Glypta haesitator host location

Glypta haesitator vertslokalisering

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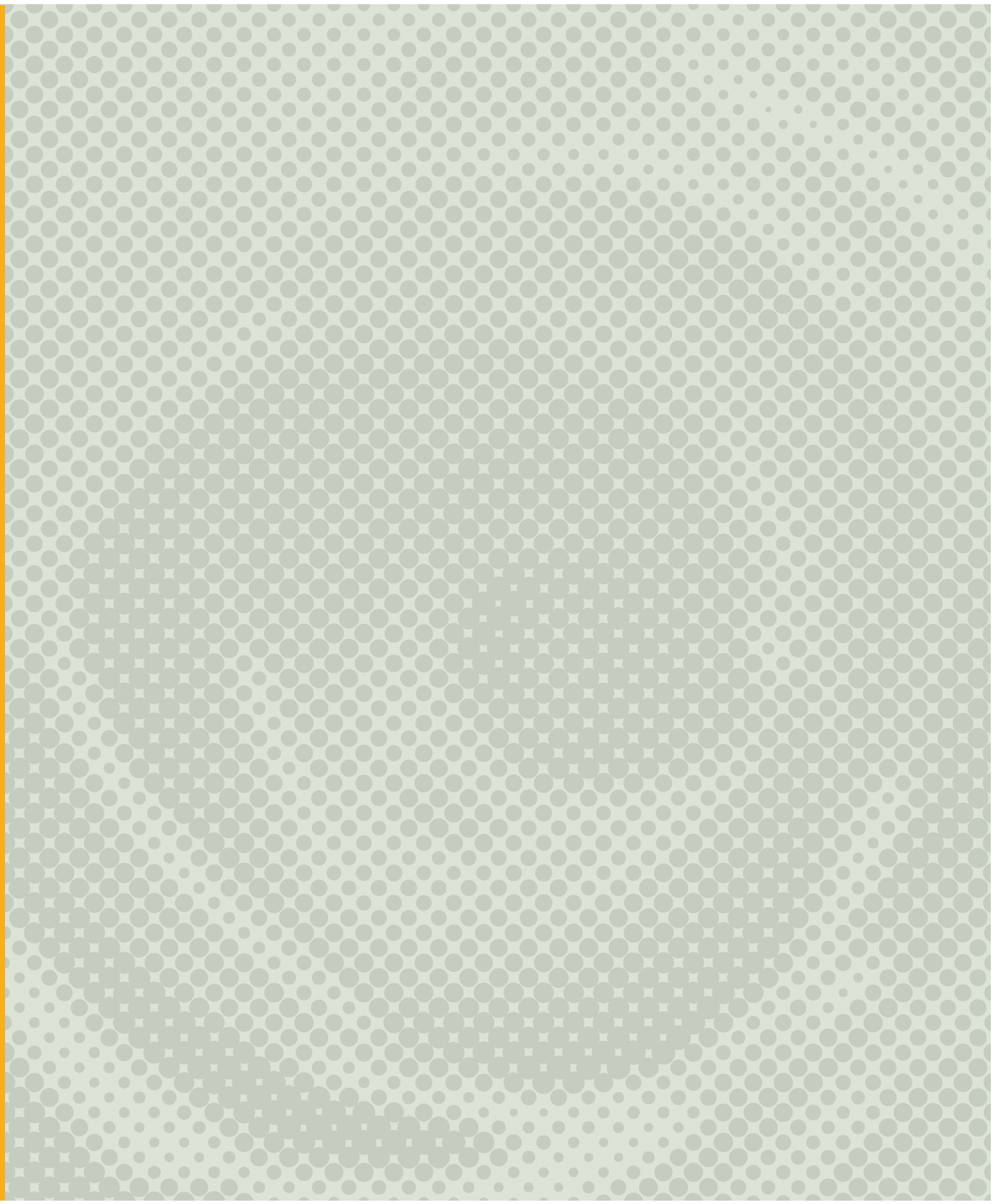

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Sammendrag

Snyltevepsen *Glypta haesitator* parasiterer erteviklerlarver (*Cydia nigricana).* Formålet med dette studiet var å identifisere luktkilder som tiltrekker seg naive *G. haesitator* hunner på søken etter et område å legge egg. Adferdsanalyser i vindtunnel viste at naive *G. haesitator* hunner er tiltrukket av erteviklerens matplante, voksne ertevikleres skall og erteviklerlarvens avføring. Infiserte erteplanter uten belger tiltrakk seg ikke naive *G. haesitator* hunner, hvilket indikerer at luktstoffer fra bakgrunnen er viktig for *G. haesitator* vertslokalisering.

