Responses to Oilseed Rape and Cotton Volatiles in Insect Herbivores and Parasitoids

Martin Jönsson
Faculty of Landscape Planning, Horticulture and Agricultural Science
Department of Crop Science
Alnarp

Doctoral thesis
Swedish University of Agricultural Sciences
Alnarp 2005

Acta Universitatis Agriculturae Sueciae
2005: 14

ISSN 1652-6880
ISBN 91-576-7013-7

Tryck: Reproenheten SLU Alnarp

Abstract

Jönsson, M. 2005. *Responses to Oilseed Rape and Cotton Volatiles in Insect Herbivores and Parasitoids*. Doctor's dissertation.
ISSN 1652-6880, ISBN 91-576-7013-7.

This thesis investigates the role of volatiles produced by oilseed rape (*Brassica napus* L.) and cotton (*Gossypium hirsutum* L.) in interactions with insects. It includes studies on release of volatiles by these two crops, as well as behavioural and electrophysiological responses to these odours by two pest insects and insect natural enemies.

The pollen beetle, *Meligethes aeneus* is an important pest of *Brassica* oilseed crops. The larvae feed only on buds and flowers of these plants. The behavioural responses of adult beetles to odours of oilseed rape in bud and flowering stage, as well as to the colours green and yellow, were shown to assist the beetles in finding host plants. Olfactory and visual stimuli were found to interact and the responses of over-wintered and summer generation beetles differed in that the summer generation had a higher preference for flower odours and for flower odours in combination with the colour yellow. We found that oilseed rape infested by pollen beetles released a volatile blend different from that released by non-infested plants.

Three of the most important parasitoids attacking pollen beetles are *Phradis interstitialis*, *P. morionellus* and *Tersilochus heterocerus*. Studies on the responses of these species to odours of bud and flowering stage oilseed rape and to colours showed that odours were used to discriminate between the plant stages, but colour was also important. Differences in host and plant stage preferences were observed in the field and such differences in behaviour are probably important for niche segregation and co-existence of the three parasitoid species. Electrophysiological responses to volatiles characteristic of damaged oilseed rape were also observed and all three species discriminated between infested and non-infested rape by odour.

The moth *Spodoptera littoralis* is a polyphagous herbivore and an important pest of cotton. Responses of female antennae to odours were investigated using two different techniques. High sensitivity to different types of cotton odours were observed, among these several compounds known to be specifically induced by larval feeding. The effects of volatiles of plants infested by conspecific larvae were tested in two types of bioassay. The results indicated that odours from infested plants deterred oviposition, but females needed to perceive other plant stimuli for this discrimination. These studies of two crop plant systems show that plant volatiles affect insects on several trophic levels and confirm that odours often interact with other plant stimuli in shaping insect behaviour.

Keywords: insecta, semiochemicals, olfaction, olfactometer, niche segregation, host plant, odour detection, Coleoptera, Hymenoptera, Lepidoptera

Author's address: Martin Jönsson, Department of Crop Science, SLU, SE-230 53, Alnarp, Sweden

Contents

Appendix

Paper I-VI

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Jönsson, M., Rosdahl, K. & Anderson, P. 2005. Comparison of responses to olfactory and visual host plant cues between over-wintered and summer generation of pollen beetles (*Meligethes aeneus*). *Manuscript.*

II. Jönsson, M., Lindkvist, A. & Anderson, P. 2005. Behavioural responses in three ichneumonid pollen beetle parasitoids to volatiles emitted from different phenological stages of oilseed rape. *Accepted for publication in Entomologia Experimentalis et Applicata.*

III. Jönsson, M., Nilsson, C., Åhman, B., Hacker, N. & Anderson, P. 2005. Micro-habitat distribution and discrimination of odours from infested oilseed rape by three pollen beetle parasitoids. *Submitted manuscript.*

IV. Jönsson, M. & Anderson, P. 2005. Emission of oilseed rape volatiles after pollen beetle infestation; Electrophysiological and behavioural responses in the parasitoid *Phradis morionellus*. *Submitted manuscript.*

V. Jönsson, M. & Anderson, P. 1999. Electrophysiological response to herbivore-induced host plant volatiles in the moth *Spodoptera littoralis*. *Physiological Entomology 24, 377-385.*

VI. Jönsson, M. & Anderson, P. 2005. Context dependent avoidance of volatiles from infested cotton plants by ovipositing *Spodoptera littoralis. Manuscript.*

Papers II and V were reprinted with kind permission from Blackwell Publishing.

Introduction

Background

Animal life cannot exist without green plants and wherever plants grow, insects are also found. Interactions between plants and insects can be both antagonistic and mutualistic (Schoonhoven, Jermy & van Loon, 1998), of which the most studied are the antagonistic relationship of insects feeding on plants and the mutualistic relationship of plant pollination by insects. Both these interactions have been important in shaping the two interacting groups of organisms throughout evolutionary time. Plants have evolved several different types of defences against herbivores and herbivores have evolved different traits to overcome these defences. The attributes of flowers advertising nectar resources for pollinating insects have also evolved to interplay with insects. When this kind of interdependent evolution leads to properties that are unique for the system, it is defined as coevolution (Allaby, 1998).

Plant chemistry is probably the most important factor regulating insect feeding and distribution (Schultz, 1988). The ability of plants to produce toxins that act as a defence against herbivorous insects and the ability of insects to detoxify plant toxins has been described as an evolutionary arms race (Ehrlich & Raven, 1964; Harborne, 1993). Plants also interact with enemies of the herbivores, *i.e.* the third trophic level (Figure 1). The mutualistic relationship between ants and acacia trees is a convincing example of coevolution. The acacia plant provides food and nesting sites, while the ants protect the plants from insect herbivores (Janzen, 1966). About twenty-five years ago, volatile plant chemicals were suggested to be important in interactions with parasitoids of the third trophic level (Figure 1) (Price *et al.*, 1980). Since then, the roles of odours in tri-trophic level interactions have been extensively studied and it has been found that very specific interactions between plants and natural enemies of herbivores can be mediated by plant volatiles (Dicke, van Poecke & de Boer, 2003; Turlings & Wäckers, 2004).

Plant stimuli in host location by insects

Plants display a multitude of different properties that characterize them, not only hundreds of different chemicals but also *e.g.* form, colour and texture (Dethier, 1982; Raguso, 2004). Recognition of plants by insects depends not only on these characters but also on the detection and coding capabilities of the insect (Dethier, 1982).

The process of finding and choosing a host plant for oviposition or feeding performed by herbivorous insects can be divided into different, but overlapping, stages: 1) orientation, 2) landing and 3) assessment (Renwick, 1989; Schoonhoven, Jermy & van Loon, 1998). In parasitoids, the behaviour types are described as: 1) habitat or host community location, 2) microhabitat location and acceptance, 3) host location and acceptance (Vinson, 1998). Clearly most, if not all, plant- and host-seeking insects use multiple sensory modalities throughout this process (Prokopy, 1986). Olfactory and visual stimuli are most important in the first steps of host location, orientation and landing, while mechano-sensory and gustatory stimuli are considered to be most important during the final assessment (Prokopy & Owens, 1983; Schoonhoven, Jermy & van Loon, 1998). Visual and olfactory stimuli are probably also involved during assessment on the plant (Bernays & Chapman, 1994).

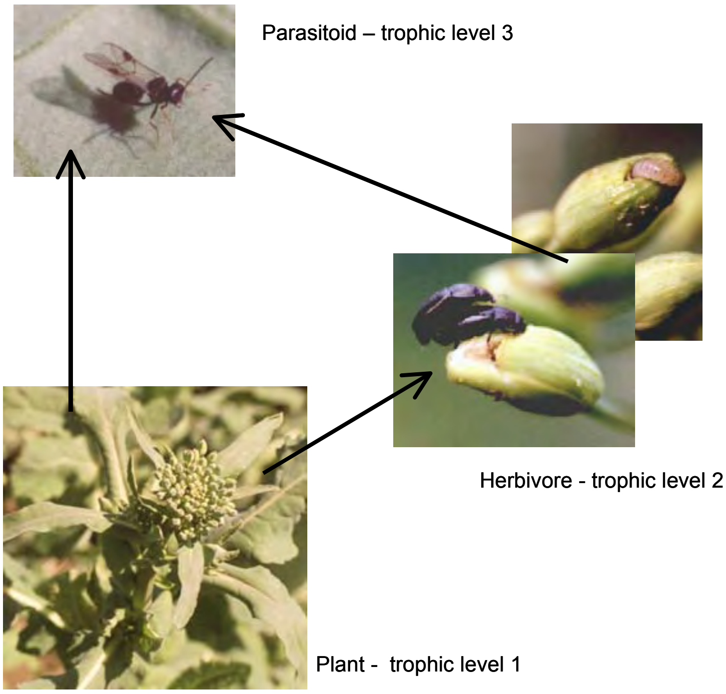

Figure 1. Examples of interactions affected by volatile signals in a community with three trophic levels, oilseed rape, pollen beetles and parasitic wasp attacking pollen beetles. The adult parasitoid oviposits in the herbivorous larvae, and the parasitoid larva feeds on its host larvae. Arrows are placed against the responding insect.

Animal behaviour is dependent not only on stimuli perceived from the environment but also on internal factors. In insects, factors such as hunger state, age and mating status influence the behavioural decisions. The response to perceived stimuli can be described as a sum of positive and negative external stimuli and internal factors processed by the central nervous system of the insect (Dethier, 1982; Miller & Strickler, 1984).

The studies included in this thesis concern insect responses to plant volatiles. Most plants release complex mixtures of volatiles (Knudsen, Tollsten & Bergström, 1993). Two major reasons for plants to actively release volatiles are for defence and for attraction of pollinators (Pichersky & Gershenzon, 2002; Raguso, 2004). Most insect-plant pollination systems have evolved as mutualistic relationships in which both the plant and the insect are rewarded; insects feed on pollen and nectar while assisting the plant in pollen transport (Kevan & Baker, 1983). Flower scents alone or in combination with visual cues can attract insect pollinators, guide the insect to the resource and elicit feeding behaviour (Blight *et al*., 1997; Raguso, 2004). Defence volatiles can act by being repellent or toxic to attacking herbivores or pathogens but also take part in plant defence by attracting enemies of the attacker (Turlings & Wäckers, 2004). The balance between pollinator attractant and herbivore repellent effects can be important in evolution of plant volatile composition (Raguso, 2004). In addition to active release, many volatiles are released passively as breakdown products of primary metabolites. This leakage is normally low but can be considerable from mechanically damaged plants. Independent of origin and primary function of different volatiles, foraging insects should evolve to explore any detectable compound increasing their success.

Plant volatiles in host location by herbivores

Many herbivorous insects are selective feeders, adapted to feed on only a limited number of plant families, and therefore need good abilities to discriminate between plant species. Furthermore, it can be important for insects to assess age, health and infestation status when searching for a suitable host plant for themselves or their offspring.

It is often suggested that defence chemicals are effective only against polyphagous insects, while specialists instead exploit these cues in location of host plants (Renwick, 2002). For example, herbivorous insects specialised on Brassicaceae are attracted to isothiocyanates (Bartlet, 1996; Smart & Blight, 2000) and the onion fly is attracted to dipropyldisulphide (Harris, Keller & Miller, 1987). Most examples of attraction to plant volatiles have been found with monophagous or oligophagous insects, but examples of attraction to plant volatiles among generalist insects, for example among moths and beetles, have also been found (Bernays & Chapman, 1994).

The induced plant volatiles, released after herbivory and attractive to natural enemies of the herbivore can also affect the herbivore itself (Turlings & Wäckers, 2004). In a few studies, herbivore-induced odours have been found to deter ovipositing Lepidoptera (Landolt, 1993; DeMoraes, Mescher & Tumlinson, 2001). There are several reasons for herbivores to avoid these volatiles. The volatiles can indicate that there is a risk of competition, that the chemical defence of the plant is activated and that the plant is probably more attractive to natural enemies of the herbivore. In an experiment by Landolt (1993), females of the generalist cabbage looper moth (*Trichoplusia ni*) were shown to be more attracted to damaged cotton plants. A higher frequency of landings was observed on damaged plants compared to undamaged. However, oviposition was significantly higher on undamaged cotton plants. In a similar test with cabbage, larval feeding decreased both attraction and oviposition. An initial attraction followed by oviposition avoidance in response to herbivore-induced volatiles was also observed by DeMoraes, Mescher & Tumlinson (2001) in similar experiments. In contrast, an attraction to induced plant volatiles has been found in some Coleoptera (Harari, Ben-Yakir & Rosen, 1994; Loughrin, Potter & Hamilton-Kemp, 1995; Bolter *et al.*, 1997). In some of these, the plant volatiles are probably used as aggregation cues. For specialised insects able to detoxify induced defence compounds and not susceptible to natural enemies, avoidance of herbivore-induced volatiles might not be important (Bolter *et al.*, 1997; Turlings & Wäckers, 2004).

Plant volatiles in host location by parasitoids

Hymenopterous parasitoids are the largest group of insect parasitoids and exhibit great diversity in life histories and strategies of host location. Hymenopterous parasitoids are known to attack all stages of hosts: egg, larva, pupa and adult. Some species are highly specific and attack only one or a few species of hosts, while other are generalists and able to develop in many different host species. Hymenopterous parasitoids visit plants not only in search of hosts but also for feeding and mating (Jervis et al., 1993) and plant volatiles are important also in location of flowers as food sources (Wäckers, 2004).

