights probably due to colder night temperature (Lindwall, Faubert, & Rinnan, 2015). Floral scents are known for the strong
diurnal activity. Scent volatiles are often produced in petal lobes of flowers (Pichersky & Dudareva, 2007), but tissue-specific scents for the stigma and stamens has been found (Burdon, Raguso, Kessler, & Parachnowitsch, 2015). Most of the floral scent volatiles are terpenoids or phenylpropanoid/benzenoid compounds, but their proportions are changing during the progress of flowering. Total floral scent emissions might be highest during the day, but also composition has differences between night and day. Burdon et al. (2015) found that day emissions dominated by monoterpenes while sesquiterpenes and other aliphatic compounds dominated night emissions. Typically, floral scent emissions peak often during the highest activity hours of the efficient pollinator species showing importance of coevolution in plant pollinator interaction (Burdon et al., 2015).

### 2.3.3 Genotypic Diversity of Plant VOCs

Plant volatile emission from flowers, fruits and vegetative parts vary strongly between plant species, but also between different genotypes within a species. Even the dominating VOC emitted by a plant species may differ between different locations. In small pine seedlings the provenance of seed material collected from a 1000 km strongly affects, if the volatile monoterpenic pool is dominated by α-pinene of Δ3-carene (Nerg et al., 1994). In agricultural plants similar variation can be found. In three-week-old carrot seedlings, myrcene is the dominating monoterpenic, but in the comparison of four cultivars the second most common monoterpenic was sabinene in one cultivar and limonene in three others (Kainulainen, Nissinen, Piirainen, Tiilikka, & Holopainen, 2002). In older seedlings the order changed and the sesquiterpene β-caryophyllene became the second most common terpene. Cultivar splendid with highest limonene and β-caryophyllene content was preferred by a generalist plant bug, Lygus rugulipennis Poppius, 1911 (Heteroptera: Miridae), in oviposition tests (Kainulainen et al., 2002).

### 2.3.4 Relationship of Plant VOCs With Climate Changes and Pollution

As long as there has been human activity leading to burning of the fuels, the levels of pollutants have increased in the atmosphere. Most important atmospheric pollutants affecting biogenic VOCs and other hydrocarbons by reacting with them in the atmosphere are ozone (O₃) and OH and NO₃ radicals (McFrederick, Kathilankal, & Fuentes, 2008). These compounds have increased substantially in the atmosphere since late 1800s and model calculation shows that this might have affected behaviour
of pollinators (McFrederick et al., 2008). There are also natural sources of these emissions, such as volcanic eruptions, wild fires and lightning, but these sources are mostly local and temporal and not so widely distributed as human activities. Recent years’ elevated concentrations of atmospheric carbon dioxide (CO2) and global climate warming—related CO2 have become even more important factors to affect ecosystems and crop production (Forkel et al., 2016).

Important for the understanding of plant communication with conspecifics and other organisms using VOCs is the fact that most of the herbivore-inducible VOCs and other volatile signalling compounds have rather short lifetime in the atmosphere with reactive air pollutants. Oxidation and other photochemical reactions in the atmosphere will lead degradation of compound originally emitted by plants (Pinto, Blande, Souza, Nerg, & Holopainen, 2010). Reduced atmospheric lifetime means also reduced signalling distance by the VOCs produced by plants (Simpraga et al., 2016). On the other hand, we do not know enough how important the specific ratio of emitted compounds is for the volatile signatures that plants and animals use to detect the specific VOC composition emitted by plants (Bruce, Wadhams, & Woodcock, 2005). Recently, it has been suggested that the ratio of the original plant-emitted volatile compound and the semivolatile degradation products might inform, e.g., for approaching parasitic wasp, the distance to the plant where the host larvae are feeding (Simpraga et al., 2016).