Flyktige stoffer avgitt av erteplanter, skall fra voksne erteviklere og erteviklerlarvens avføring ble prøvetatt med SPME-fiber eller headspace oppsamling. Prøveanalyser med gasskromatograf koblet til massespektrometer indikerte at de flyktige stoffene avgitt fra erteplantene, larve-avføringen og erteviklerskallene er vanlig å avgi for planter i mange ulike slekter. Det betyr at *G. haesitator* sannsynligvis bruker ratiospesifikk luktgjenkjennelse. Mer forskning er nødvendig for å fastslå om erteviklere og *G. haesitator* er tiltrukket av like stoffer i lik ratio.

I dette studiet parasiterte *G. haesitator* 22% av innsamlede, overvintrede erteviklerkokonger fra en erteåker. Totalt var 70% av kokongene parasitert av tilsammen tre ulike arter. Fordi erte-vikleren og *G. haesitator* begge tiltrekkes av erteplanter, kan bruk av semiokjemikalier fra erteplanten til bruk i erteviklerbekjempelse redusere *G. haesitators* parasitering. En slik uønsket effekt vil trolig hindre bruk av semiokjemikalier til bekjempelse av skadedyr. Effekten flyktige, organiske stoffer har på parasitering bør derfor vurderes før stoffene implementeres i skadedyr-bekjempelse.

Abstract

Glypta haesitator is a larval parasitoid of the pea moth (*Cydia nigricana)*. The objective of this study was to identify semiochemical sources exploited by naive, female *G. haesitator* looking for an oviposition site. In a wind tunnel bioassay *G. haesitator* showed attraction to pea moth larval frass, pea moth scales and pea moth host plants. Infected pea plants without pods did not elicit *G. haesitator* attraction, indicating that the background odour is important for *G. haesitator* host location.

Volatile compounds emitted from pea moth larval frass, pea moth scales and pea plants were sampled by SPME-fibre or headspace collection. Gas-chromatograph coupled with mass spectrometer analyses of the volatiles indicated presence of compounds commonly emitted by plants in many genera. The results indicate that *G. haesitator* use ratio-specific odour recognition. Further research is needed to investigate if pea moths and *G. haesitator* are attracted to similar compounds in similar ratio.

G. haesitator parasitizised 22% of the collected overwintered pea moth cocoons from a pea field. The combined parasitization rate by three parasitoids was 70%. Because both the pea moth and *G. haesitator* are attracted to pea plants, the use of semiochemicals from the pea plant in pea moth pest management could reduce *G. haesitator* parasitization rate. Such adverse effects are likely to hamper the utilization of semiochemicals in pest management. The impact of a volatile organic compound on parasitization should therefore be considered prior to its implementation in pest management.

Terminology

Anemotaxis	Orientation with respect to wind.
Biological control	The action of living natural enemies in suppressing the abundance or activity of pests.
Conservation biological control	Enhancement of natural enemy populations through provisioning of limiting resources or alteration of crop production practices.
IPM	Integrated pest management. Utilization of all suitable techniques in as compatible manners possible. Maintains the pest population below a threshold of economic injury.
Kairomone	A semiochemical. Its information benefits a receiving individual of another species, not the emitter.
Koinobiont parasitoid	A parasite whose host is allowed to grow and metamorphose beyond the stage attacked.
Optomotor	The use of visual images by flying or swimming insects to maintain orientation to a current flow.
Parasitoid	Insects which in their larval stage feeds exclusively on the body of another arthropod, eventually killing it.
Scotophase	The dark phase in a cycle of light and dark.
Semiochemical	Chemical signal that mediates interactions between organisms.
SPME	Solid phase micro extraction. A sample preparation technique.
Synomone	A semiochemical. Its information benefits a receiving individual of another species and the emitter.
VOC	Volatile organic compound.