Generally, cues derived directly from the host are difficult to detect from a distance, as herbivores have evolved strategies to avoid detection. Instead, more easily detectable indirect cues derived from another stage of the host, from faeces or from plants can be used by parasitoids (Vet, Lewis & Cardé, 1995; Vinson, 1998). The value of a stimulus for a searching parasitoid is dependent on the reliability in indicating host presence and the detectability of the stimuli. The reliability and detectability of a stimulus in general are expected to be inversely correlated (Vet & Dicke, 1992; Vet, Lewis & Cardé, 1995). Some parasitoids are attracted to odours of undamaged plants normally attacked by their host (Elzen, Williams & Vinson, 1983; Benrey, Denno & Kaiser, 1997), but such cues can be considered to be relatively unreliable in indicating host presence. Plants are often more attractive after artificial damage (Whitman & Eller, 1990; Mattiacci, Dicke & Posthumus, 1994; Potting, Poppy & Schuler, 1999). In such cases, the probability of finding a host is possibly higher but the cues are still not very reliable in indicating host presence. In a number of plants, the volatile blend emitted after herbivory can be a more specific indicator of host presence and attractive for parasitoids (Dicke, van Poecke & de Boer, 2003; Turlings & Wäckers, 2004). The possibility to explore herbivore-induced plant volatiles and sexual pheromones of the host shows that the detectability and reliability of a stimulus are not always negatively correlated (Vinson, 1998). In cases where cues that are both reliable and detectable are absent, associative learning can be important. By associating highly reliable but less detectable host cues with plant-derived stimuli, the parasitoid can increase the host encounter rate and thereby its fitness (Vet, Lewis & Cardé, 1995).

Most studies of parasitoids exploring plant odours have involved leaf-feeding lepidopteran larvae, but herbivores with a concealed lifestyle can also be revealed by plant volatiles. For example, maize plants infested by stem borers release parasitoid attractants (Potting, Vet & Dicke, 1995). For egg parasitoids, indirect cues such as plant odours have been considered to be of minor importance due to the absence of plant damage (Vet, Lewis & Cardé, 1995). However in a few systems, effects of oviposition have been found. In these cases, a wound in combination with eggs triggers the response (Meiners & Hilker, 1997; Hilker, Rohfritsch, & Meiners, 2002). In bean plants attacked by a heteropteran species, egg deposition needs to be combined with feeding for the plant odours to be attractive to egg parasitoids (Colazza, McElfresh & Millar, 2004).

Many parasitoids have a good ability to associate odours with both host and food cues (Turlings *et al.*, 1993). Learning can be adaptive when the environment is unpredictable between generations but predictable during the lifetime (Vet, Lewis & Cardé, 1995) and is an important factor for foraging success in many parasitoid species (Turlings *et al.*, 1993). The degree to which responses to odours can be modified by experience can be related to the degree of specialisation of the parasitoid on both first (plant) and second (herbivore) level (Vet, Lewis & Cardé, 1995).

Plant volatiles in defence

An important part of plant chemical defence consists of non-volatile compounds that primarily act as toxins or feeding deterrents. However, volatile compounds are probably also important. Toxic substances can very likely be detected as odours before ingestion, *i.e.* act against herbivores in the gaseous, liquid and/or solid state (Langenheim, 1994).

It is generally accepted that expression of defence has a cost for the plant (Cipollini, Purrington & Bergelson, 2003) and depending on life traits, plants invest differently in different kinds of defence, reproduction and growth. In many plants, both a *constitutive* and an *inducible defence* against herbivores or pathogens are present. The constitutive defence is always present, while the inducible defence is not activated until the plant is attacked. A large investment in inducible defence compared to constitutive, according to theory, be favourable when the attack by herbivores is unpredictable and does not kill the plant, and when the defence is costly compared to growth and reproduction (Karban, 1993, Zangerl, 2003).

A constitutive release of volatiles is probably of minor importance in plant defence and apart from flower odours undamaged plants normally release only minute amounts of volatiles. In addition to the production cost, a drawback for a plant continuously releasing volatiles is that it is revealing its presence to herbivores, in particular specialised herbivores (Zangerl, 2003). However, many undamaged plants can contain constitutive reserves of volatiles that can be released immediately upon damage and probably act in defence (Langenheim, 1994; McAuslane & Alborn, 1998). Defence traits that act directly against attackers by being repellent, deterrent or toxic are part of the plant's direct defence, while those plant defences that affect the herbivore by interaction with their antagonists are called indirect defence.

Direct inducible defence

Several examples of herbivore-induced production of non-volatile chemicals can be found. One example is production of glucosinolates after herbivory in oilseed rape (Bartlet *et al*., 1999) and another is toxic terpenoid aldehydes produced in response to larval feeding on cotton (McAuslane, Alborn & Toth, 1997). Recent results indicate that odours can also be involved in direct inducible defence. Odours emitted by tobacco after damage cause avoidance by ovipositing female moths (DeMoraes, Mescher & Tumlinson, 2001), and in a natural setting volatiles from wild tobacco were found to deter oviposition (Kessler & Baldwin, 2001).

Indirect inducible defence

Since the pioneering studies on the role of herbivore-induced odours in tri-trophic plant-arthropod interactions (Dicke & Sabelis, 1988; Turlings, Tumlinson & Lewis, 1990), attraction of natural enemies to this kind of odour has been reported in more than 15 plant species (Degenhardt *et al*., 2003). It has been questioned whether attraction of the enemy of the herbivore is a real defence, increasing plant fitness. In contrast to predators, parasitoids do not kill their victims. Parasitised larvae continue to feed on the plant and plant damage might not be reduced (Turlings & Wäckers, 2004). However, reduced herbivory due to predators has been demonstrated (Kessler & Baldwin, 2001) and both *Arabidopsis* and maize plants fed upon by parasitised larvae set more seeds than plants fed upon by unparasitised larvae (Fritche-Hoballah & Turlings, 2001; van Loon, De Boer, & Dicke, 2000).

The induced volatile release can be specific (Turlings *et al*., 1995). Different blends are released from mechanically and herbivore damaged plants in *e.g.* cotton, maize and lima bean (Dicke *et al*., 1990; Turlings *et al*., 1990; Röse *et al*., 1996). In several plant species including maize (Turlings & Tumlinson, 1992) and cotton (Röse *et al*., 1996), it has also been found that undamaged parts release attractive chemicals, *i.e.* the response is systemic. Specificity in timing of volatile emission has been described by Loughrin *et al*., (1994), with the peak in release of herbivore-induced volatiles coinciding with the peak in parasitoid activity. Turlings *et al*. (1998) showed that volatiles released from maize plants differed depending on the herbivore species that was feeding on the plant. The specificity of the plant response is also supported by the finding that it is substances in the oral secretions of feeding larvae that trigger the plant responses (Mattiacci, Dicke, & Posthumus, 1995; Alborn *et al*., 1997). Two biosynthetic pathways, the octadecanoid pathway with jasmonic acid as a key compound and the shikimic acid pathway with salicylic acid as the key compound, are important in production of the induced odours. The octadecanoid pathway seems to be the most important for inducible defence against insects and mites (Dicke, van Poecke & de Boer, 2003).

Plant volatiles

The volatile blend released from a plant may consist of more than hundreds of different components (Raguso, 2004). Some of them are unique for a plant family, but many compounds can be found in the blends of many different and unrelated plants (Knudsen, Tollsten & Bergström, 1993). Single volatiles among these commonly occurring compounds are unlikely to mediate specific plant-insect interactions. However, the relative abundance, *i.e.* ratios of general compounds in a blend, can elicit specific behaviour (Roseland *et al.*, 1992; Raguso, 2004). The majority of volatiles collected from plants can be placed in the following four groups: terpenoids, fatty acid derivatives, benzenoids and nitrogen-containing compounds (Figure 2) (Knudsen, Tollsten & Bergström, 1993; Dudareva & Pichersky, 2000).

Terpenoids are biosynthesised via two different pathways (mevalonate and methylerythritol-4-phosphate pathway) but in both these pathways two different five-carbon units (isopentenyl diphosphate and dimethylallyl diphosphate) are coupled in enzymatically controlled condensation reactions (Paré & Tumlinson, 1999; Degenhardt *et al.*, 2003). These units can be merged in numerous different ways and functional groups can be added. The terpenoids are classified according to size. The monoterpenes and sesquiterpenes are the most volatile and responsible for the typical smell of many plants. Numerous examples of toxic and deterrent functions of both monoterpenoids and sesquiterpenoids can be found (Langenheim, 1994; Tsao & Coats, 1995).

Among the *fatty acid derivatives* aliphatic alcohols, aldehydes and esters are found. The six-carbon compounds in this group together with some of their derivatives, *e.g.* acetates, are often referred to as green leaf volatiles (GLVs). These are breakdown products formed by oxidation of plant lipids and dominate the bouquet of newly damaged green plants (Hatanaka, 1993).

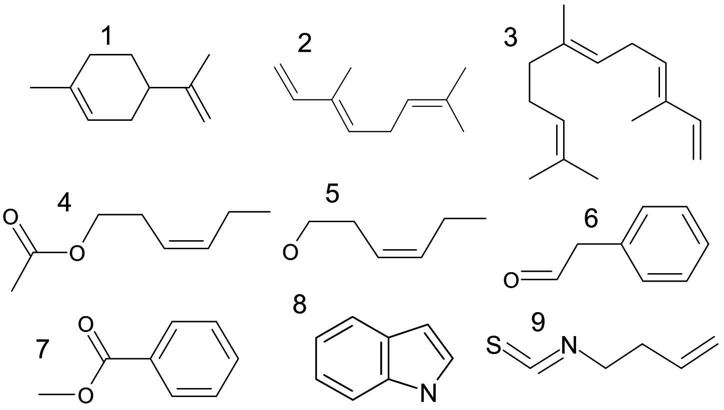

Figure 2. Molecular structure of some commonly found volatiles: 1. limonene 2. (*E*)-β-ocimene (monoterpenes), 3. (*E,E*)-α-farnesene (sesquiterpene), 4. (*Z*)-3-hexenyl acetate, 5. (*Z*)-3-hexenol (fatty acid derivatives), 6. phenylacetaldehyde 7. methylbenzoate (benzenoids), 8. indole, and 9. the more rarely found 3-butenyl isothiocyanate.

Benzenoids are often released from flowers (Dobson, Groth & Bergström, 1996; Verdonk *et al*., 2003). They are biosynthesised from phenylalanine via benzoic acid (Dudareva & Pichersky, 2000) and have a benzene ring on which a carbon chain with alcohol, aldehyde or ester functionality can be attached.

The *nitrogen-containing compounds* is a diverse group including compounds with a biosynthetic origin from amino acids. Indole is a compound emitted from many different plants (Raguso, 2004). Plants in the mustard family (Brassicaceae) and some plants in a few other families can release isothiocyanates that are volatile breakdown products of glucosinolates (Halkier & Du, 1997).

Insect olfaction

The antennae are the primary olfactory organs, but odour receptors can also be found on the mouthparts of several insects (Keil, 1999). Insect antennae have in many cases evolved into sophisticated shapes, such as feather-like and club-shaped structures, to maximize the area carrying odour detecting organs (Keil, 1999). Olfactory receptor neurons (ORNs) are housed within small sensilla. Apart from the ORNs, the olfactory sensilla consist of a number of auxillary (or enveloping) cells that have supportive functions and are involved in development of the sensilla during ontogeny. Depending on the cuticular structure, the sensilla are classified into different types (Hallberg & Hansson, 1999). The most common are (1) trichoid, long hair-like sensilla (Figure 3), (2) basiconic sensilla, also hair-like but normally shorter and thicker than the trichoid, (3) placoid sensilla, plate-like and (4) coeloconic sensilla, which are short peg-like structures situated in a pit (Hallberg & Hansson, 1999). Trichoid sensilla are the dominating sensillum type on the antennae of many insect species. In most insects this sensillar type is innervated by 2-3 neurons, but in Hymenoptera sensilla trichodea often contain around 10 neurons (Hallberg & Hansson, 1999). Another character typical for Hymenoptera is a high abundance of sensilla placodea (Hallberg & Hansson, 1999; Bleeker *et al*., 2004).

Odour molecules enter the sensilla through pores in the wall (Figure 3). The odour molecules are transported through the sensillum lymph by water-soluble odourant binding proteins (OBPs). The selectivity of insect odour detection is thought to depend partly on selectivity in these odour-OBP bindings, but mainly on specificity of receptor sites of the odour receptor neuron (Stengl *et al*., 1999). The mechanisms involved in the transformation of chemical signals to electrical signals are only partly understood (Stengl *et al*., 1999). Briefly, the odour-OBP complex interacts with receptor proteins in the dendritic membrane of the odour receptor neuron. These interactions give rise to a change in receptor potential and if the potential is above a threshold, action potentials are triggered in the neuron (Todd & Baker, 1999). After receptor activation the odour-OBP complex is deactivated and dissolved, followed by degradation of the odour molecule by enzymes in the lymph (Stengl *et al*., 1999). The action potentials of different odour receptor neurons have different amplitudes and waveforms, which often allow discrimination between recorded neurons within one sensillum (Todd &

Baker, 1999). However, it is the frequency of action potentials rather than their size and shape that reflects the strength of the response and affects the behaviour. These action potentials are transmitted in the olfactory axons of the antennal nerves ending in the antennal lobe, where the olfactory signal is processed before being transferred to higher integrative centres of the brain (Todd & Baker, 1999).