When signalling compounds are destroyed in the polluted atmosphere, it has been found to reduce this signalling value of VOCs in plant—plant communication (Blande, Holopainen, et al., 2010), in floral pollinator cues (Farre-Armengol et al., 2016), and in attraction of natural enemies on herbivore-damaged plants (Himanen et al., 2009; Pinto, Nerg, & Holopainen, 2007). Blande, Holopainen, et al. (2010) observed that in ozone-rich air (80 ppb O3) spider mite—induced VOCs from lima bean—induced defences (production of extra floral nectar) in intact plants only in 20 cm distance while in ambient air response was induced also in 70 cm distance. Homoterpenes E-DMNT, TMTT and monoterpenes β-ocimene were the induced compound, which were significantly reduced at 80 ppb ozone. Elevated ozone concentrations also reduced several herbivore—induced VOCs in cabbage and lima bean plants damaged by Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae) and Tetranychus urticae Koch, 1836 (Trombidiformes: Tetranychidae), respectively (Pinto, Blande, et al., 2007). However, this did not reduce the efficiency of
parasitoid wasps and predatory mites to orientate towards herbivore-damage plant in olfactometric tests. It was concluded that parasitoid wasp probably detected lower concentration of some key volatiles of damaged plant than the used CG—MS system (Pinto, Blande, et al., 2007). This result also suggests that some of the individual herbivore-induced volatile compounds even in trace concentration might be more important cues for natural enemies than the changes in ratios of emitted compounds.

Elevated CO2 and other greenhouse gas emissions to the atmosphere are the main reason for global climate warming. As CO2 is a major atmospheric gas taken up by plants and it has significant effects on plant growth and carbon—nitrogen balance. Elevated atmospheric CO2 concentrations have also effects on constitutive and induced emissions of plant VOCs. In their literature analysis, Penuelas and Staudt (2010) found that the increases in atmospheric CO2 led lower emission rates of all type of VOC, but warming had an opposite effect with increases of VOC emissions, most clearly in the emissions of different terpenes. They also found that the results for biogenic VOC emissions in response to increasing atmospheric CO2 concentrations varied depending on the species, phenology and other environmental conditions. Explanation to reduced VOC emissions could be that increasing CO2 concentration might uncouple isoprene emission from photosynthesis (the carbon source for VOC synthesis) and inhibit isoprenoid (terpenes) emissions at leaf level (Penuelas & Staudt, 2010). As elevating temperature activate biosynthesis of VOCs and warming increases diffusion of VOCs from plant tissues to the atmosphere (Widhalm et al., 2015) the numerous observations of elevated emission rates of isoprenoids under warming is a logical consequence.

2.3.5 Mechanism of Plant—Plant Communication

Plant volatiles in plant—plant communication are expected to evolve from the use of volatile compounds as molecular signals in unitary plant development (Holopainen & Blande, 2013). This view is also supported by results, which have shown plant—plant signalling to be more efficient between clonal cuttings of the same plant than between nonclonal conspecifics (Karban, Shiojiri, Ishizaki, Wetzel, & Evans, 2013). This indicates a degree of self or kin recognition to occur in receiver plants. Although currently we do not know any specific plant organs aimed to sense the volatile signals received by plants (Simpraga et al., 2016), plants have dense network of stomata in their leaves with a capacity of gas transfer and known of uptake of atmospheric plant volatiles (Niinemets, Fares, Harley, & Jardine, 2014).
This allows for volatile compound an access to cellular interspaces of leaf mesophyll cells. It is known that plants have several salicylic acid-binding proteins (Manohar et al., 2015) which could be related (e.g., to perception volatile methyl salicylate signals). On the other hand, glycosylation of key volatile signalling compounds, such as glycosylation of (Z)-3-hexenol in receiver plant, is proposed to be one of the mechanisms involved in the reception volatile signalling in plants (Sugimoto et al., 2014).

Evidence of communication by VOCs within plant is gathered especially from woody plant species (Baldwin, Halitschke, Paschold, von Dahl, & Preston, 2006) where volatile signalling between damaged plant parts and intact branches results in intact branches being better protected against subsequent attacks by herbivores (Frost et al., 2007; Heil & Karban, 2010; Shiojiri, Karban, & Ishizaki, 2009). Signalling between individual conspecific plants has shown that volatiles emitted by a herbivore-attacked plant will cause activation of various defence in the receiver plants. These include, for example, activation of defence genes (priming) resulting in more vigorous response in primed plants under herbivore-attacked when compared to nonprimed plants (Arimura et al., 2000; Frost et al., 2008). Also increased emission rates of typical herbivore-induced volatile compounds that are known to attract natural enemies of herbivores are found in the neighbouring intact lima plants when focal plant is attacked by spider mites (Blande, Holopainen, et al., 2010). Induced production of extra floral nectar, which is known to keep the predatory mites on plant foliage, was also found from these neighbouring plants (Blande, Holopainen, et al., 2010). Current evidence in literature suggests that plants have a capacity to activate efficient defences in neighbouring plants, but applications for pest control are still needed. An important result in most of the studies is that the plant–plant communication by volatile signals seems to be efficient usually at less than 1 m distance.