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Introduction

Background

Research on insect herbivore host and mate location by plant derived volatile organic compounds (VOC) and pheromones has provided new tactics of managing insect pests: Mass trapping (Light et al., 2001; Woodford et al. 2003), push-pull (Khan et al., 1997), mating disruption (Witzgall et al., 2008) and application of insecticides based on monitoring (Wall et al., 1987; Light et al., 2001; Woodford et al., 2003; Witzgall et al., 2008). As a result, semiochemicals are today implemented in applied plant protection worldwide (Witzgall et al., 2010). Biological control agents like predators and parasitoids are commercially utilized in modern greenhouse industry (van Lenteren et al., 1997; Brandsæther and Birkenes, 2006). In field-crops attention to conservation biological control has begun to increase from a previously relatively low level (Landis et al., 2000). Together, the tactics based on semiochemicals and biological control agents address the challenge to reduce pesticide usage and implement integrated pest management (IPM). Implementing plant VOC in pest management may however have unknown effects on biological control agents. Natural enemies of arthropod herbivores and plants are mutualistic (Price et al., 1980; Dicke and Sabelis, 1988; Vinson, 1999; Schoonhoven et al., 2005a; Vet and Godfray, 2008). Many of them are attracted to VOC from the prey food plant (Vinson, 1976, 1981; Whitman and Eller, 1990; Vet and Dicke, 1992; Godfray, 1994a; Barbosa and Benrey, 1998). Thus, if a plant VOC utilized for mass trapping attracted both the insect pest and its parasites, it could facilitate a net increase in the pest population. Such adverse effects are likely to hamper the utilization of semiochemicals in pest management. The impact of a VOC on parasitization should therefore be considered prior to its implementation in pest management.

Glypta haesitator biology

Information on the biology of *Glypta haesitator* Gravenhorst (Hymenoptera: Ichneumonidae: Banchinae) is based on the papers by Cameron (1938) and Wright and Geering (1948), and the writers own observations. *G. haesitator* is a larval, koinobiont, solitary endoparasitoid of the pea moth *Cydia nigricana* Fabricius (Lepidoptera: Tortricidae). Other hosts recorded include amongst others *Spilonota ocellana* Fabricius (Lepidoptera: Tortricidae) and a tortrix larva on *Myrica gale* Linne' (Myricaceae). The parasitoid is established in Canada, the U.K., Scandinavia, most of continental Europe and northern Africa (McLeod, 1962; Zwakhals, 2009). In the U.K. adults emerge from the start of July to the start of august. Males emerge mainly in the first part of July. The sex-ratio at birth is two females to one male. Mating occurs soon after emergence, lasting for approximately 45 seconds. Gravid females encountering infested pea pods start

tapping the pea pod surface rapidly with the antenna. Next, the ovipositor is withdrawn from its sheath and bent forward underneath the abdomen, inserted into the pod, and a single egg laid in the pea moth larva inside. The entrance hole of the host is utilized for oviposition. After three to four days the egg hatches. The *G. haesitator* larva moults three times: The first during autumn, the second during February and the last during May. A cocoon of white, translucent silk is made when the host has been devoured. The pre pupal stage lasts for a few days. The parasitoid then voids its faeces and enters the pupal stage, which lasts for a week at 25°C and 60% RH. Adult *G. haesitator* have under laboratory conditions been kept alive no more than a month. Adults show positive phototropism in the laboratory.

Pea moth biology

The pea moth is a major, native pest of *Pisum sativum* Linne' (Fabaceae) in Europe (Capinera, 2001; Aarvik et al., 2011). The pest is also established throughout northern United States, southern and coastal Canada, central to western Asia and Japan (Cameron, 1938; Capinera, 2001). Less suitable hosts are found within the family of *Fabaceae*, in the genera *Vicia, Lathyrus, Lupinus, Phaseolus* and *Cytisus* (Hanson and Webster, 1936; Wright and Geering, 1948; Sawar, 1973). There is generally a single generation per year (Capinera, 2001; Brandsæther et al., 2009). Pupation occurs in the upper soil layer during spring (Capinera, 2001; Brandsæther et al., 2009). After a threshold day length is achieved, a temperature accumulation of 515.0 - 728.8ºC is needed before the adults eclose (Thöming and Saucke, 2011). Most of the adult pea moths mate at the emergence site (Macaulay et al. 1985). Adults have a peak activity between 4 and 6 p.m., when temperatures are above 18°C (Wright and Geering, 1948). Pea moths fly from the middle of May until the beginning of August. Oviposition happens in June and July 5-11 days after mating (Wright and Geering, 1948). Females lay on average 91 eggs during a lifetime of 16-21 days (Lewis and Sturgeon, 1987). First instar larva (L1) hatch after approximately seven days (at 21°C), and within 24 hours move to the emerging pea pods to penetrate them (Wright and Geering, 1948; Thöming and Saucke, 2011). The larvae develop (L1–L5) inside the pea pod, where they feed on the seeds. The L5 instar migrate into the soil and spin a thick silken cocoon coated with soil particles (Wright and Geering, 1948). Inside the cocoon the larva gets a deeper, yellow colour and becomes shortened and more distended (Cameron, 1938). The larva then overwinters and waits for satisfactory environmental conditions to begin the cycle anew.