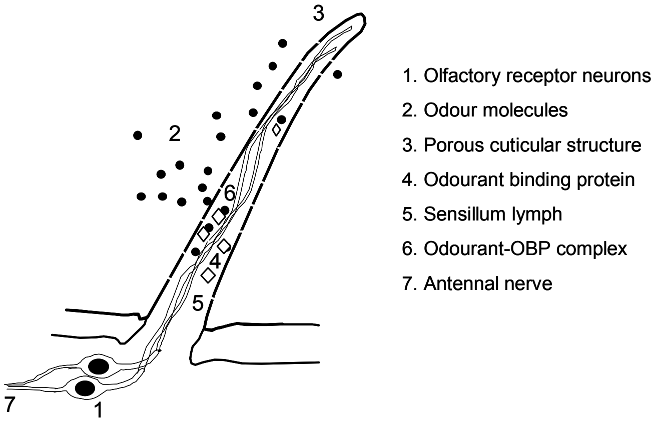

Figure 3. Schematic illustration of an insect trichoid sensillum with two olfactory neurons (1) and a proposed mechanism of odour detection. Odour molecules (2), *e.g.* plant volatiles can pass the porous cuticula (3) of the sensillum. Odourant binding proteins (4) in the sensillum lymph (5) binds to the odour molecule and a complex is formed (6). This complex interacts with a receptor site on the dendrite of the neuron and action potentials are formed in the neuron. The action potentials are transmitted to the central nervous system via the antennal nerve (7).

Insect vision

The information perceived by the visual sense can be described as brightness (intensity of perceived light), hue (dominant wavelength), and saturation (spectral purity). The spatial distribution of perceived light provides information about shape, size, distance and motion (Prokopy & Owens, 1983). Among insects, three types of light-detecting organs exist: ocelli, stemmata and the compound eye. It is only the compound eye that can detect colours and true images (Gullan & Cranston, 1994). In general, the insect compound eye detects wavelengths between 300-650 nm, which in comparison with human vision means that insects are more sensitive to short (UV) wavelengths but less sensitive to the long (red) wavelengths (Prokopy & Owens, 1983).

Species studied

Oilseed rape

Oilseed rape (*Brassica napus* L. (Brassicaceae)) is an annual, typically 1-2 m in height (Figure 4a). The foliage is bluish-green and the four-petalled flowers are yellow. Oilseed rape is an important crop in Europe, grown mainly for the oil that is extracted from the seeds. Today, the double-zero varieties with seeds low in erucic acid and glucosinolates dominate. A number of studies have compared the preferences of pest insects between traditional and zero-cultivars without finding any differences (Milford *et al*., 1989; Alford, 2003). This has been explained by levels of the acid and the glucosinolates differing mainly in the seeds and not in the vegetative parts fed upon by test insects (Milford *et al*., 1989). Both the winter and spring oilseed rape used in our experiments were double low varieties. Flowering oilseed rape is known to release a complex mixture of volatiles, including fatty-acid derivatives, terpenoids, benzenoids and nitrogen-containing compounds (Tollsten & Bergström, 1988; Jakobsen *et al*., 1994). Some of these compounds are probably involved in attraction of pollinators, rewarded with nectar of rape (Westcott & Nelson, 2001). Insect pollination is not crucial (Williams, 1978), but can increase seed set (Westcott & Nelson, 2001). A number of specialised herbivorous pest insects are attracted to oilseed rape odours (Bartlet, 1996). Odours released during larval feeding on leaves can attract parasitoids (Potting, Poppy & Schuler, 1999).

The pollen beetle

Several species of pollen beetles (*Meligethes spp.* (Coleoptera: Nitidulidae)) occur throughout Europe (Figure 1). In most areas, including southern Sweden, *M. aeneus* Fabricius is predominant (Alford, Nilsson & Ulber, 2003). This species reproduces solely on brassicaceous plants and is a pest of both winter and spring rape. The over-wintered adult beetles migrate into the oilseed rape fields in spring and oviposit in buds that are at least 3mm. Both adults and the two larval instars (Osborne, 1965) feed on the buds and flowers. Pollen is important but not essential for larval development (Cook *et al*., 2004). Fully developed, larvae drop to the ground and pupate in the soil. The development from egg to adult normally takes 40-50 days (Nilsson, 1988). There is only one generation per year and the new generation of beetles feeds on flowers before hibernation starts in August (Hokkanen, 1993). The oilseed rape crop is most susceptible to pollen beetles at the early bud stage and becomes less and less sensitive as the crop develops (Alford, Nilsson & Ulber, 2003). Pollen feeding adult beetles are possibly valuable for pollination of oilseed rape (Crowson, 1988). During years when the plant develops fast in spring, *i.e.* when the bud stage is short in time, pollen beetles are only a minor or no problem in winter rape. In years with longer bud time, however, problems occur. Spring rape is always susceptible and is routinely sprayed against pollen beetles, normally with pyrethroids. In some areas of Europe, including parts of Sweden, the intensive use of insecticides has led to increased resistance to pyrethroids (Nilsson *et al*., 2003). Pollen beetles are known to be attracted to yellow, as well as to odours of bud and of flowering stage oilseed rape (Ruther & Thiemann, 1997; Blight & Smart, 1999).

Three pollen beetle parasitoids

At least nine species of hymenopteran parasitoids of pollen beetles exist in Europe, all of which are considered to be larval parasitoids. Most of them belong to Ichneumonidae or Braconidae. The ichenumonid parasitoids *Phradis interstitialis* (Thomson), *P. morionellus* (Holmgren) and *Tersilochus heterocerus* (Thomson) are among the most frequent species in northern and central Europe (Figure 4b, c) (Nilsson & Andreasson, 1987; Billqvist & Ekbom, 2001; Büchi, 2002). These three species are univoltine and specialised on *Meligethes spp*. They are koinobiont, *i.e*. the host continues to develop with the parasitoid inside. Their larval development and pupation take place inside the host larvae. Imagines over-winter in cocoons and emerge from the soil during the following spring and summer (Osborne, 1960). These solitary parasitoids often multi-parasitise (Osborne, 1960; Nilsson & Andreasson, 1987), but only one parasitoid will develop to adult within each larva. Clearly, the species are competitors.

Figure 4. Species studied: a) Oilseed rape b) *Tersilochus heterocerus* c) *Phradis interstitialis* d) *Spodoptera littoralis* e) Cotton (*Photo by courtesy of USDA NRCS*).

The parasitoids often show partial temporal separation, with *P. interstitialis* being most abundant early in the season, while *P. morionellus* is most abundant in late season. *T. heterocerus* is relatively abundant throughout the whole season except for very early and very late (Figure 5) (Nilsson, 1985; Ferguson *et al*., 2003). However, in the region of this study, all three species occur together during a major proportion of the season. Their host stage preferences are not fully described, but *P. interstitialis* has been suggested to oviposit in eggs and first instar larvae by penetrating the bud wall (Osborne, 1960). Based on the parasitation levels of the first and second instar pollen beetle larvae, it has been concluded that *T. heterocerus* prefers to oviposit in second instar larvae (Winfield, 1963; Nilsson & Andreasson, 1987). The host-stage preference of *P. morionellus* has not been studied, but due to their late occurrence it has been suggested that they have similar preferences to *T. heterocerus* and attack larvae in the flowers (Winfield, 1963; Nilsson, 2003).

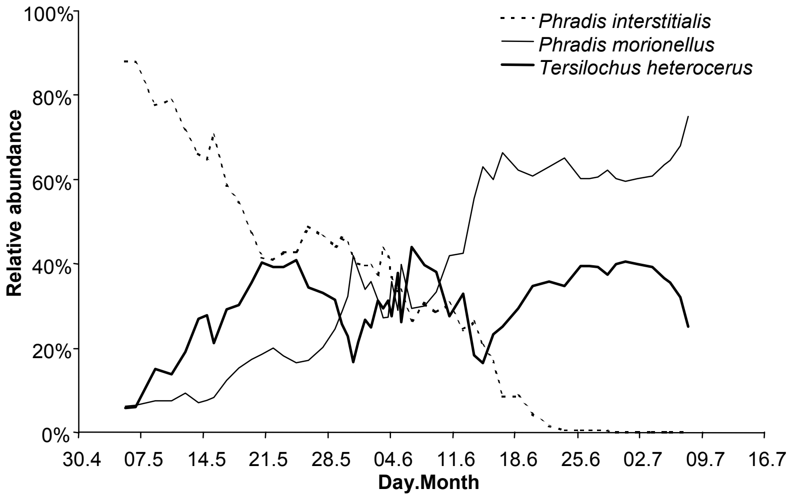

Figure 5. Relative abundance of females of the three pollen beetle parasitoids studied. Sweep net collections were made in fields of winter rape (May-June) and spring rape (June-July) at the university farm in 2002 and 2003. The figure is based on data from Jönsson, Nilsson & Anderson (2004).

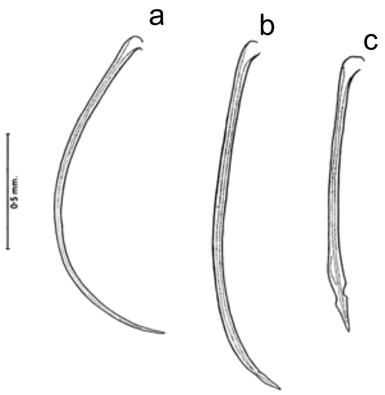

Figure 6. Ovipositor form a) *Phradis interstitialis* b) *P. morionellus* c) *Tersilochus heterocerus*. From Osborne (1960) with kind permission from Cambridge University Press.

The most important character for identification and separation of the species is differences in ovipositor shape. *P. interstitialis* have a long and thin ovipositor. The *T. heterocerus* ovipositor is relatively short and thick, whereas the *P. morionellus* ovipositor is intermediate (Figure 6). Such differences in ovipositor size and shape can reflect a specialisation in attacking hosts in different micro-habitats (Price, 1972) and this possibly indicates that *P. morionellus* are able to parasitise larvae inside the buds. The mechanisms of host location both from a distance and on the plant have not previously been studied. It has been observed that they are attracted to yellow and it has been suggested that plant volatiles may play a role (Nilsson, 2003).

Cotton

The most important plant grown for fibre production is upland cotton (*Gossypium hirsutum* L. (Malvaceae)). Cotton is a perennial shrub but is grown as an annual crop (Figure 4e). The first study on the effects of herbivory on the volatile blend emitted from cotton plants was made by McCall *et al.* (1994). From this and subsequent studies (*e.g.* Loughrin *et al.*, 1994; Röse *et al.*, 1996), it is clear that feeding by insects induces significant changes in volatile emission. The amounts of volatiles released increases dramatically under the impact of constant larval feeding. Constitutive volatiles stored and released immediately upon damage and others that are synthesised and released in elevated amounts as an induced response to larval feeding have been identified (Paré & Tumlinson, 1999). It has also been shown that the response to damage in cotton is systemic, *i.e.* the inducible emission of volatiles is not restricted to the damaged site, but released from the whole plant (Röse *et al.*, 1996).

The Egyptian cotton leaf worm

The Egyptian cotton leafworm (*Spodoptera littoralis* (Boisd.), (Lepidoptera: Noctuidae)) (Figure 4d) occurs in the Mediterranean area, Africa and the Middle East and is an important pest of cotton in *e.g.* Egypt (Hosny, 1980) and Spain (Rubio & Sanchez, 1986). The larvae are highly polyphagous and have been found to feed on plants from a very large number of plant families (Brown & Dewhurst, 1975).

The response to odours in *S. littoralis* has been studied on both peripheral receptor neurons (Anderson, Hansson & Löfqvist, 1995) and interneurons in the antennal lobe of the brain (Anton & Hansson, 1994; Carlsson, Galizia & Hansson, 2002). On the filiform antenna, two different types of sensilla trichodea are found, long and short (Ljungberg, Anderson & Hansson, 1993). The long sensilla are common on the male antennae and contain receptor neurons that respond to sex pheromones. Responses to sex pheromones are almost absent in the neurons of the short sensilla, the dominant sensilla on the female antennae (Anderson, Hansson & Löfqvist, 1995). Behavioural studies have shown that ovipositing female *S. littoralis* distinguish between non-infested plants and plants previously infested by conspecifics (Anderson & Alborn, 1998). In these experiments, it was not determined whether olfactory cues, contact chemoreception or both mediated the observed behaviour. It is known that the behaviour of both adults and larvae of *S. littoralis* is affected by plant odours (Salama, Rizk & Sharaby, 1984; Carlsson *et al*., 1999). However, the importance of the damage-indicating volatiles in host plant selection has not been studied.

Objectives

As outlined, plant odours affect the behaviour of many insects, such as herbivores and natural enemies of these. In addition, plant odours often interact with other stimuli when shaping insect behaviour. For diurnal insects, visual stimuli are often involved in plant location. As the behavioural output of a stimulus is affected by interaction between different sensory modalities, it can be necessary to study the behaviour in different contexts and to relate laboratory data to field observations. Knowledge about the roles of different stimuli is important not only for understanding the basics of insect biology and ecology, but also for development of environmentally-friendly crop management strategies (Agelopoulos *et al*., 1999). The main aim of the present work was to investigate the roles of odours and visual stimuli for pollen beetles and parasitoids in discrimination between different growth stages of rape. A further aim was to study electrophysiological and behavioural responses to damage-indicating odours in both pollen beetle parasitoids and *S. littoralis*. Finally, we aimed to characterise the volatile blends of plants used in behavioural and electrophysiological tests and to compare laboratory results on parasitoids with field observations.