3. PLANT–INSECT CHEMICAL INTERACTION IN REPRODUCTION

The host plant location is more often the burden of the mated females excepted for the specialized species for which the host plant is the “rendezvous” place for mating. In polyphagous insects an extensive host range is associated with a less delicacy link between the possible host plants and the insect. The host plant chemical signals become less necessary for reproduction and mate finding relies exclusively on pheromone signal.
3.1 Host Plant Chemical Signal and Reproduction

For phytophagous insect, plant is principally the food resource for the larval stage and occasionally for adults in some species. Host plants are also a place where adults mate. For some species the larval host plant is required for mating whereas not for others. In this case, the adults move to other places and vegetation sometimes completely different from the native host plant.

Some literature highlighted the role of host plant chemical on reproduction. The host plants release compounds affecting several important steps of reproductive behaviour, such as pheromone production (Raina, Kingan, & Mattoo, 1992), and pheromone releasing behaviour (Landolt & Phillips, 1997; McNeil & Delisle, 1989a, 1989b; Tamhankar, 1994).

A research question arising from the past decades is: how the insects find a partner in a constant changing olfactory environment or in the middle of the host plant complex odours? Few studies have demonstrated the action of host plants on male behaviour attraction. In most cases, modifications in the male attraction are due to increase of female pheromone production (Reddy & Guerrero, 2004). Emelianov, Drés, Baltenweiler, and Mallet (2001) and Emelianov, Simpson, Narang, and Mallet (2003) described female calling behaviour improvement of Zeiraphera diniana Guénée, 1845 (Lepidoptera: Tortricidae) in relation with host plant. The two host races of Z. diniana develop on larch and pine respectively. The male attraction is improved by female calling on the host of conspecific. Males find more females on host plant or a synergy occur between calling behaviour, pheromone production and host plant volatiles. That being, reproductive isolation and host plant adaptation can be associated with mate finding. For a polyphagous noctuid feeding on maize no change in male and female reproductive behaviour was found in presence and absence of the host plant (Félix, Smail, & Frérot, 2013), whereas a synergistic effect on male attraction behaviour was shown in the tortricid moth Eupoecilia ambiguella (Hübner, 1796) (Lepidoptera: Tortricidae) (Schmidt-Büsser, von Arx, & Guerin, 2009) although the species is not specialized on grape.

Electrophysiology experiments on Heliothis zea (Boddie, 1850) (Lepidoptera: Noctuidae) males showed that olfactory receptor neuron response is synergized when plant compounds are presented in a mixture with major female pheromone component. The increase in male attraction to H. zea female placed on host plant is probably also due to synergy...
between pheromone components and plants volatiles (Ochieng et al., 2002). Namiki, Iwabuchi, and Kanzaki (2008) have shown the synergy at the level of antennal lobe neurons. The main information available about the processing of mixtures of plant odours and pheromone at the level of the antennal lobe is that the neurones located at that level of the brain are not so specific than postulated. It is now accepted that several peripheral neurones respond as well to a pheromone component and a plant odour compound (Anton & Hansson, 1995; Trone, Anfora, Bengtsson, Witzgall, & Ignell, 2010).

Recently on *H. virescens*, an extensive study including behaviour and neuroperception studies demonstrate that under natural conditions the olfactory system of the male moth appears to be well adapted to follow the female pheromone without interference from plant-emitted odours (Badeke, Haverkamp, Hansson, & Sachse, 2016).