Pea moth pest management

Ways of managing pea moths have included classical biological control, early pea varieties, partly resistant varieties, soil tillage, early harvesting, crop rotation and insecticides (Cameron, 1938; Wright and Geering, 1948; McLeod, 1962; Capinera, 2001; Huusela-Veistola and Jauhiainen, 2006; Brandsæther et al., 2009). Unfortunately, these methods are for several reasons often not applicable or sufficient. In the absence of natural enemies, pea moth infestation has accounted for a crop loss of up to 75% (Cameron, 1938). In a classical biological control program described by McLeod (1962), *Ascogaster quadridentata* Wesm. (Hymenoptera: Braconidae) and *G. haesitator* were obtained in England and released in Canada. Both *A. quadridentata* and *G. haesitator* became established, and *A. quadridentata* became an important factor in the control of pea moth in British Columbia.

A successful crop rotation should include an area of at least 1.5 km distance from the field, often more, due to the pea moth's mobility (Huusela-Veistola and Jauhiainen, 2006). To achieve sufficient crop rotation effects a group of producers would normally need to cooperate. If spraying insecticides, the first 24 hours after hatching is the window of opportunity for the insecticides to kill the larva (Macaulay et al., 1985). The effect of a too early spray will wear off before the larva hatches, and the larva is protected after entering the pod. The identification of a male attracting sex pheromone (Greenway, 1984) has led to the reliable timing of insecticide applications based on a monitoring system (Wall et al., 1987). However, a satisfactory coating of insecticides may prove difficult when pea vines become matted. Substantial mechanical damage to the crop and soil is inevitable when spraying. Most importantly, pesticide application is the least preferred pest management method according to the European Union IPM principles.

Research on mating disruption by pheromones and pest management strategies using kairomones could provide powerful tools for future management of the pea moth (Witzgall et al., 1996; G. Thöming, personal communication[1]). Currently, the efficacy of mating disruption is limited by immigrated, mated females (Balasus et al., 2008). Since parasitization can account for as much as 60% of the pea moth mortality (Cameron, 1938), mating disruption and mass trapping methods should be assessed with regards to their impact on the third trophic level. Also, the obtained knowledge of parasitoid attractants could be used to monitor parasitoid populations, or prime parasitoids and spray crops to increase parasitization rates through increased patch residence times (Gross, 1981; Mills and Wajnberg, 2008).

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Role of olfaction in insect behaviour

Insect behaviour can be stimulated by olfactory (volatile chemicals), gustatory (non-volatile chemicals), visual (shape, size, colour, texture), mechano-sensory (movement, structure and sound) and heat cues (Vinson, 1976; Weseloh, 1981; Prokopy and Owens, 1983; Bernays and Chapman, 1994a; Godfray, 1994a; Gullan and Cranston, 2010). The cues reveal refuges, hosts, competitors, predators, diseases and environmental conditions (Vinson, 1998). Intra specific intrinsic variation in parasitoid responses to stimuli is common, caused by genetic diversity, phenotypic plasticity and the physiological state of the parasitoid (Lewis et al., 1990; Bernays and Chapman, 1994b; Lewis et al., 1998; Vinson, 1998). However, Vet et al. (1990) observed that for a foraging parasitoid the strong responses were less variable than the weak ones. And, for naive parasitoid females, host derived stimuli served as key stimuli in associative learning of other stimuli.