Materials and methods

Bioassays for responses to odours

Insect responses to odours can be studied in various laboratory bioassays and in the field by direct observations or as catches in odour traps. The insects studied can either be laboratory-reared or collected in the field. However, pollen beetles are not easily reared (Bromand, 1983), so the beetles and the parasitoids used in the present study were collected in the field. In order to avoid effects of changes in environment, the tests were mainly performed outdoors with newly collected insects. The moth studied (*S. littoralis*) was laboratory-reared and the experiments performed in a greenhouse. It can be advantageous to study insect behaviour in the laboratory as experimental conditions can be kept constant and variation in responses thereby minimized (Hare, 1998). On the other hand, laboratory experiments can be misleading due to the absence of factors important for natural behaviour. A similar dilemma exists between using laboratory-reared and field-collected insects. Using reared insects offers the advantage that physiological factors such as age, mating status and hunger state can be controlled. However, reared insects sometimes respond differently from wild (Hare, 1998). When behavioural studies are conducted to explain fundamental questions about insect natural behaviour, laboratory studies always need to be related to field observations (van Alphen & Jervis, 1996; Hare 1998).

Olfactory bioassays can be divided into *moving-air* and *still-air* types. Two types of *moving-air bioassays* were used in the studies included in this thesis. An oviposition tube was used with the moth and a Y-tube with beetles and parasitoids. A Y-tube allows the insects to choose between two airstreams carrying odours of different treatments or purified air (Figure 7a). An early description of a Y-tube olfactometer was published by McIndoo (1926). In these experiments, the first evidence for insect attraction to plant odours was demonstrated in tests with Colorado beetle and potato plant odours. Both McIndoo (1926) and the present study used a light source behind the fork to stimulate insect movement. A disadvantage with the Y-tube is that choice is no longer possible when the insects has passed the decision line in one of the arms (van Alphen & Jervis, 1996) and the effects of non-odour based decisions can be large if the insects are strongly attracted to the light source. In our experiments, parts of the tubing before the decision line were shaded to decrease the photo-tactic response in favour of odour-guided decisions. The oviposition tube consisted of a straight Plexiglas tube with the ends covered with net and connected to two odour sources (Figure 7b). Air was pulled out from the centre of the tube and thereby two fields with different odours were created in the two halves of the tube. Single insects were allowed to oviposit and the amounts of eggs laid in the two fields were compared.

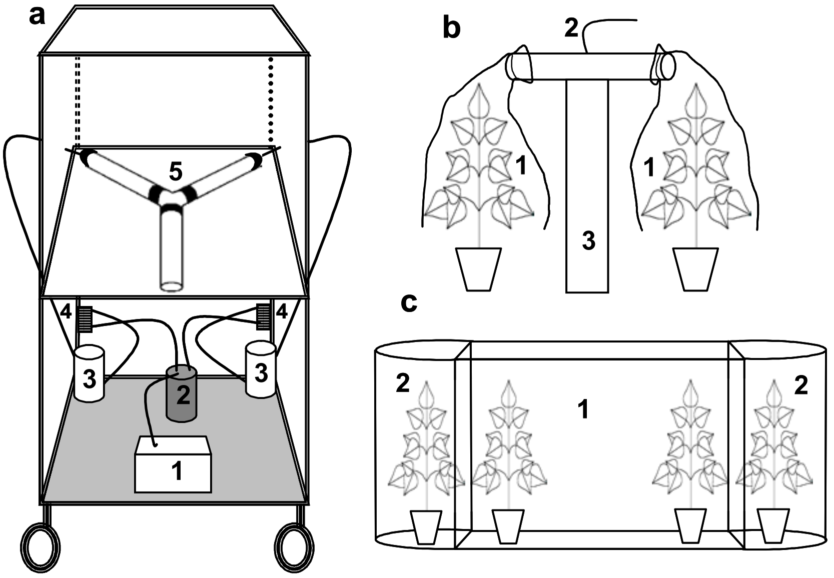

Figure 7. Behavioural assays used. a). Mobile outdoor olfactometer used with beetles and parasitoids. 1) pump, 2) charcoal filter, 3) bottles with stimuli source 4) flow-meters 5) Y-tube. b) oviposition tube-olfactometer used in *Spodoptera* oviposition experiments. 1. plants in plastic cooking bags. 2) air-outlet 3) support. c) cage-assay used in *Spodoptera* oviposition experiments, 1) cage 2) paper-screens enclosing plants used as odour sources.

A still-air type bioassay consisting of a cage with plants inside and odour sources placed outside the two ends of the cage were used for oviposition experiments (Figure 7c). The amounts of eggs laid on plants close to the two odour sources were evaluated. Similar cage oviposition experiments have been performed previously with moths (Landolt, 1993; Anderson & Alborn, 1998; DeMoraes, Mescher & Tumlinson, 2001). In two of these, olfactory location mechanisms were confirmed either by direct observation of first landing (Landolt, 1993; DeMoraes, Mescher & Tumlinson, 2001) or by introducing dispensers emitting synthetic plant volatiles within the cages (DeMoraes, Mescher & Tumlinson, 2001).

Collection and identification of volatiles

The most frequently used method to concentrate volatiles in ecological studies is headspace collection (Millar & Sims, 1998). With this method, the volatiles in the air surrounding the odour source are trapped on an adsorbent. The odour source, for example a plant or an insect, is placed within an aeration chamber. Often different glass chambers are used, but these can be difficult to handle when larger plants are enclosed (Millar & Sims, 1998). As an alternative, we used plastic cooking bags in the present study. When collecting odours from part of a larger plant, it is often not possible to completely seal the bags. To avoid contamination from the laboratory air, purified air can be pumped into the bags and only a portion of the output sampled (Agelopoulos *et al.*, 1999). Different adsorption materials are available, and the choice depends on compounds of interest and on desorption method used (Agelopolous & Pickett, 1998; Raguso & Pellmyr, 1998). In our collections we used a polymer mesh (Super Q, Alltech) as adsorbent and volatiles were desorbed with hexane.

Collected volatiles were separated and analysed by gas chromatography (GC) equipped with a capillary column. In the column, the sample (a mixture of volatiles) is swept through the column by a gas stream. The components of the mixture separate as, depending on volatility and chemical bonding, they partition differently in the mobile gas and in the stationary film covering the inside of the column. The most volatile components and those with low degree of chemical interaction reach the detector first. The components volatilising at higher temperatures and those interacting most with the stationary phase reach the detector last (Jennings, 1987). Consequently, the time required for a component to pass through the column can provide some information about molecular structure. The most commonly used detector in GC-analysis of organic compounds is the flame ionisation detector (FID), which is useful for quantification but does not provide details about molecular structures. A more specific detector is the mass spectrometer (MS). With GC-MS, the sample components are introduced into the ion source of the MS, where they are bombarded with electrons and thereby fragmented and ionised. The amounts of differently sized ions are continuously scanned. The recorded fragmentation pattern, the mass spectrum, can be specific for the compound and analysed manually or by computerised comparisons with standard spectra in commercial or user-created mass spectra databases.

Electrophysiology on insects

Electrophysiological experiments on insect antennae can be performed both as physiological studies on the function of the olfactory sense and as a tool in identifying behaviourally active odours. The most important techniques include the electro antennogram (EAG) technique for recording from whole antennae and single-cell recording (SCR) from odour receptor neurons (Bjostad, 1998).

With the EAG technique, the voltage change (0.1-10 mV) in response to odours is measured over the whole antenna (Figure 8). We used two ringer-filled glass electrodes with silver wires inside to contact the antennae, but other methods can also be used (Bjostad, 1998).

With the SCR technique, responses of individual olfactory receptor neurons can be recorded. This technique is often more difficult to perform than the EAG technique but provides more detailed information. We used electrolytically sharpened tungsten electrodes inserted at the base of sensilla as recording electrodes. The signals from EAG and SCR is amplified and then monitored visually on an oscilloscope or PC. The volatile stimulus is delivered in a purified and humidified air stream continuously passing over the mounted antennae. The compound to be tested is normally placed on a small piece of filter paper inside a Pasteur pipette; a puff of air through the pipettes brings the odour molecules into the air-stream passing over the antenna.

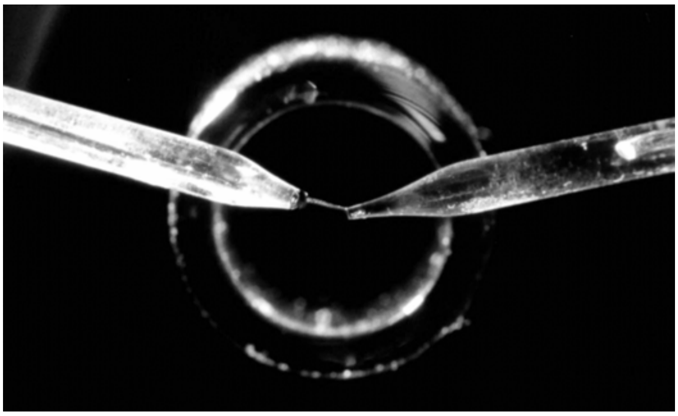

Figure 8. Photo of parasitoid head and antennae mounted in two glass electrodes in front of the air-outlet carrying test odours.

With both these techniques odours can be delivered from a GC, then named GC-EAD (electroantenno detection) and GC-SCR. These combined techniques can be powerful tools to identify biologically active compounds in a natural mixture of volatiles. Although a recorded response provides no information about effects on behaviour, the number of compounds to test in behavioural assays can be reduced. The GC-EAD technique has been very useful in identification of the sex pheromones in many species (Bjostad, 1998).

Results and discussion

Pollen beetle responses to host plant cues (Paper I)

It is well known that both the colour yellow and oilseed rape odours are attractive to pollen beetles (Blight & Smart, 1999 and references therein). Over-wintered pollen beetles searching for host plants arrive in the oilseed crop before flowering and both mating and oviposition start during the green bud stage. The summer generation is known to feed in flowers before hibernation. Attraction to odours of oilseed rape in the bud stage (Ruther & Thiemann, 1997) and odours of flowering rape (Evans & Allen-Williams, 1994) has previously been observed in experiments with starved over-wintered beetles. The response to odours has been less studied with the summer generation, but one experiment indicates that they are attracted to flower odours (Charpentier, 1985). We wanted to examine whether the known differences in demands and behaviour between the generations are reflected in different responses to bud and flower stimuli. As colour stimuli are known to be important for pollen beetles, we evaluated the effects of combining visual stimuli with plant odours.

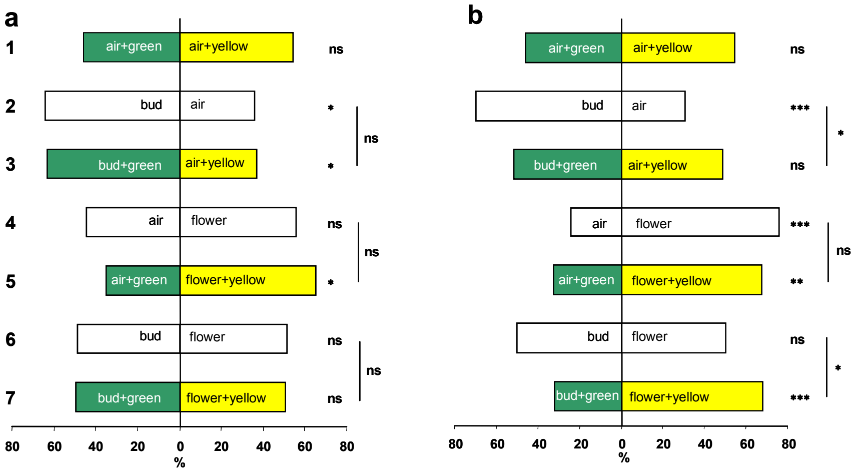

Figure 9. The response of pollen beetles (*M. aeneus)* from the a) over-wintered generation and b) summer generation in Y-tube experiments to odours from oilseed rape at bud and flowering stages, to colour stimuli (green and yellow) or to these stimuli combined. The left side shows experiment number, while the right side shows significant deviation from equal preference in each experiment (* = p<0.05, ** = p<0.01, *** = p<0.001, ns=non significant, G-tests). Outside of these are the results of statistical tests for effects of adding colours, G-test. The numbers of beetles tested in the experiments were 67-97.

In Y-tube experiments we found a higher preference for bud stimuli, *i.e.* green colour and bud odours, in the over-wintered beetles compared with the summer generation (Figure 9). The summer population showed a high preference for flower odours and for flower odours in combination with the colour yellow. With the over-wintered generation, colour had no significant effect on preferences, but for the summer generation effects of colour were found. This can indicate that odours were most important for the over-wintered beetles and be explained as an adaptation to enable location of host plants in the bud stage among other green plants.

Visual stimuli seem to be important for discrimination between the two plant stages for the summer generation. A high preference for flower stimuli in the summer generation is in agreement with the biology of pollen beetles, as food intake is important for this generation and has a strong impact on their winter survival (Hokkanen, 1993). These results demonstrate the dynamics of insect responses to odours. Adding visual stimuli can change the response to odours and the role of a stimulus can shift from one generation to the next.

Parasitoid plant and host stage preferences (Papers II and III)

We investigated whether known differences in the temporal occurrence and preferred host stage of the parasitoids are reflected by differences in their response to olfactory and visual plant stimuli provided by plants in bud and in flowering stage. Furthermore, field observations were made to get more detailed knowledge of plant and host stage preferences of the three parasitoid species tested.

In Y-tube experiments we examined the responses of female parasitoids to odours of bud and flowering stage oilseed rape, to green and yellow, and to odours and colours combined. All three species were attracted to odours from the bud stage of oilseed rape (Figure 10). *T. heterocerus* was attracted to odours of flowering rape, but the two *Phradis* species avoided flower odours. However, when the odours of flowering rape were combined with yellow and odours of the bud stage were combined with green, *P. interstitialis* was equally attracted to both stimuli and *T. heterocerus* also showed an increased preference for flower odours when combined with yellow, while no significant effect of colour could be found in *P. morionellus*. These results are in agreement with the known biology of *P. interstitialis* and *T. heterocerus*. The former is known to attack the host inside the buds (Nilsson, 2003), but can be expected to visit flowers for feeding. The latter has been observed to prefer to oviposit in older larvae, often found in the open flowers (Winfield, 1963; Nilsson & Andreasson, 1987). However, the similarity in response by the *Phradis* species was unexpected, as *P. morionellus* is suggested to be similar to *T. heterocerus* in host stage preference (Winfield, 1963; Nilsson, 2003). To find explanations for these results, the distribution and behaviour of female parasitoids on buds and flowers were observed in the field. During the first observation period in June, *P. interstitialis* and *T. heterocerus* were the dominant species. *P. interstitialis* was frequently observed to parasitise buds, while *T. heterocerus* was only found on flowers.