What is less clear for most of the insect species is whereabout do they mate? It has been demonstrated that the *O. nubilalis* however specialized on maize or on other plants; whatever the pheromone strain it was, mate in grassy area out of the host plant spot (Showers, Reed, Robinson, & Derozari, 1976). Scouting in maize field during mating period reveals the absence of both males and females whereas pairs were observed in the “rendezvous” grassy area (Ponsard et al., 2004). *M. brassicae*, a noctuid moth specialized on cabbages for oviposition and larval feeding did not mate in the field planted with cabbages. Pheromone trapping of males is less efficient in the cabbage field than in wooden bush. Scouting after artificial release of males and females in the field showed that the adult insects did not stay in the cabbage field (Frérot, unpublished data), only the mated females come back for oviposition. Few examples on mating location are available whereas a foisonnante literature appeared during the last decades on the role of host plant VOCs on pheromone perception by male and on female pheromone production. Such basic knowledge is missing for most of the pest insects and will be a challenge for developing efficient treatment with sex pheromone. The basic knowledge of the mating procedure is indeed fundamental for using mating disruption or mass trapping of males. For instance there is no reports on mating place for *Cydia pomonella*, nor for *Lobesia botrana* (Denis & Schiffermüller, 1775) (Lepidoptera: Tortricidae) main pest in orchards and vineyard respectively. Could we imagine that they do not mate in their respective cultivated areas where mating disruption is applied?
3.2 Species for Which the Host Plant Is the “Rendezvous” Place

Phytophagous insects are generally adapted to their native host plant via behavioural and/or physiological adjustments. Some of them become through evolution highly specialized to a host plant family and by extension to a single host within a family.

To illustrate the case, the leek moth *Acrolepiopsis assectella* (Walker, 1864) (Lepidoptera: Yponomeutidae) is an example. Thibout (1974) and Auger and Thibout (1983) demonstrated the importance of the host plant in the mate recognition process. The host plant induced pheromone production and act on mating performances. The host plant is a “rendezvous” place. Such a tight link between a plant and an insect is not rare and plenty of examples are available within all the insect orders. Another case where the plant attracts either one or both sexes and the pheromone become a co-attractant of the plant signal as in *Rhynchophorus palmarum* (Linnaeus, 1758) (Coleoptera: Curculionidae) where the host plant volatiles synergize the sex pheromone attraction (Rochat, Gonzalez, Mariau, Villanueva, & Zagatti, 1991).

3.3 Species for Which Host Plants Are Sex Pheromone Precursor

In Lepidoptera, the female sex pheromone is biosynthetised de novo and bears no relation with the host plant. In some species males carry hair pencils associated with glands that produced a pheromone used in courtship behaviour. The male pheromone originated from sequestration of alkaloids or other compounds from larval food as in some Arctiid moths (Conner, Eisner, Vander Meer, Guerrero, & Meinwald, 1981) or from male foraging intakes on specific plants like in nymphalids (Meinwald, 1986; Pliske, 1975). The reproduction is linked to the availability of host plants. In Coleoptera, the bark beetles need to feed on the host plant to produce the sex and aggregation pheromone (Blomquist et al., 2010). For such insects the reproduction relies on the availability of the host plants.

4. PLANT–INSECT CHEMICAL INTERACTION IN HOST FINDING FOR OVIPOSITION

In most of the species that did not rely on trial-error strategy, mated females have to locate the suitable host plant for their brood. Few examples reported active host seeking by larvae. The choosy behaviour of the ovipositing female is well described in several species and there is no doubt
that the mated females in some species are attracted by the host plant. After landing, they exhibited sophisticated probing of the substratum leading to evaluation of the chemical signal and the physical properties (Calatayud et al., 2008; Frérot & Robert, 1998).

Locating a host plant is crucial for herbivore to find the suitable site for the brood to develop on. The sensory cues that elicit or inhibit host location have an important role in survival of the offspring (Renwick & Chew, 1994). When an insect is searching for host plant, it may use different senses, including olfaction, vision, tactile and gustation (Bernays & Chapman, 1994). At the first stages of selection, olfaction and vision are the most important senses because they operate at long distances whereas at short distance the gustatory and tactile cues become more important.

The oviposition choice is based on a complex set of external and internal stimuli and responses in which plant volatiles play an essential role (Miller & Strickler, 1984; Visser, 1986). They are emitted by plants and diffused in the air and mixed with volatiles of different sources to form an aerial soup (Cardé & Willis, 2008) in which the insects are capable to locate their potential host at distance even when the host plant is hidden among an array of other plants and buried in the background noise (Schröder & Hilker, 2008).