A general framework for the host selection chain has been accepted: Host habitat location, host location and host acceptance (Doutt, 1959; Vinson, 1976, 1981, 1998; van Alphen and Vet, 1986). Visual and olfactory stimuli are generally most important during habitat and host location, while mechano-sensory and gustatory stimuli are also considered important during the final assessment of the host (Prokopy and Owens, 1983; Bernays and Chapman, 1994a; Schoonhoven et al. 2005b). Olfaction seems to be the most common sensory mode (van Alphen and Vet, 1986), and olfactory stimuli the easiest to learn (Schoonhoven et al. 2005c). The insect's olfactory apparatus is extremely sensitive, capable of operating on a remarkably low signal/noise ratio (Leal, 2005). The recognition of a specific plant is achieved by sensing a particular ratio between a few key volatiles (Bruce et al., 2005). Placement of different olfactory receptor neurons closely together enables the insect to distinguish between odour sources located as close as 1mm in space and 0.001 s in time (Baker et al. 1998; Bruce et al. 2005). When exposed to an attractive olfactory cue, flying insects usually start positive optomotor anemotaxis and zigzag counterturns up the odour plume (Murlis et al., 1992; Bernays and Chapman, 1994a; Bell et al., 1995). However, flying insects may also walk upwind and/or orient while on the ground (Bernays and Chapman, 1994a). Olfactory cues exploited by parasitoids can emanate from host faeces (Leong and Oatman, 1968; Turlings et al., 1991), host food (Camors and Payne, 1972; Mattiacci et al., 2000), lepidopteran scales (DeLury et al. 1999), host sex pheromones (Milonas, 2009), symbiotic host micro organisms (Madden, 1968), gland secretions (Mudd and Corbet, 1973), interactions between gland secretions and the plant (Turlings et al., 1990; Meiners and Hilker, 2000) and combinations of different semiochemical sources (Kaiser et al., 1989).

Objectives

The objective of this study was to identify semiochemical sources exploited by female *G. haesitator* looking for an oviposition site. The results were used to assess how semiochemical cues attractive to the pea moth could impact *G. haesitator* parasitization.

Materials and methods

Plants

P. sativum cv. Avola was sown in substrate (Plantejord, Plantasjen Norge AS, Kolbotn, Norway»), one batch a week, from January 23. until March 26. Watering was done 1-2 times per week. All plants were grown in environmental chamber 1 (70% RH, 15°C ± 2˚C, 14:10 h L:D and 100-150 µmol/m^2/s white fluorescent light) until the first flower buds were visible. The plants were then transferred to environmental chamber 2 (65% RH, 18°C ± 2˚C, 18:6 h L:D and 25-50 µmol/m^2/s white fluorescent light).

A cylindrical chamber (diameter 48 cm, height 65cm) inside environmental chamber 2 was used to infect pea plants. Flowering pea plants stayed inside the infection chamber for two weeks together with four to ten pairs of 3-7 days old pea moths. Infected pea plants had a height of 35-55cm and minimum 2 pods. All pea pods were dissected after the wind tunnel bioassays to confirm or disprove infection. Infected pods were stored in a ventilated plastic box inside environmental chamber 2 to rear larvae.

Insects

Infected pea pods were collected during July 2011 from pea fields (*P. sativum* cv. Santana) in Neu-Eichenberg, North of Hesse, Germany (51˚38'N, 9˚91'E). The fields had been managed organically for more than 10 years. Wooden boxes (72x45x12cm) filled with dry sand (8cm depth) were used to obtain pea moth material from the harvested pods. The upper side of the boxes was covered with chicken mesh (1.2cm mesh size). Infected pods were placed on the top of the chicken mesh. When the L5 instar left the pods, they dropped to the sand and spun a cocoon to overwinter. The boxes were stored until the end of January in outdoor conditions, protected against rainfall. The sand was sieved 30.1.2012 to extract the cocoons.

The cocoons were placed in plastic boxes with a 2-cm thick layer of slightly moist sand above and below the cocoons. The removable lid and the lower side of the box had ventilation openings covered with gauze. The boxes were then transferred to the University of Life Sciences, Ås, Norway and cultivated at 4 ± 2˚C, 60-70% RH and 0:24h L:D. From 03.02.2012 3-4 boxes containing approximately 300-600 cocoons were used as a replicate, and transferred to

environmental chamber 2 once a week for 18 weeks. All animals emerging from the cocoons were recorded daily. The amount of day degrees from transfer to environmental chamber 2 until first emergence, 50% emergence and 100% emergence were calculated for the pea moth, *G. haesitator, A. quadridentata* and an unknown parasitoid in each replicate.