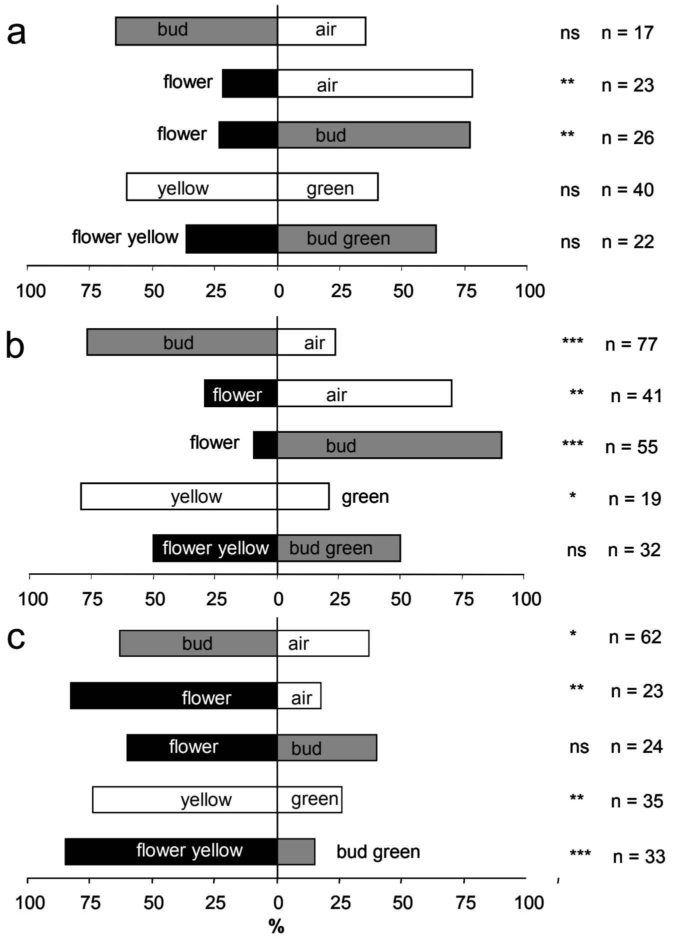

Figure 10. The response of female pollen beetle parasitoids in Y-tube experiments to odours from oilseed rape in bud and flowering stages, to colour stimuli or to these stimuli combined. The three species tested were: a) *Phradis morionellus,* b) *P. interstitialis* and c) *Tersilochus heterocerus*. Asterisks indicate statistically significant differences (ns=not significant * $P<0.05$; **$P<0.01$;***$P<0.001$, G-test, n=number of parasitoids tested).

In July we found that *T. heterocerus* and *P. morionellus* were the dominant species, both abundant on flowers. During this period *P. morionellus* was frequently observed to parasitise on buds, while *T. heterocerus* only rarely did. Thus, the fact that *T. heterocerus* has a higher preference for flower odours than *P. morionellus* can partly be explained by differences in plant stage preferences. The field distribution is also in agreement with ovipositor morphology (Figure 6). *P. morionellus* was intermediate in its preference for parasitising the host inside the buds and is intermediate in ovipositor length. Examination of the pollen beetle eggs found in buds probed by *P. interstitialis* showed that this species is capable of, and specialises in, attacking the egg stage of pollen beetles (Figure 11). The differences in host stage attacked, as well as the higher relative abundance of *P. morionellus* on flowers compared to *P. interstitialis* was not reflected by differences in behaviour in the Y-tube experiments.

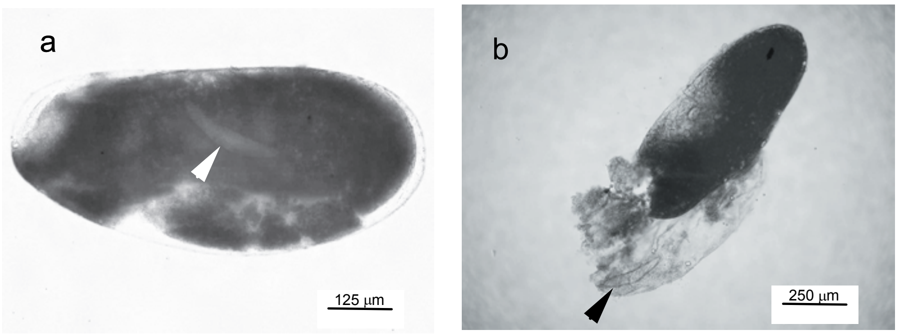

Figure 11. Pollen beetle eggs with parasitoid egg viewed under microscope a) Parasitoid egg appears as a brighter structure in contrast to the pollen beetle egg. b) Pollen beetle egg chorion and parasitoid egg separated from the pollen beetle egg during preparation, *Photo: M.Sjöholm, B. Åhman, M. Jönsson, SLU.*

Volatiles released from oilseed rape (Papers II and IV)

In order to further understand the role of odours in insect-plant interactions, two studies were carried out of the volatiles released from oilseed rape. In the first (II), volatiles were collected from field-grown oilseed rape in bud and in flowering stages, corresponding to the plants used in behavioural experiments with parasitoids (Figure 10). In the second study (IV), collections were made from pot-grown non-infested and infested rape, corresponding to the behavioural assays with *P. morionellus*. Both studies show that the volatile blends released by the plants compared were clearly different and provided the insects with odour cues that could be used to discriminate between the plants used in behavioural experiments.

The comparison between oilseed rape in the bud and the flowering stages showed that the majority of the compounds identified were released at a significantly higher rate from flowering plants (Figure 12) (II). The terpenes sabinene, myrcene, limonene and (*E,E*)-α-farnesene were the dominant volatiles in both bud and flower headspace. A number of benzenoids, including benzaldehyde, phenyl acetaldehyde and 2-phenyl ethanol, as well as the nitrogen-containing compounds indole and benzyl cyanide, were almost exclusively released from the flowering rape.

When the volatile blends of non-infested and infested rape in the flowering stage were compared (Figure 13) (IV), release of the two GLVs increased most due to infestation. These compounds are general indicators of damage and are released from most plants after all types of mechanical damage (Hatanaka, 1993). In similar volatile collections in the bud stage, these two green leaf volatiles were also found to be released at increased rates from pollen beetle-infested plants (Lindkvist, 2003).

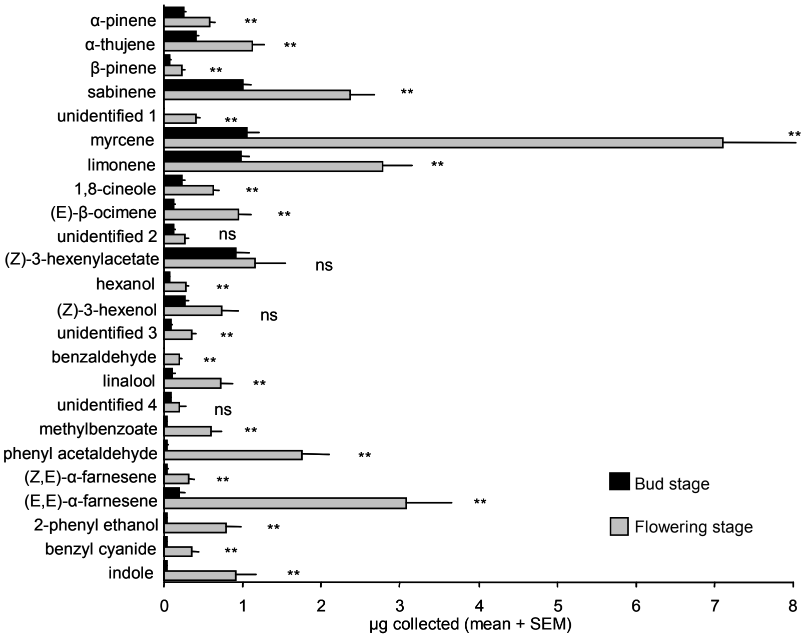

Figure 12. Volatiles collected from bunches of filed grown winter oilseed rape in bud stage and in mid-flowering stage. P-values were calculated using Mann-Whitney U-test (ns = not significant, **P<0.01).

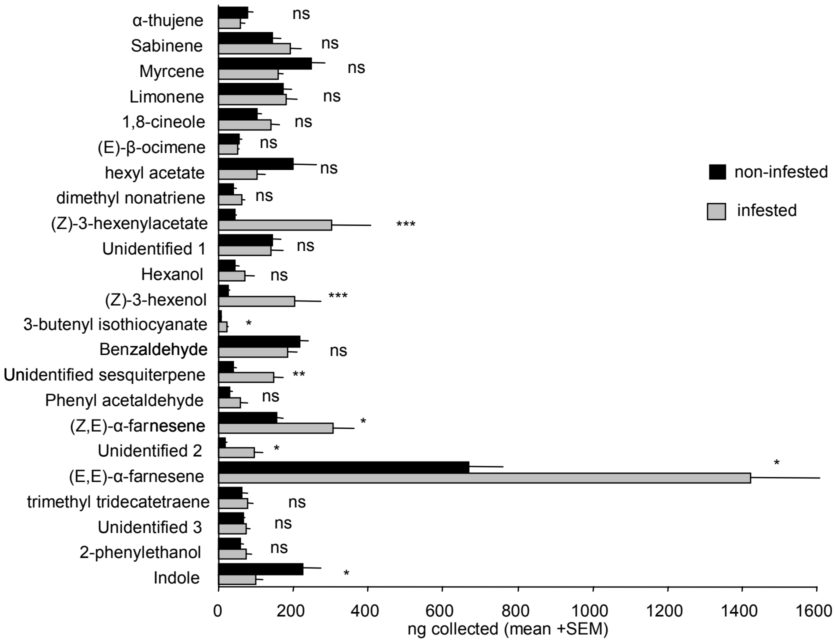

Figure 13. Volatiles collected during 24 hrs from the upper part of non-infested and pollen beetle-infested pot-grown oilseed rape in flowering stage. P-values were calculated using Mann-Whitney U-test (ns = not significant,*P<0.05; **P<0.01; ***P<0.001).

An isothiocyanate (3-butenyl isothiocyanate) was emitted in small but increased amounts from infested flowering plants. This volatile compound is released due to tissue damage when 3-butenyl glucosinolate comes into contact with the enzyme myrosinase (Halkier & Du, 1997). This volatile is typical for Brassicaceae and can therefore be important both in location of plant species and as an indicator of host presence. The third type of compounds that increased in emission due to pollen beetle feeding were the sesquiterpenes. It is possible that these, in contrast to the GLVs, are more specific indicators of the type of damage inflicted to the plant.

GC-EAD responses in parasitoids (Paper IV)

As a step in the process of identifying behaviourally active compounds in the volatile blends, electrophysiological tests were made on the parasitoid antennae. In experiments using volatiles collected from infested rape, thirteen compounds elicited responses from antennae of female *P. morionellus.* Responses were found to compounds of the three dominating classes of volatiles, GLVs [(*Z)*-3-hexenyl acetate, (*Z*)-3-hexenol)], terpenoids [limonene, (*E,E*)-α-farnesese] and benzenoids [methyl benzoate, phenyl acetaldehyde, 2-phenyl ethanol] as well as to 3-butenyl isothiocyanate and indole. This confirms that the parasitoid can detect compounds characteristic for flowering rape as well as pollen beetle-infested rape. The GC-EAD experiments did not indicate that a single compound or a limited group of compounds alone is responsible for the behavioural responses to plant odours. An ability to detect several compounds in all these different classes has also been found in *P. interstitialis* and *T. heterocerus* (Lindkvist, 2003). Detection of a broad spectrum of different types of plant odours has previously been observed in electrophysiological experiments and seems to be general among hymenopterous parasitoids (*e.g*. Li, Dickens & Steiner, 1992; Smid *et al*., 2002).

Parasitoid responses to infested oilseed rape (Papers III and IV)

As described above, the volatile blend of infested oilseed rape is different from that of non-infested rape and the antennae of the parasitoids are able to detect volatiles indicating damage (IV). Clearly, the olfactory cues released provide a possibility for the parasitoids to discriminate between non-infested and infested plants. The ability to do so was tested in two studies, firstly in outdoor Y-tube experiments using cut upper parts of both bud and flowering stage oilseed rape (III) and secondly in indoor experiments using intact potted plants in the flowering stage (IV). The preferences for non-infested versus infested plants in bud stage were studied for all three parasitoids (III). The bud stage oilseed rape in these experiments was used directly after a two-day period of infestation by adult beetles and contained only the egg stage of the host. We observed a higher number of all three parasitoids choosing the arm with odours of infested plants (Figure 14a). However, only the two *Phradis* species showed a significant preference.

As the adult beetles were taken off the plants just prior to the start of experiments, the release of behaviourally active compounds could have been triggered either by the eggs themselves, the damage made by biting beetles during oviposition or feeding, and/or a combination of these factors. In a few other systems, effects of oviposition on attraction to odours of the third trophic level have been studied (Hilker, Rohfritsch & Meiners, 2002; Colazza, McElfresh & Millar, 2004). In these studies, the presence of eggs alone was not enough for attraction of egg parasitoids, while eggs in combination with mechanical damage induced attractive volatiles. The same mechanisms are possibly involved in our system.