Plant cues that guide gravid females include long distance attractants that act during search of oviposition site and close range attraction. Compounds with host plant specific distribution as well as ubiquitous plant volatiles and primary metabolites have been found to act on the oviposition choice (Simmonds, 2001). By now, there are two major hypotheses for host plant recognition based on olfactory cues: (a) plant-specific odour recognition and (b) ratio-specific odour recognition.

In the plant specific odour recognition, the host plant recognition relies on highly specific volatiles cues that are not found in unrelated plant species. For numerous specialist insects the host plant recognition is guided by chemoreception of token stimuli (Dethier, 1982; Fraenkel, 1959). Pieris butterflies are specialist on cruciferous plants and use glucosinolates, secondary plant metabolites chemotaxonomically characteristic for this plant family, as token stimuli (Huang & Renwick, 1994). This nonvolatile glucosinolates were shown to stimulate the oviposition of other crucifer-adapted insect species (Giamoustaris & Mithen, 1995; Griffiths et al., 2001; Hopkins et al., 1997; Mewis, Ulrich, & Schnitzler, 2002). On the contrary, rejection of nonhost plant for oviposition is linked to contact
chemoreception of secondary plant metabolites that have repulsive action on females. Compounds present in some plant family that are token stimuli to specialist insects can have inhibitory effect to nonassociated generalist (Huang & Renwick, 1993).

In the ratio-specific odour recognition the host plant recognition is provided by a specific ratio of mostly ubiquitous volatiles and not by a single compounds or a class of plant-specific volatiles. Wide range of herbivore insects are tuned to detect ubiquitous plant volatiles and the specificity of the signal relies on recognition of particular blends of compounds. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), uses a ratio-specific blend of green leaf volatiles to locate its host plant and a subtle alternation of the blend ratios switches off attraction (Visser & Avé, 1978). Moreover the blend composition of host plant compounds is critical since ratio-specific blends are more attractive than individual compounds (Piñero, Galizia, & Dorn, 2008).

Herbivore insects are attracted or repelled by volatiles cues emitted from plants (Foster & Harris, 1997) but also by compounds released by others herbivores. In some case, the primary cue that leads to oviposition site is not the plant volatiles, but the chemical signal released by a conspecific insect. In many bark beetles species (i.e., “true weevil” family Curculionidae), males choose the host plant and attract females at distance with a pheromone, thus the females are lured by the interplay of pheromones and host plant cues (Wood, 1982). Some female flies, e.g., the cabbage root fly, *Delia radicum* (Linnaeus, 1758) (Diptera: Anthomyiidae), mark the host plant with an oviposition deterring substance to discourage other females from laying eggs on the same host (De Jong & Städler, 2001). As well, it was shown also that the gravid female in search of oviposition site, avoid actively host plants that release volatiles induced by conspecific larvae (De Moraes, Mescher, & Tumlinson, 2001). On the contrary, some species like the leaf miner species, *Liriomyza trifolii* (Burgess, 1880) (Diptera: Agromyzidae), and the beetle *Plagiometriona clavata* (Fabricius, 1798) (Coleoptera: Chrysomelidae) showed a strong oviposition preference for conspecific-infested plants (Facknath, 2012; Viswanathan, Narwani, & Thaler, 2005).

### 4.1 Case of Insects That Do Not Mate on the Host Plant

In many herbivorous lepidopteran species, the imago does not feed and uses the energy accumulated from the food eaten by the larval stage. Depending on the species, the adult may live from few days to few weeks.
The adult stage is very short compared to the overall lifecycle of the insect and the main concern of the insects at this stage is reproduction and oviposition. In some species, adults do not stay on the host plant after emerging from the pupae stage. They leave the larval host plant and mate in a different biotope. For example, the European corn borer (O. nubilalis) adults leave the maize field and mate in dense grassy areas where they rest and hide during the day (Showers et al., 1976). After mating, only the nocturnal-behaving gravid females fly back to maize field to lay eggs. Field observations clearly show that the gravid O. nubilalis females reach maize fields by oriented flights from the resting area, flying up the prevailing wind carrying maize field odours (Leppik & Frérot, 2014). As the females mate far from their host plant, they have a formidable task to find a suitable oviposition site in a complex and changing odorscape. The gravid females discriminate the right host plant at a precise time of the night just after the sunset.