Moths and *G. haesitator* were caged separately, within 24 hours after emergence, in transparent plastic cages (30x30x34cm) inside environmental chamber 2. Males and females were caged together to mate. *G. haesitator* emerging 1-6 and 5-11 days before the tests were caged separately. Moths and *G. haesitator* were offered 5% honey water and tap water through soaked cotton wads and filter paper. The parasitoids had no positive or negative memory connected to oviposition or host location prior to wind tunnel tests. However, pea moths were present when *G. haesitator* emerged. The parasitoids had thus experienced moth scales prior to host location, which is also the situation in the field.

Wind tunnel hardware and protocol

A detailed description of the wind tunnel hardware is provided in Aak et al. (2010). Wind tunnel dimensions were 250 cm long, 88 cm wide and 55 cm height, with 50-60% RH, 21 ± 1˚C and a wind speed of 30 cm/s. The flight tunnel was transparent and illuminated with white light from below (diodes) and above (fluorescent). Light intensity from above increased gradually from 5 μmol/m^2/s at the initiation point, to 10 μmol/m^2/s at the landing point. Light intensity from below increased gradually from 2 μmol/m^2/s at the initiation point, to 8 μmol/m^2/s at the landing point. Sources of stimuli were positioned inside the 30 cm section upwind of the flight arena. The *G. haesitator* female started inside an upwind open glass tube (diameter 2.2cm, length 17 cm), on top of a 26 cm high metal rack 190 cm downwind of the source of stimuli. Glassware had been heated to 500ºC for a minimum of 2h before use.

Bioassays were conducted on Tuesdays from week 13 until week 21, between 6 hours prior to scotophase and start of scotophase. Naive *G. haesitator* females were acclimatized to wind tunnel conditions 2-3 hours prior to testing. In order to prevent the animals from dehydrating, the glass tubes were regularly moisturised on the inside. The bioassay lasted for 3 minutes per insect. Five distinct and successive behavioural steps could be recognized in host location in the wind tunnel. 1) Initiation: Movement to a location outside of the glass tube. 2) Upwind oriented flight: Take off from the glass tube while orienting upwind. 3) Halfway flight: Flying, in the odour plume, to a position halfway or nearer to the stimulus. 4) Full distance flight: Flying, in the odour plume, the full length of the flight section. 5) Landing on a predefined area: Landing directly on a plant, or a glass plate (15x10cm) on a rack 26 cm above the floor. If a wasp landed

outside the predefined area, it was relocated to the initiation area.

Experimental design

A control stimulus and 7 combinations of stimuli were tested, by random order, inside the wind tunnel. The control stimulus consisted of a 26 cm high metal rack, with a green sheet covered by a transparent glass plate (15x10cm) and a cotton wad placed on top. All stimuli, including the control, were tested on minimum 50 naive *G. haesitator* females of unknown mating status. All stimuli with pea plants also contained plastic pots with substrate and a spruce stick for mechanical support. When plant material was used as a part of a stimulus, a pair of pea plants in the pod development stage (57 and 64 days after sowing) were used together, in order to reduce genetic, developmental and infection variation. The 7 stimuli tested are listed as follows:

1) Healthy pods and larvae - To test a combination of host food and the host. A pod was cut from two healthy pea plants. The pods were split in two similar halves and six larvae (instar 1-5) were inserted into the pods. Pods were placed like the cotton wad in the control.
2) Healthy pea plants - To test the host food without hosts, host faeces nor scales.
3) Infected pea plants without pods - To test a host plant with scales, without host food, host faeces or hosts. Pods were cut off one minute before the plants were placed inside the wind tunnel.
4) Frass - To test the faeces of the host. A mixture of new and old frass collected from infected pods stored in a ventilated plastic box inside environmental chamber 2 was used. The frass was placed like the cotton wad in the control.
5) Infected pods without larvae - To test a combination of host food and frass. A pod was cut from two infected pea plants. The pods were split in two similar halves. Peas and larvae were removed with tweezers. Pods were placed like the cotton wad in the control test.
6) Moth scales - To test a combination of wing and body scales from male and female pea moths. Moths from the infection chamber (3-8 pairs) were shaken inside a glass vial. A cotton wad was inserted into the vial to collect the moth wing scales. The cotton wad with wing scales was placed like the cotton wad in the control test.
7) Infected pea plants - To test the host plant complex.