As mentioned, oilseed rape buds damaged by ovipositing pollen beetles release the general damage indicators (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenol. These compounds are most likely also released after larval feeding and are possibly attractive to the larval parasitoids, even though larvae are absent. These and other green leaf volatiles singly or blended are known to attract parasitoids (Whitman & Eller, 1990).

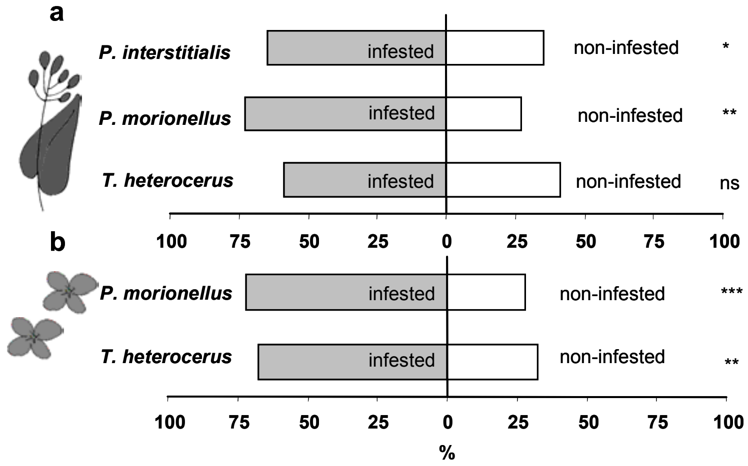

Figure 14. The response of three pollen beetle parasitoids in Y-tube experiments to odours from infested and non-infested oilseed rape in a) the bud stage and b) in the flowering stage. Asterisks indicate statistically significant differences (ns=not significant; *=P<0.05; **=P<0.01; ***=P<0.001; G-test).

The two species known to search for host larvae in the flowers, *P. morionellus* and *T. heterocerus*, were tested in the same type of experiments with flowering stage oilseed rape. Both these species showed a preference for odours of rape with feeding larvae (Figure 14b). Similar results were obtained in the indoor experiments with *P. morionellus* (IV) Parasitoid preference for odours of plants with feeding larvae is often observed (Turlings & Wäckers, 2004) but most of the earlier studies have been made with leaf feeding larvae. The present study shows that a similar response takes place when the reproductive parts are attacked and that oilseed rape odours can be involved in locating preferred host stage on plants by all three parasitoids.

S. littoralis responses to odours of cotton (Papers V and VI)

In two studies we examined the possibility of odour guidance in discrimination between non-infested cotton plants and plants infested by conspecifics in female *S. littoralis.* First, the capacity to detect cotton volatiles was tested with two different electrophysiological techniques and with special focus on herbivore-induced odours (V). Secondly, we tested how odours of damaged cotton plants known to be detected by *S. littoralis* affected the oviposition site choice in two different contexts (VI).

GC-EAD responses to more than ten volatiles collected from plants subjected to larval feeding were found. Using the single sensillum technique, the responses of short sensilla trichodea on the antennae of *S. littoralis* females to cotton and some general plant volatiles were recorded from 108 ORNs. Several neurones activated by herbivore-induced cotton volatiles were found. For example, a neuron type responding to two homoterpenes [(*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene and (*E*)-4,8-dimethyl-1,3,7-nonatriene] and to (*E,E*)-α-farnesene was frequently found. Furthermore, very sensitive neurones responding specifically to the herbivore-induced volatiles linalool and indole at a stimulus load of 10 pg were also observed. Such sensitivity clearly demonstrates that concentrations found in nature can be detected. In addition, specific neurones were found for constitutive cotton volatiles such as caryophyllene, as well as to GLVs which are released after mechanical damage of cotton. These results show that females of *S. littoralis* have receptor neurones to detect and discriminate between damaged and undamaged cotton by odours. A number of compounds were detected only with the GC-EAD technique, while others were found only with the SCR technique. An explanation for absence of response in SCRs is that only one sensillum type and only a selection of these were tested. Explanations for the opposite observation, absence of GC-EAD response, especially to those compounds for which receptor neurons are abundant, are not as clear. However, similar differences between the techniques have been found previously (Blight *et al*., 1995; Wibe, 2004) and illustrates that the techniques are complementary.

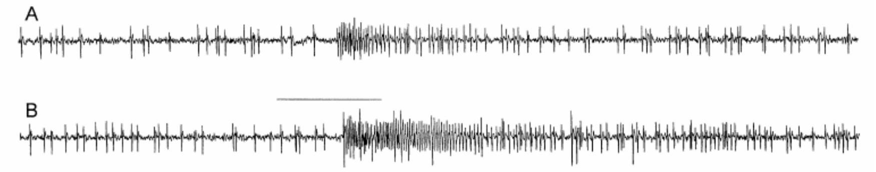

Figure 15. Responses of a receptor neuron in *S. littoralis* female to (A) 0.01 ng and (B) 0.1 ng stimulus load of linalool. Stimulus bar=200 ms.

In the first type of bioassay, the oviposition tube, females could choose to oviposit in the presence of odours from non-infested or infested cotton within the tube (VI). In the other bioassay, the cage experiments, females were allowed to oviposit on undamaged plants placed either close to infested plants emitting damage-indicating volatiles or placed close to non-infested plants. No discrimination was observed in the tube experiments while the odours of plants infested by conspecifics were avoided by ovipositing *Spodoptera* females in the cage experiments. Avoidance was displayed although the plants to which the females had direct access were undamaged. This suggests that the damage-indicating odours can be used in avoidance of infested plants or a habitat with infested plants. However, other cues, *e.g.* mechanosensory, chemosensory contact or perhaps visual stimuli from plants, are probably essential and interact with the damage-induced odours in the decisions preceding oviposition. This is in agreement with the finding that host plant location as well as other behaviour displayed by insects is dependent on inputs from more than one sensory system (Harris & Foster, 1995; Renwick, 1989). Other noctuids also avoid odours of plants infested by conspecifics (Landolt, 1993; DeMoraes, Menscher & Tumlinson, 2001). A possible explanation is that these odours may indicate that the plants' direct chemical defence is activated and that the plants could be more toxic or have lower nutritional value (Turlings & Wäckers, 2004). Feeding by *Spodoptera* larvae on cotton is known to induce the production of terpenoid aldehydes (McAuslane, Alborn & Toth, 1997) probably involved in feeding deterrence (McAuslane & Alborn, 1998; Anderson, Jönsson & Mörte, 2001). Avoidance of damage-indicating odours, as observed in this and other studies with lepidoptera, can be important for ovipositing females to escape parasitation or predation, as well as competition.

Concluding remarks and possibilities for applications

In this thesis the behaviour of all studied insects were affected by odours from an associated plant species. The responses to plant signals were complex and plastic. Electrophysiological experiments showed that plant volatiles could be detected with high sensitivity and selectivity, but responses to odours were influenced by interactions with other sensory modalities. In addition, as found for the pollen beetles, the behavioural responses to plant stimuli can shift depending on insect generation tested.

Although only few compounds were unique for any of the compared treatments of oilseed rape, *i.e.* observed differences were more quantitative than qualitative, the insects were able to differentiate between test plants. This indicates that either amounts or ratios of volatiles were sufficient for odour discrimination or that minor, more specific not identified compounds were important. The electrophysiological experiments also indicated that a mixture of volatiles, probably including compounds of different chemical groups, could be important. Both the parasitoids and the moth showed electrophysiological responses to a broad spectrum of different types of plant volatiles. These antennal responses to a wide spectrum of different plant odours, and the plasticity in behavioural responses to plant odours, are different from responses to sex pheromones, which in general are less variable and stronger. This can be explained by the fact that intraspecific interactions take place between only two actors with well-defined common interests, which can agree on a specific communication, while interspecific responses, *e.g.* insect-plant interactions, often are less specific, involving many actors with widely differing agendas (Hare, 1998).

The effects of context observed in the experiments with the moth stress the importance of relating laboratory data to field observations to explain observed behaviour. In the studies with insects collected in the wild under near natural conditions, it is more likely that behaviour relevant in the field is observed. The observations of the natural behaviour of the parasitoids in the field were useful in explaining the results of the bioassay experiments.

These combined laboratory and field studies expanded our knowledge about how the three closely related parasitoids are niche-segregated. Clearly, the three species not only differ in temporal occurrence, but also in host and plant stage preferences. It was possible to relate these preferences to differences in responses to oilseed rape odours and colours. These differences between the species are probably essential in shaping the realised niches of the three species and in facilitating co-existence. This kind of basic knowledge about the biology of the natural enemies of pest insects is one of the first, but very important, steps in an active promotion of their effectiveness in biological control (Cortesero, Stapel & Lewis, 2000). In a pest management programme, it can be essential to include several species of natural enemies, as a succession of parasitoids attacking different stages of the pest can be necessary for effective biological control (Bottrell, Barbosa & Gould, 1998).

A goal for the future could be to identify volatiles essential for the observed behaviour. Synthetic blends attractive to herbivorous insects have been composed (Roseland *et al.*, 1992; Smart & Blight, 2000; Ansebo et al. 2004). A few examples where synthetic mimics of the herbivore-induced odour blend attract hymenopterous parasitoids can be found (Turlings *et al.*, 1991; James, 2003). However, current knowledge about specific compounds and ratios of these that are actually important for attraction of natural enemies is small (Degenhardt *et al.*, 2003). A relatively large number of volatiles must probably be included in correct ratios. Identifying these blends can be a complex and time-consuming task, even though electrophysiological data are present. An easier way to identify volatiles important for the natural behaviour could be to add potential defence volatiles to the background odour bouquet of intact plants (Pettersson, Birgersson & Witzgall, 2001).

Different ways of applying plant odours in pest management have been suggested (Agelopuolos *et al.*, 1999). Clearly, plant volatiles with repellent effects can be effective directly against several insect pests (Tsao & Coats, 1995). One important problem is the formulation of volatiles for release in the field. A possible but probably less applicable method has been to intercrop with plants known to produce and release active volatiles (Khan *et al.*, 2000). However, apart from lack of knowledge about optimal blends, there are also other problems with usage of synthetic natural enemy attractants. One is that many of the attracted natural enemies will not find a host and probably associate the signal with absence rather than presence of hosts (Lewis *et al.,* 1997). Another is that natural enemy populations possibly decrease due to misleading and unsuccessful host seeking (Degenhardt *et al*., 2003). Plants developed, by breeding or molecular genetics, to continuously release increased amounts of natural enemy attractants would probably face similar problems. A solution to these problems could be a herbivore-induced release also in modified plants (Degenhardt *et al*., 2003). The increasing knowledge about plant volatile biosynthesis and the genes involved makes it possible, in principle, to engineer crop plants with a modified volatile release. When manipulating the volatile release for enhancement of the effectiveness of natural enemies, herbivores should also be studied to avoid attraction of these. However, the complexity and plasticity of insect responses to plant odours probably make it unrealistic to build a pest management strategy against most types of insects based on one type of odour. However, using combined strategies with crops having optimised odour blends, combined with trap crops in push-pull systems, could be a possibility for the future (Agelopoulos *et al*., 1999; Khan *et al*., 2000). Integrating such odour-based systems with other strategies to support beneficial insects and suppress pest insects could be valuable in future pest management strategies.

References

Agelopoulos, N., Birkett, M.A., Hick, A.J., Hooper, A.M., Pickett, J.A., Pow, E. M., Smart, L. E., Smiley, D.W.M., Wadhams, L.J. & Woodcock, C.M. 1999. Exploiting semiochemicals in insect control. *Pesticide science* 55, 225-235.

Agelopoulos, N.G. & Pickett, J.A. 1998. Headspace analysis in chemical ecology: Effects of different sampling methods on ratios of volatile compounds present in headspace samples. *Journal of chemical ecology* 24, 1161-1172.

Agelopoulos, N.G., Hooper, A.M., Maniar, S.P., Pickett, J.A. & Wadhams, L.J. 1999. A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. *Journal of chemical ecology* 25, 1411-1425.

Alborn, H.T., Turlings, T.C.J., Jones, T.H., Stenhagen, G., Loughrin, J.H. & Tumlinson, J.H. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276, 945-949.

Alford, D.V. 2003. The oilseed rape crop. In: *Biocontrol of oilseed rape pests* (Ed. D.V. Alford), Blackwell Science, Oxford, UK, pp 1-8.

Alford, D.V. Nilsson, C. & Ulber, B. 2003. Insect pests of oilseed rape Crops. In: *Biocontrol of oilseed rape pests* (Ed. D.V. Alford DV),.Blackwell Science, Oxford, UK, pp 9-41.

Allaby, M. (1998) *Dictionary of ecology*. Oxford University Press, Oxford.

Anderson, P. & Alborn, H. 1998. Effects on oviposition behaviour and larval development of *Spodoptera littoralis* by herbivore-induced changes in cotton plants. *Entomologia experimentalis et applicata* 92, 45-51.

Anderson, P., Hansson, B.S. & Löfqvist, J. 1995. Plant-odour receptor neurons on the antennae of female and male *Spodoptera littoralis*. *Physiological entomology* 20, 189-198.

Anderson, P., Jönsson, M. & Mörte, U. 2001 Variation in damage to cotton affecting larval feeding preference of *Spodoptera littoralis. Entomologia experimentalis et applicata* 101, 191-198.