The chemical signature of the maize field evolves through diel change linked with photosynthetic activity between the light and dark time. In the maize field odorscape, the ratios of monoterpenes, sesquiterpenes and some green leaf volatiles change between the day and night and consequently the chemical signal encounter by nocturnal flying insect is specific to their oviposition period (Leppik, Tammaru, & Frérot, 2014). Navigating in a complex and ever-changing odorscape, gravid females need to be constantly tuned into their host plant volatiles and avoid the background odours emitted by other plants. A quick view to what is released by a woody area compared with maize field evidences specific blend released by each biotope (Leppik & Frérot, 2014).

O. nubilalis is present in Europe with two pherotypes specialized on different host plants (Pelozuelo et al., 2004). Z-pherotype feed and lay eggs on maize (Zea mays L.), whereas the E-pherotype does on wild plants such as mugwort (Artemisia vulgaris L.) or hop (Humulus lupulus L.). These three plants have a distinctive volatile signature based on ubiquitous volatiles present in specific ratios (Leppik & Frérot, 2012). Gravid females, based on plant volatile cues specificity, make the host plant selection. The Z-pherotypes females are attracted to the maize volatile blend, whereas the E-pherotype is attracted to the mugwort and hop volatile blend (Bengtsson et al., 2006; Leppik & Frérot, 2012; Molnár, Tóth, Fejes-Tóth, Dekker, & Kárpáti, 2015). Wide range of herbivore insects are tuned into detecting ubiquitous plant volatiles and the specificity of the signal relies on recognition of particular blends of compounds (Bruce & Pickett, 2011). Moreover
the blend composition of the host plant is critical because ratio-specific blends are more attractive than individual compounds (Piñero et al., 2008). The use of VOC in oviposition site recognition is the most prevalent mechanism in most herbivore insects that rely on gravid females to locate and choose the host plant.

4.2 Case of Specialized Insects that Mate and Oviposit on the Host Plant

The chemical signature of the plant changes with the development stages. Therefore, the airborne host plant signals encountered by the insects that colonize the crop at young leaf stage or at flowering stage are different. The different development stages of the plant may act on the different phases of specialized herbivore life such as host location, mating and oviposition. The broad bean weevil, *Bruchus rufimanus* (Boheman, 1833) (Coleoptera: Chrysomelidae), is a specialist pest of *Faba* bean (*Vicia faba* L.). The insects hibernate in woody areas away from *Faba* bean fields (Balachowsky, 1962). Early in the spring, the weevils come out of hibernation places and have to locate the host plant newly planted. The first weevils observed in the field coincide with the development of flower buds. Behavioural tests in the wind tunnel confirm that the weevils are only attracted to their host plant at the flower bud stage, not before at leaf stage (Leppik, Pinier, & Frérot, 2014). For oviposition, only the pod stage of the *Faba* bean is attractive to gravid females. Chemical analyses on the *Faba* bean VOCs show a clear separation of leaf, flower and pod stage. The chemical signature of all the three development stages of *Faba* bean is composed of ubiquitous plant volatiles; the specificity is ensured by change of ratios in the blend (Leppik, Pinier, et al., 2014). The nonattractive leaf stage is dominated mainly by monoterpenes. The flowering stage, when weevils arrive massively in the field, is characterized by a blend of monoterpenes and sesquiterpenes (Bruce, Martin, Smart, & Pickett, 2011). At the pod stage when the females search for oviposition sites, the chemical signature of *Faba* bean is an original blend of green leaf volatiles and monoterpenes (Leppik & Frérot, unpublished data).

5. CONCLUSION

All the advances in understanding the plasticity of the chemical signal released by insects and plants were made possible because of the technological contribution and the improvement of the analytical equipment.
For example, solid phase microextraction (SPME) and headspace gas chromatography mass spectrometry (HS-GC-MS) have allowed the detection of Lepidoptera pheromone blend from a single female (Frérot, Malosse, & Cain, 1997) leading to the vision of individuals belonging to defined populations. The computer and dedicated software allow a quick identification by comparison of compounds spectra with the assistance of improved database.