Volatile sampling and analysis

Six infected and three healthy pea plants in the pod development stage (58 and 65 days after sowing) were selected for headspace collection. The pea plants were cut at the third nodium and

placed, with the stem in a glass vial of water, inside a 2000 ml glass chamber. All samples were aerated for 3 hours at room temperature. A water aspirator drew charcoal-filtered air at 220 ml/minute through the chamber and a connected glass column filled with Super Q (35 mg; 80/100 mesh; Alltech, Deerfield, IL, USA). Volatiles were eluted from the Super Q with 0,3 ml of hexane (>99% pure; Sigma-Aldrich), and mixed with 500 ng heptyl-acetate (99.4 % pure; Chiron AS) and 500 ng undecanal (97% pure; Sigma-Aldrich) as internal standard. After volatiles had been eluted, filters were immediately rinsed with first 6 ml hexane (>99% pure; Sigma-Aldrich), then 6 ml methanol (> 99 % pure; Sigma-Aldrich) and then 6 ml hexane again. Filters were stored and reused for the next headspace collections. The eluted volatiles were stored at -80°C before injection into a gas-chromatograph (GC) coupled with a mass spectrometer (MS).

Solid phase microextraction (SPME) (Pawliszyn, 1997) was used to sample volatile compounds from frass and moth wings. Samples were made from female and male moths, before and after infecting pea plants. The moths were chilled in a freezer for 5 minutes. Wings were then cut off with a scalpel. Three wings, eight wings or 0.01 gram of larval frass, were inserted per 2 ml vial and stored overnight for the volatile compounds to saturate. A red 100 µm polydimethylsiloxane fibre (Sigma-Aldrich) was inserted into the vial for 25 minutes at room temperature, to absorb volatile compounds. One fibre was used for all samples.

The SPME-fibre and samples diluted with hexane were injected into a Hewlett Packard 6890 Gas Chromatograph (GC) coupled with a Hewlett Packard 5973 mass selective detector (MS). The GC had an Agilent DB-wax capillary column (30,0mX250µmX0,25µm), and used helium as a carrier gas with a flow of 1,4ml/min. and a velocity of 43 cm/s. Retention time was locked to heptylacetate. Injection temperature for SPME samples was 250˚C. The temperature program started with 2 minutes at 40˚C, increasing by 6.9˚C/minute until 160˚C, increasing 21.5˚C/minute until 250˚C, then staying at 250˚C for 3.6 minutes. Molecules were ionized by electron ionization at 70eV. The quadrupole mass analyzer scanned from 35.00 amu – 350.00 amu.
The Automatic Mass Spectral Deconvolution and Identification System (AMDIS) (Stein, 1999; D'arcy & Mallard, 2004) was used to locate and extract significant spectra and retention times from the GC/MS data files, and then compare them against mass spectra and retention times from the National Institute of Standards and Technology (NIST) mass spectral database and a database prepared by Bioforsk Plantehelse. Compounds detected in one sample or more were

accepted as present. Relative amounts of target compounds were estimated by comparing average total peak areas of the same compounds in different SPME samples taken during similar sampling conditions.

Statistical analysis

Statistical calculations were performed by the software «R» version 2.14.1 (R Core Team, 2012). All significant differences were at a 5% level unless otherwise specified.

The *G. haesitator* response to stimuli in each behavioural step was analyzed with a binary logistic regression model (McCullagh and Nelder, 1989) with stimulus as explanatory variable. If behavioural response is observed Y=1. If else Y=0. S=1 if the *G. haesitator* is tested on stimuli i (i=1,2,3,4,5,6,7), if else S=0. Probability$(Y=1|S) = 1/(1+\exp(-\alpha-\beta_i S))$, α is the effect of the control stimulus, $\beta_i S$ is the effect of stimulus i relative to the control stimuli. In the calculations, minimum number of observations of behavioural response to the control stimulus is 1.