Ansebo, L., Coracini, M.D.A., Bengtsson, M., Liblikas, I., Ramírez, M., Borg-Karlsson, A.K., Tasin, M. & Witzgall, P. 2004. Antennal and behavioural response of codling moth *Cydia pomonella* to plant volatiles, *Journal of applied entomology* 128, 488-493

Anton, S. & Hansson, B.S. 1994. Central processing of sex pheromone, host odour and oviposition deterrent information by interneurons in the antennal lobe of female *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of comparative neurology* 305, 199-214.

Bartlet, E. 1996. Chemical cues to host-plant selelection by insect pests of oilseed rape. *Agricultural zoology reviews* 7, 89-116.

Bartlet, E., Kiddle, G., Williams, I. & Wallsgrove, R. 1999. Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. *Entomologia experimentalis et applicata* 91, 163-167.

Benrey, B., Denno, R.F. & Kaiser, L. 1997. The influence of plant species on attraction and host acceptance in *Cotesia glomerata* (Hymenoptera: Braconidae). *Journal of insect behaviour* 10, 619-630.

Bernays, E.A. & Chapman, R.F. 1994. *Host-plant selection by phytophagous insects*. Chapman and Hall, London.

Billqvist, A. & Ekbom, B. 2001. The influence of host plant species on parasitism of pollen beetles (*Meligethes spp.*) by *Phradis morionellus. Entomologia experimentalis et applicata* 98, 41-47

Bjostad, L.B. 1998. Electrophysiological methods. In: *Methods in chemical ecology, vol 1, chemical methods,* (Eds. J.G. Millar and K.F. Haynes), Kluwer Academic Publishers, Boston, Dordrecht, London, pp. 339-375.

Bleeker, M.A.K., Smid, H.M., van Aelst, A.C., van Loon, J.J.A. & Vet, L.E.M. 2004. Antennal sensilla of two parasitoid wasps: A comparative scanning electron microscopy study. *Microscopy research and technique* 63, 266–273.

Blight, M.M. & Smart, L.E. 1999. Influence of visual cues and isothiocyanate lures on capture of the pollen beetle *Meligethes aeneus* in field traps. *Journal of chemical ecology* 25, 1501-1516.

Blight, M.M., Le Métayer, M., Delégue, M.P., Pickett, J.A., Marion-Poll, F. & Wadhams, L.J. 1997. Identification of floral volatiles involved in recognition of oilseed rape flowers, *Brassica napus* by honeybees, *Apis mellifera*. *Journal of chemical ecology* 23, 1715-1727.

Blight, M.M., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. 1995. Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera Curculionidae). *Journal of chemical ecology* 21, 1649-1664.

Bolter, C.J., Dicke, M., van Loon, J.J.A., Visser, J.H. & Posthumus, M.A. 1997. Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of chemical ecology* 23, 1003-1023.

Bottrel, D.G., Barbosa, P. & Gould, F. 1998. Manipulating natural enemies by plant variety selection and modification: A realistic strategy?*Annual review of entomology*, 43, 347-367.

Bromand, B. 1983. Possibility of continuous rearing of *Meligethes aeneus* Fabr. (Col.), *Zeitschrift für angewandte Entomologie* 96, 419-422.

Brown, E.S. & Dewhurst, C.F. 1975. The genus Spodoptera (Lepidoptera: Noctuidae) in Africa and near east. *Bulletin of entomological research* 65, 221-265.

Büchi, R. 2002. Mortality of pollen beetle (*Meligethes spp.*) larvae due to predators and parasitoids in rape fields and the effects of conservation strips. *Agriculture, eccosystems and environment* 90, 255-263.

Carlsson, M.A., Anderson, P., Hartlieb, E. & Hansson, B.S. 1999. Experience-dependent modification of orientational response to olfactory cues in larvae of *Spodoptera littoralis*. *Journal of chemical ecology* 25, 2445-2454.

Carlsson, M.A., Galizia, C.G. & Hansson, B.S. 2002. Spatial representation of odours in the antennal lobe of the moth *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Chemical senses* 27, 231-244.

Charpentier, R. 1985. Host plant selection by the pollen beetle *Meligethes aeneus*. *Entomologia experimentalis et applicata* 38, 277-285.

Cipollini, D., Purrington, C.B. & Bergelson J. 2003. Costs of induced responses in plants. *Basic and Applied ecology* 4, 79-89.

Colazza, S., McElfresh, J.S. & Millar, J.G. 2004. Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on bean spp., that attract the egg parasitoid *Trissolcus basalis*. *Journal of chemcial ecology* 30, 945-964.

Cook, S.M., Murray, D.A. & Williams, I.H. 2004. Do pollen beetles need pollen? The effect of pollen on oviposition, survival, and development of a flower feeding herbivore. *Ecological entomology* 29, 164–173.

Cortesero, A.M., Stapel, J.O. & Lewis, W.J. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biological Control* 17, 35-49.

Crowson, R.A. 1988. Meligethinae as possible pollinators (Coleoptera: Nitidulidae). *Entomologia Generalis* 14, 61-62.

Degenhardt, J., Gershenzon, J., Baldwin, I.T. & Kessler, A. 2003. Attracting friends to feast on foes: Engineering terpene emission to make crop plants more attractive to herbivore enemies. *Current opinion in biotechnology* 14, 169-176.

DeMoraes, C.M., Mescher, M.C. & Tumlinson, J.H. 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410, 577-580.

Dethier, V.G. 1982. Mechanism of host-plant recognition. *Entomologia experimentalis et applicata* 31, 49-56.

Dicke, M. & Sabelis, M.W. 1988. How plants obtain predatory mites as bodyguards. *Netherlands journal of zoology* 38, 148-165

Dicke, M., van Beek, T.A., Posthumus, M.A., Ben Dom, N., van Bokhoven, H. and De Groot, A.E. 1990. Isolation and identification of volatile kairomone that affects acarine predator-prey interaction, involvement of host plant in its production. *Journal of chemical ecology* 16, 381-396.

Dicke, M., van Poecke, R.M.P. & de Boer, J.G. 2003. Inducible indirect defence of plants: From mechanisms to ecological functions. *Basic and applied ecology* 4, 27-42.

Dobson, H.E., Groth, I. & Bergström, G. 1996. Pollen advertisement: Chemical contrasts between whole-flower and pollen odors. *American Journal of Botany* 83, 877-885.

Dudareva, N. & Pichersky, E. 2000. Biochemical and molecular genetic aspects of floral scents. *Plant physiology* 122, 627-633.

Ehrlich, P.R. & Raven, P.H. 1964. Butterflies and plants: A study in coevolution. *Evolution* 18, 586-608.

Elzen, G.W., Williams, H.J. & Vinson, S.B. 1983. Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: Implications for host habitat location. *Environmental entomology* 12: 1873-1877.

Evans, K.A. & Allen-Williams L.J. 1994. Laboratory and field response of the pollen beetle, *Meligethes aeneus*, to the odour of oilseed rape. *Physiological entomology* 19, 285-290.

Ferguson, A.W., Campbell, J.M., Warner, D.J., Watts, N.P., Schmidt, J.E.U. & Williams, I.H. 2003. Spatio-temporal distributions of *Meligethes aeneus* and its parasitoids in an oilseed rape crop and their significance for crop protection. *Proceedings of the 11th International rapeseed congress* 4, 1057-1059.

Fritche-Hoballah, M.E. & Turlings, T.C.J. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids, *Evolutionary ecology research* 3, 553-565.

Gullan, P.J. & Cranston, P.S. 1994. Insects, An outline of entomology, Chapman & Hall, London.

Halkier, B.A. & Du, L. 1997. The biosynthesis of glucosinolates. *Trends in plant science* 2, 425-431.

Hallberg, E. & Hansson, B.S. 1999. Arthropod sensilla: Morphology and phylogenetic consideration. *Microscopy research and technique* 47, 428-439.

Harari, A.R., Ben-Yakir, D. & Rosen, D. 1994. Mechanism of aggregation behaviour in *Maladera matrida* Argaman (Coleoptera: Scarabidae). *Journal of chemical ecology* 20, 361-371.

Harborne, J.B. 1993. *Introduction to Ecological Biochemistry*. 4th edition. Academic Press. London.

Hare, J.D. 1998. Bioassay methods with terrestrial invertebrates. In: *Methods in Chemical Ecology, vol 2, Bioassay Methods*, (Eds. J.G. Millar & K.F. Haynes), Kluwer Academic Publishers, Boston, Dordrecht, London, pp. 212-269.

Harris & Foster 1995. Behaviour and integration. In: *Chemical of Ecology Insects 2*. (Eds. R.T. Cardé & W.J. Bell). Chapman and Hall, New York, pp. 3-46.

Harris, M.O., Keller, J.E. & Miller, J.R. 1987. Responses to n-dipropyl disulfide by ovipositing onion flies: effects of concentration and site of release. *Journal of chemical ecology* 13, 1261-1277.

Hatanaka, A. 1993. The biogeneration of green odour by green leaves. *Phytochemistry* 34, 1201-1218.

Hilker, M., Rohfritsch, O. & Meiners, T. 2002. The plant's response towards insect egg deposition. In: *Chemoecology of Insect Egg and Egg Deposition.* (Eds. M. Hilker and T. Meiners). Blackwell Publishing, Berlin, pp 205-233.

Hokkanen, H.M.T. 1993. Overwintering survival and spring emergence in *Meligethes aeneus*: Effects of body weight, crowding, and soil treatment with *Beauveria bassiana*. *Entomologia experimentalis et applicata* 67, 241-246.

Hosny, M.M. 1980. The control of cotton pests in Egypt. *Outlook on agriculture* 10, 204-205.

Jakobsen, H.B., Friis P., Nielsen, J.K. & Olsen, C.E. 1994. Emission of volatiles from flowers and leaves of *Brassica napus in situ*. *Phytochemistry* 37, 695-699.

James, D.G. 2003. Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. *Environmental entomology* 32, 977-982.

Janzen, D.H. 1966. Coevolution of mutualism between ants and Acacias in Central America. *Evolution* 20, 249-275.

Jennings, W. 1987. *Analytical gas chromatography*, Academic Press, London.

Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T. & Dawah, H.A. (1993) Flower-visiting by hymenopterous parasitoids, *Journal of natural history* 27, 67-105.

Jönsson, M., Nilsson, C. & Anderson, P. 2004. Occurrence of pollen beetle parasitoids in the south of Sweden. *Integrated Protection in Oilseed Crops IOBC/wprs, Bulletin* 27, 241-244.

Karban, R. 1993. Costs and benefits of induced resistance and plant density for a native shrub *Gossypium thurberi*. *Ecology* 74, 9-19.

Keil, T.A. 1999. Morphology and development of the peripheral olfactory organs. In: *Insect olfaction*. (Ed. B.S. Hansson). Springer-Verlag Berlin. pp. 5-47.

Kelber, A., Balkenius, A. & Warrant, E.J. 2002. Scotopic colour vision in nocturnal hawkmoths. *Nature* 419, 922-925.

Kessler, A. & Baldwin, I.T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291, 2141-2144.

Kevan, P.G. & Baker, H.G. 1983. Insects as flower visitors and pollinators. *Annual review of entomology* 28, 407-453.

Khan, Z.R., Pickett, J.A., van den Berg, J., Wadhams, L.J. & Woodcock, C.M. 2000. Exploiting chemical ecology and species diversity: Stem borer and striga control for maize and sorghum in Africa. *Pest management science* 56, 957-962.

Knudsen, J.T., Tollsten, L. & Bergström, G. 1993. Floral scents - A checklist of volatile compounds isolated by headspace techniques. *Phytochemistry* 33, 253-280.

Landolt, P.J. 1993. Effects of host plant leaf damage on Cabbage Looper moth attraction and oviposition. *Entomologia experimentalis et applicata* 67, 79-85.

Langenheim, J. H. 1994. Higher plant terpenoids: A phytocentric overview of their ecological roles, *Journal of chemical ecology* 20, 1223-1280.

Lewis, W.J., van Lenteren, J.C., Phatak, S.C. & Tumlinson, J.H. 1997. A total system approach to sustainable pest management, *Proceedings of the national academy of sciences USA* 94, 12243-12248.

Li, Y., Dickens, J.C. & Steiner, W.W.M. 1992. Antennal olfactory responsiveness of *Microplitis croceipes* (Hymenoptera: Braconidae) to cotton plant volatiles. *Journal of chemical ecology* 18, 1761-1773.

Lindkvist, A. 2003. *Behaviour and Antennal Responses of Pollen Beetle Parasitoids to Volatiles Emitted from Oilseed Rape.* Honours thesis, Department of Crop Science, Swedish University of Agricultural Sciences, Alnarp.

Ljungberg, H., Anderson, P. & Hansson, B.S. 1993. Physiology and morphology of pheromone-specific sensilla on the antennae of male and female *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of insect physiology* 39, 253-260.

Loughrin, J.H., Manukian, A., Heath, R.R., Turlings., T.C.J. & Tumlinson, J.H. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. *Proceedings of the national academy of sciences of the United States* 91, 11836-11840.

Loughrin, J.H., Potter, D.A. & Hamilton-Kemp, T.R. 1995. Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese Beetle (*Popilia japonica* Newman). *Journal of chemical ecology* 21, 1457-1467.

Mattiacci, L., Dicke, M. & Posthumus, M.A. 1994. Induction of parasitoid attracting synomone in Brussels sprouts plants by feeding of *Pieris brassicae* larvae: Role of mechanical damage and herbivore elicitor. *Journal of chemical ecology* 9, 2229-2247.

Mattiacci, L., Dicke, M. & Posthumus, M.A. 1995. ß-Glucosidase: An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proceedings of the national academy of sciences of the United States* 92, 2036-2040.

McAuslane, H.J. & Alborn, H.T. 1998. Systemic induction of allelochemicals in glanded and glandless isogenic cotton by *Spodoptera exigua* feeding. *Journal of chemical ecology* 24, 399-416.