In conclusion, significant variation in moth sex pheromone blends can be found, not only geographically, but also after being in different experimental conditions, showing that the pheromone composition is likely to be more plastic than previously assumed (Butlin & Trickett, 1997; Groot, Classen, et al., 2010; Löfstedt, 1993). Such variations can directly affect reproduction (e.g., when attraction of conspecific males is reduced), or when other closely related and/or sympatrically occurring moth species with similar pheromone blends are attracted. Such mating reductions or interactions could result in either divergence of moth populations or convergence through hybrid speciation (Harrison, 2012). The plasticity of the pheromone signal makes the mate recognition system able to evolve and thus to preserve the assortative mating even in case of accidental introduction of alien insects in a specific habitat. Plasticity also contributes to adaptation to new host plant and to diversification of species via the specific mate recognition system.

The chemical signals produced by the plants and acting on the insect behaviour are based on the same principle as pheromones. The insects respond to a specific blend composed of a mixture of compounds being released in different quantities. The chemical profile is therefore made of major and minor components and changes in ratio of these compounds have significant information value. The concentration is also a component of the efficient chemical signal as at longer distances from the emission source concentration of many plant VOCs in the atmosphere decreases fast. The insects are tuned to these specific blends and as for pheromone they respond very quickly by a behaviour. The plant chemical signals evolve with the plant development, with the diel periodicity, with the environmental conditions and atmospheric pollution and are often species and variety specific.

The understanding of what are the key compounds for insects may be used by breeders to select new varieties missing the chemical information attractive for herbivorous species but also enhance information on what attracts natural enemies of pest insects on the crop plants.
Odorscape Definition

Odorscape is a new concept that considers the general atmospheric bouquet of VOCs emitted from all the organisms: the flora, the fauna, the soil, bacteria and fungus, developing in a biotope.

**Odorscape is the odorant description of a landscape.** An agrobiocoenose in which biodiversity is reduced can be defined by an odorscape made of a specific mixture of VOCs (Fig. 2). Specificity of the odorscape relies on the chemical structures of the VOCs and on the ratio of the compounds forming the mixture. The dose is also specific, with landscape releasing different amount of VOCs.

Odorscape is characterised by a blend made of one or more main compounds associated with several minor compounds. The action on insects depends of all the components. The odorscape complexity is related with the plant species, the physiological stages of the plants, the biodiversity.

Technique of the Odorscape Collection

Odorscape can be collected by dynamic collection on adsorbent or by passive adsorption on SPME. SPME-HS/GC—MS is relatively simple nondestructive sampling method for collecting and characterizing the composition of the plant.
volatiles. Since the sampling is done on undamaged plants in situ, the SPME-HS analysis gives a realistic picture of plant volatile profile released by plants and allowed comparison of biocoenosis chemical signature. The volatile profile obtained by this method is pertinent for many ecological applications. For an absolute view of the odorscape or of the plant volatile profile, the fibres need to be calibrated with a series of chemicals belonging to different classes, i.e., alcohol, aldehyde, terpenes and sesquiterpenes. As well the mass spectrometry detection should be known for each relevant chemical.

Methodological Considerations of Plant VOC Sampling and Analysis

Sampling of VOCs from living plant material should be conducted so that the sampling method does not change the authentic emission profiles or concentration of emitted compounds significantly. This does not allow, for example, picking the flowers, leaves or twigs from plants for VOC sampling. The studied plant or part should be enclosed in a sampling cuvettes made of glass or inert plastic or even inside transparent plastic bag (Stewart-Jones & Poppy, 2006) so that the enclosed organ is keeping its normal physiological status during VOC sampling. The sampling environment can be the natural environment in the field or if potted, plants can be transferred into the laboratory where the environmental condition can be controlled better. Vuorinen et al. (2005) compared VOC emission of the same branches of potted silver birch seedlings before and after detachment from the seedling and found that detached branches (base in a water container after detachment) had significant increase in emission rates GLV compounds. For instance, emission of (Z)-3-hexenol was 13 and 23 fold higher and emission of (Z)-3-hexenyl acetate was 5 and 28 fold higher after detachment in two different birch genotypes, respectively. However, detachment did not have significant effect on mono-, sesqui- or homoterpene emission rates from the same plants (Vuorinen et al., 2005). If focusing just on analysis of special group or compounds, such as terpenes of essential oil, the separation of specific organs from the original plant does not necessarily change the scent composition significantly, but it does not represent the full volatile signature, which has ecological relevance.