McAuslane, H.J., Alborn, H.T. & Toth, J.P. 1997. Systemic induction of terpenoid aldehydes in cotton pigment glands by feeding of larval *Spodptera exigua*. *Journal of chemical ecology* 23, 2861-2879.

McCall, P.J., Turlings, T.C.J., Loughrin, J., Proveaux, A.T. & Tumlinson, J.H. 1994. Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *Journal of chemical ecology* 20, 3039-3050.

McIndoo, N.E. 1926. An insect olfactomer. *Journal of economic entomology* 19, 545-571.

Meiners, T. & Hilker, M. 1997. Host location in *Oomyzus gallerucae* (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). *Oecologia* 112, 87-93.

Milford, G.F.J., Porter, A.J.R., Fieldsend, J.K., Miller, C.A., Leach J.E. & Williams, I.H. 1989. Glucosinolates in oilseed rape (*Brassica napus*) and the incidence of pollen beetles (*Meligethes aeneus*). *Annuals of applied biology* 115, 375-380.

Millar, J. G. & Sims, J.J. 1998. Preparation, cleanup, and preliminary fraction of extracts. In: *Methods in Chemical Ecology, vol 1, Chemical methods*, (Eds. J.G. Millar & Kenneth, F. H.), Kluwer Academic Publishers, Boston, Dordrecht, London, pp. 1-37.

Miller, R.M. & Strickler, K.L. 1984. Finding and accepting host plants. In: *Chemical Ecology of Insects*. (Eds. W.J. Bell & R.T. Cardé). Chapman and Hall, London, pp. 127-153.

Nilsson, C. & Andreasson, B. 1987. Parasitoids and predators attacking pollen beetles (*Meligetes aeneus* F.) in spring and winter rape in southern Sweden. *IOBC/WPRS Bulletin* 10, 64-73.

Nilsson, C. 1985. Impact of ploughing on emergence of pollen beetle parasitoids after hibernation. *Zeitschrift für angewandte Entomologie* 100, 302-308.

Nilsson, C. 1988. The pollen beetle (*Meligethes aeneus* F.) in winter and spring rape at Alnarp 1976-1978. III. Mortality factors. *Växtskyddsnotiser* 52, 145-150.

Nilsson, C. 2003. Parasitoids of pollen beetles. In: *Biocontrol of Oilseed Rape Pests*. (Ed. D.V. Alford), Blackwell Science, Oxford, UK, pp 73-85.

Nilsson, C., Åhman, B., Gustafsson G. & Djurberg, A. 2003. Resistens mot pyretroider hos rapsbaggar (*Meligethes sp*). *DJF Report* no. 89, 143-149.

Osborne, P. 1960. Observations on the natural enemies of *Meligethes aeneus* (F.) and *M. viridescens* (F.) [Coleoptera: Nitidulidae]. *Parasitology* 50, 91-110.

Osborne, P. 1965. Morphology of the immature stages of *Meligethes aeneus* (F.) and *M. viridescens* (F.) (Coleoptera, Nitidulidae). *Bulletin of Entomological Research* 55, 747-759.

Paré, W. & Tumlinson, J.H. 1999. Plant volatiles as a defence against insect herbivores. *Plant Physiology* 121, 325-332.

Pettersson, E.M., Birgersson, G. & Witzgall, P. 2001. Synthetic attractants for the bark beetle parasitoid *Coeloides bostrichorum* Giraud (Hymenoptera: Braconidae). *Naturwissenschaften* 88, 88-91.

Pichersky, E. & Gershenzon, J. (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current opinion in plant biology* 5, 237-243.

Potting, R.P.J., Poppy, G.M. & Schuler, T.H. 1999. The role of volatiles from cruciferous plants and pre-flight experience in the foraging behaviour of the specialist parasitoid *Cotesia plutellae*. *Entomologia experimentalis et applicata* 93, 87-95.

Potting, R.P.J., Vet, L.E.M. & Dicke, M. 1995. Host microhabitat location by stem-borer parasitoid *Cotesia flavipes*: the role of herbivore volatiles and locally and systemically induced plant volatiles. *Journal of chemical ecology* 21, 525-539.

Price, P.W. 1972. Parasitoids utilizing the same host: Adaptive nature of differences in size and form. *Ecology* 53, 190-195.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual review of ecology and systematics* 11, 41 - 65.

Prokopy, R.J. & Owens, E.D. 1983. Visual detection of plants by herbivorous insects. *Annual review of entomology* 28, 337-364.

Prokopy, R.J. 1986. Visual and olfactory stimulus interaction in resource finding by insects. In: *Mechanisms in Insect Olfaction* (Eds. T.L. Payne, M.C. Birch & Kennedy, C.E.), Oxford University Press, New York, pp. 81-89.

Raguso, R.A. & Pellmyr, O. 1998. Dynamic headspace analysis of floral volatiles: A comparison of methods. *Oiokos* 81, 238-254.

Raguso, R.A. 2004. Why do flowers smell? The chemical ecology of fragrance-driven pollination. In: *Advances in Insect Chemical Ecology* (Eds. R.T. Cardé & J.G. Millar). University Press Cambridge, pp. 151-178.

Renwick, J.A.A. 1989. Chemical ecology of oviposition in phytophagous insects. *Experientia* 45, 223-228.

Renwick, J.A.A. 2002. The chemical world of crucivores: lures, treats and traps. *Entomologia experimentalis et applicata* 104, 35-42.

Röse, U.S.R., Manukian, A., Heath, R.R. & Tumlinson, J.H. 1996. Volatile semiochemicals released from undamaged cotton leaves, a systemic response of living plants to caterpillar damage. *Plant Physiology* 111, 487-495.

Roseland, C.R., Bates, M.B., Carlson, R.B. & Oseto, C.Y. 1992. Discrimination of sunflower volatiles by the red sunflower seed weevil. *Entomologia experimentalis et applicata* 62, 99-106.

Rubio, M.E.O. & Sanchez, Y.L.S. 1986. Datos sobre apareamiento y puesta de *Spodoptera littoralis* (Boisd.). *Graellsia* XLII, 189-203.

Ruther, J. & Thiemann, K. 1997. Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. *Entomologia experimentalis et applicata* 84, 183-188.

Salama, H.S., Rizk, A.F. & Sharaby, A. 1984. Chemical stimuli in flowers and leaves of cotton that affect behaviour in the Cotton Moth *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Entomologia Generalis* 10, 27-34.

Schoonhoven, L.M., Jermy, T. & van Loon, J.J.A, 1998. *Insect-Plant Biology*, Chapman and Hall, London.

Schultz, J.C. 1988. Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology* 69, 896-897.

Smart, L.E. & Blight, M.M. 2000. Response of the pollen beetle, *Meligethes aeneus*, to traps baited with volatiles from oilseed rape, *Brassica napus*. *Journal of chemical ecology* 26, 1051-1064.

Smid, H.M., van Loon, J.J.A., Posthumus, M.A. & Vet, L.E.M. 2002. GC-EAD-analysis of volatiles from Brussels sprouts plants damaged by two species of Pieris caterpillars:

Olfactory receptive range of a specialist and a generalist parasitoid wasp species. *Chemoecology* 12, 169-176.

Stengl, M., Ziegelberger, G., Boekhoff, I. & Krieger, J. 1999. Perireceptor events and transduction mechanisms in insect olfaction. In: *Insect Olfaction*. (Ed. B.S. Hansson). Springer-Verlag Berlin. pp. 49-66.

Todd, J.L. & Baker, T.C. 1999. Function of peripheral olfactory organs. In: *Insect Olfaction*. (Ed. B.S. Hansson). Springer-Verlag Berlin. pp. 67-96.

Tollsten, L. & Bergström, G. 1988. Headspace volatiles of whole plants and macerated plant parts of *Brassica* and *Sinapis*. *Phytochemistry* 27, 2073-2077.

Tsao, R. & Coats, J. R. 1995. Starting from nature to make better insecticides, *Chemtech* July, 23-28.

Turlings, T.C., Wäckers, F.L., Vet, L.E.M., Lewis, J.L. & Tumlinson, J.H. 1993. Learning of host-finding cues by hymenopterous parasitoids. In: *Insect Learning*. (Eds. D.R. Papaj and Lewis A.C.), Chapman and Hall, New York.

Turlings, T.C.J., Bernasconi, M., Bertossa, R., Bigler, F.,Caloz, G. & Dorn, S. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biological control* 11, 122–129.

Turlings, T.C.J. & Tumlinson, J.H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proceedings of the National Academy of Sciences USA* 89, 8399-8402.

Turlings, T.C.J. & Wäckers, F. 2004. Recruitment of predators and parasitoids by herbivore-injured plants. In: *Advances in insect chemical ecology*. (Eds. R.T. Cardé & J.G. Millar). University Press Cambridge, pp. 21-75.

Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Röse, U.S.R, Lewis, W.J. & Tumlinson, J.H. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States* 92, 4169-4174.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. 1990. Exploitation of herbivore induced plant odors by host-seeking parasitic wasps, *Science* 250, 1251-1253.

Turlings, T.C.J., Tumlinson, J.H., Heath, R.R., Proveaux, A. T. & Doolittle, R. E. 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *Journal of chemical ecology* 17, 2235-2251.

van Alphen, J.J.M. & Jervis, M.A. 1996. Foraging behaviour. In: *Insect Natural Enemies, Practical Approaches to Their Study and Evaluation*. (Eds. M. Jervis & N. Kidd), Chapman & Hall, London, pp. 1-62.

van Loon J.J.A., de Boer, J.G. and Dicke, M. 2000. Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia experimentalis et applicata* 97, 219-227.

Verdonk, J.C., Ric de Vos, C.H., Verhoeven, H.A., Haring, M.A., van Tunen, A.J. & Schuurink, R.C. 2003. Regulation of floral scent production in petunia revealed by target metabolomics. *Phytochemistry* 62, 997-1008.

Vet, L.E.M. & Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual review of entomology* 37, 141-172.

Vet, L.E.M., Lewis, W.J. & Cardé, R.T. 1995. Parasitoids foraging and learning. In: *Chemical of ecology insects 2*. (Eds. R.T. Cardé & W.J. Bell). Chapman and Hall, New York, pp. 65-104.

Vinson, S.B. 1998. The general host selection behaviour of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological control* 11, 79-96.

Westcott, L. & Nelson, D. 2001. Canola pollination: an update. *Bee world* 82, 115-129.

Whitman, W.W. & Eller, F.J. 1990. Parasitic wasps orient to green leaf volatiles. *Chemoecology* 1, 69-75.

Wibe, A. 2004. How the choice of method influence on the results in electrophysiological studies of insect olfaction. *Journal of insect physiology* 50, 497–503.

Williams, I.H. 1978. The pollination requirements of swede rape (*Brassica napus* L.) and of turnip rape (*Brassica campestris* L.). *Journal of agricultural sciences, Cambridge*. 91, 343-348.

Winfield, A.L. 1963. A study on the effects of insecticides on parasites of larvae of blossom beetles (*Meligethes aeneus* F., Coleoptera: Nitidulidae). *Entomologia experimentalis et applicata* 6, 309-318.

Wäckers FL (2004) Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. *Biological Control* 29: 307-314

Zangerl, A.R. 2003. Evolution of induced plant responses to herbivores. *Basic and applied ecology* 4, 91-103.

Acknowledgements

Först och främst ett stort tack till min handledare Peter Anderson, du har varit ett stort stöd och en god samarbetspartner från början till slutet, alltid redo för en diskussion.

Tack till mina biträdande handledare – Christer Nilsson, din erfarenhet och positiva inställning har varit mycket värdefull, Mats Gustafsson och Bill Hansson tack för ert stöd.

Mina tankar går till Jan Löfqvist som inspirerade mig att börja i Alnarp och var ett viktigt stöd under de första åren.

Nanna, Anna, Kristina, Helena och Ulf – tack för gott samarbete och för glatt sällskap både regniga och soliga dagar i fält och på lab.

Tack till Mattias Larsson och Göran Birgersson för att ni tog av er jultid för att läsa mina manuskript.

Tack till Britt Åhman och Bertil Christensson för hjälp med steklar och med raps.

Tack till personalen på biblioteket och på repro i Alnarp och på Lönnstorp.

Tack till Elisabeth och Rita för alla stora och små problem ni hjälpt mig med under åren.

Many people in the chemical ecology group have contributed to my work by being helpful with all kinds of things, but most importantly contributed to the nice and friendly atmosphere – Marcus (=hög standard på kontoret), Lena, Per M, Micke, Marcus, Ylva (tack för skjutsen), Anna-Karin, Marco (see you in Kirseberg), Fredrik, Peter W, Marie, Jocelijn, Teun, Miryan, Takuma, Filipe, Malin, Rickard, Medhat, Per N, Siju, Niels, Nelia, Marie-Louise, Majid, Maryam, Jörgen, Yitbarek, and all those mentioned elsewhere and those not mentioned - I wish you all the best.

Tack till många andra på Växtvetenskap och i Feromongruppen, Lund.

Thanks to Ted Turlings and co-workers in Neuchatel for your hospitality. Many thanks also to Sam Cook and Darren Murray, Rothamsted for useful discussions about pollen beetles and statistics.

Vissa har varit extra viktiga för att göra livet där hemma lättare och ofta mycket trevligt – Pappa och Mamma, Lasse och Karin, Ola och Lisa och Åsa – tack för att ni alltid ställer upp.

Sist men allra mest tack till Lotta, Oda, Tage och Tore ni är det bästa, förhoppningsvis kommer jag vara mindre rapsbaggetokig i fortsättningen.