Tholl et al. (2006) listed plant VOC sampling methods in three categories: (1) Static headspace sampling, (2) Dynamic headspace sampling, these both are for gas chromatography—mass spectrometry (GC–MS) sampling and (3) fast, GC-independent analysis [e.g., for proton transfer reaction —mass spectrometry (PTR-MS)]. Main difference between static and dynamic headspace sampling is
that dynamic sampling has controlled air flow in the sampling cuvette, which allows to quantify the emission rates, i.e., calculation of VOC emission per leaf area or dry weight. This allows, for example, calculation of proportion of VOC fluxes of total net carbon exchange, which can account for up to 5—10% particularly in stressed condition (Penuelas & Staudt, 2010) and comparison of the emission. Dynamic head space sampling could disadvantage the low molecular weight compounds such as isoprene, eluated by the air flow passing through the adsorbent trap. Also the replacement air in dynamic sampling needs additional filtering to exclude volatile contaminants and an ozone scrubber to remove atmospheric ozone, which can otherwise degrade most reactive plant VOCs in the sample tube. Static headspace sampling is suitable for qualitative analyses of, for example, flower emissions. Static, noncirculated air allows enrichment of many compounds, which might stay below detection level in dynamic sampling. In the third category, PTR-MS sampling, the cuvettes have often higher air flow rate than dynamic sampling for GC—MS. PTR-MS instrument is connected directly to the sampling cuvette or bag for online sampling with real time analysis having few seconds time resolution (Brilli et al., 2011; Maja et al., 2014). This is important for proper detection of some volatile compounds that are highly reactive in the atmosphere after release from the plant.

Also GC—MS sampling can be done with near real time in fast GC 1—10 min time resolution (Materic et al., 2015). More common in static and dynamic sampling is off-line/storing sampling, which allows storing of VOC samples adsorbent for several weeks and transportation of samples for long distances to the GC—MS analysis laboratory. In static headspace sampling with the SPME method is used. It is a fast and simple method allowing collection of volatiles at detection limits in the ppbv (parts per billion by volume) range (Tholl et al., 2006). SPME is based on ad/absorption and desorption of volatiles inside the static on an inert fibre coated with different types of ad/absorbents.

VOCs are most conveniently collected in situ from undamaged plants. Nonetheless, sometimes it is necessary to collect volatiles from plant parts or organs, for example, to distinguish the VOCs profiles of reproductive organs and vegetative parts. In that case, volatiles are collected either from cut plant parts or preferably in situ from enclosed plant organs to avoid supplementary emission of volatiles due to injuring effects.

The plant is tightly enclosed into a Teflon bag or a vessel to form a headspace. The air surrounding the plant is static, i.e., with no airflow. As the plant continues to release volatile compounds, the static air around the plant becomes enriched with volatile compounds. A small hole is pierced to the Teflon bag and an SPME fibre is inserted into the headspace. The volatile compounds emitted by plant are passively adsorbed on the fibre coating according with the affinity of the fibre for the compounds. Following equilibration between the SPME fibre and the enriched headspace, the SPME fibre is retracted and packed in
aluminium foil or analyzed directly in GC—MS. When properly stored, SPME samples can be analyzed days later without significant loss of volatile compounds. The adsorbed volatiles are thermally desorbed from the SPME fibre without further preparation in a common GC injector. Thermal desorption of VOCs from the SPME fibre eliminates the need for solvents and allowed detection of very volatile compounds, usually undetected due to the solvent peak. Furthermore, the technique is extremely sensitive and enables to detect trace compounds with detection limits down to parts per trillion (ppt) (pg/mL) levels for certain compounds. The same SPME fibres can be reused nearly 100 times.

SPME has some inconveniences: the fibres provide semiquantitative information for a fraction of the plant volatiles, since the adsorbed amount depends on the fibre-coating affinity for the compound and the coating-free sites where compounds are adsorbed (Pawliszyn, 1997), in addition to their concentration in the plant headspace. As a result, the plant volatile composition may misrepresent some volatiles and over represent others. However, by desorbing the entire volatile sample into the injector, no repeated injections of the sample are possible.

In dynamic sampling for GC—MS sample, air is sucked through adsorbent powder inside a glass or steel tube. The sample air-flow through sample tube allows calculation of air volume and concentration of compound in that air volume. Both SPME and adsorbent tube sampling methods use thermodesorption systems to transfer VOC sample for final GC—MS analysis (Tholl et al., 2006).

REFERENCES