Stimuli had no effect on initiation, while age had a significant effect on initiation. Age had no significant effect on the other behaviours. Animals not initiating (14.2%) were therefore regarded as not interested in host location and thus excluded from the model.

Paired t-tests were used to calculate differences in daydegrees until emerging between *C. nigricana* and the respective parasitoids. Confidence intervals for parasitization rates and daydegrees until emergence were calculated by: $\bar{y} \pm t_{0.025,n-1} * SE(\bar{y})$ (Montgomery, 2009).

Results

Emergence from overwintered cocoons

Table 1 shows that *G. haesitator* is the second most common parasitoid of the pea moth in the sampled area. Of the overwintered cocoons 22.07% were parasitized by *G. haesitator*. *A. quadridentata* parasitized 41.99%, and an unknown parasitoid accounted for 6.58% of the emerged animals. Hence, the total amount of parasitization obtained was 70.64%.

According to Table 1, *G. haesitator* and *A. quadridentata* life cycles are synchronized to the life cycle of the pea moth, while the life cycle of the unknown parasitoid is not. The unknown parasitoid had a much earlier first emergence, 50% emergence and 100% emergence than all the other species. *G. haesitator* and *A. quadridentata* had first emergence, 50% emergence and 100% emergence at equal amounts of day degrees. The pea moth had first emergence and 50% emergence at 43-70 day degrees before *G. haesitator* and *A. quadridentata,* while at 100% emergence these three species required an approximately equal amount of day degrees.

Table 1. Percentage of species emerged from overwintered cocoons and day degrees until emergence, with 95% confidence intervals. The three bottom lines show day degrees until emergence relative to C. nigricana*, with 95% confidence intervals.*

	Percentage emergence from cocoons and 95% c.i. (n=18)	**Daydegrees and 95% c.i. (n=18)**		
		First emergence	**50% emergence**	**100% emergence**
C. nigricana	29.36 ± 2.01	512 ± 10.73	643 ± 19.85	907 ± 42.45
G.haesitator	22.07 ± 1.97	579 ± 25.36	706 ± 25.05	889 ± 35.69
A.quadridentata	41.99 ± 2.32	582 ± 17.64	686 ± 20.11	913 ± 24.33
Unknown parasitoid	6.58 ± 1.6	254 ± 11.02	339 ± 14.48	590 ± 96.78
Sum parasitization	70.64 ± 2.02			
G.haesitator – C.nigricana		67 ± 22.31	63 ± 16.32	-18 ± 41.31
A.quadridentata – C.nigricana		70 ± 12.99	43 ± 9.28	6 ± 33.91
Unknown parasitoid. - *C.nigricana*		-258 ± 14.07	-304 ± 15.62	-317 ± 100.06

Behavioural evidence for attraction to stimuli

In the wind tunnel bioassay three female *G. haesitator* landed on a leaf of an infected pea plant (Table 2). All three parasitoids intensively tapped the plant surface with their antennal tips after landing, and two parasitoids walked to the nearest pod. One *G. haesitator* female inserted her ovipositor into the larval entrance hole.

Observations of upwind oriented flight (Figure 1) in the wind tunnel bioassay showed that frass, pea moth scales, infected pea plants and infected pods without larvae were significantly attractive to female *G. haesitator* relative to the control treatment. Analyses of halfway flight observations (Figure 2) showed that infected pods without larvae, moth scales, frass and healthy

plants were significantly attractive relative to the control treatment. No significant attraction relative to the control treatment was found in the analyses of full distance flight (Figure 3) nor landings (Figure 4). Hence, attractive stimuli emanated from frass, moth scales and healthy plants.

Table 2. Numerical summary of G. haesitator *tested in the wind tunnel bioassay. Amount of animals tested, and observations of initiation, upwind oriented flight, halfway flight, full distance flight and landings when exposed to 8 different stimuli.*

Stimulus	Tested	Initiated	Upwind oriented flight	Halfway	Full distance	Landed
Control	50	42	10	2	0	0
Healthy pods and larvae	50	42	11	3	1	0
Healthy pea plants	50	44	13	9	5	0
Infected pea plants without pods	50	45	14	1	0	0
Frass	50	42	20	9	3	0
Infected pods without larvae	50	41	22	12	4	1
Moth scales	51	47	26	10	3	1
Infected pea plants	50	41	23	7	5	3
Sum	401	344	139	53	21	5

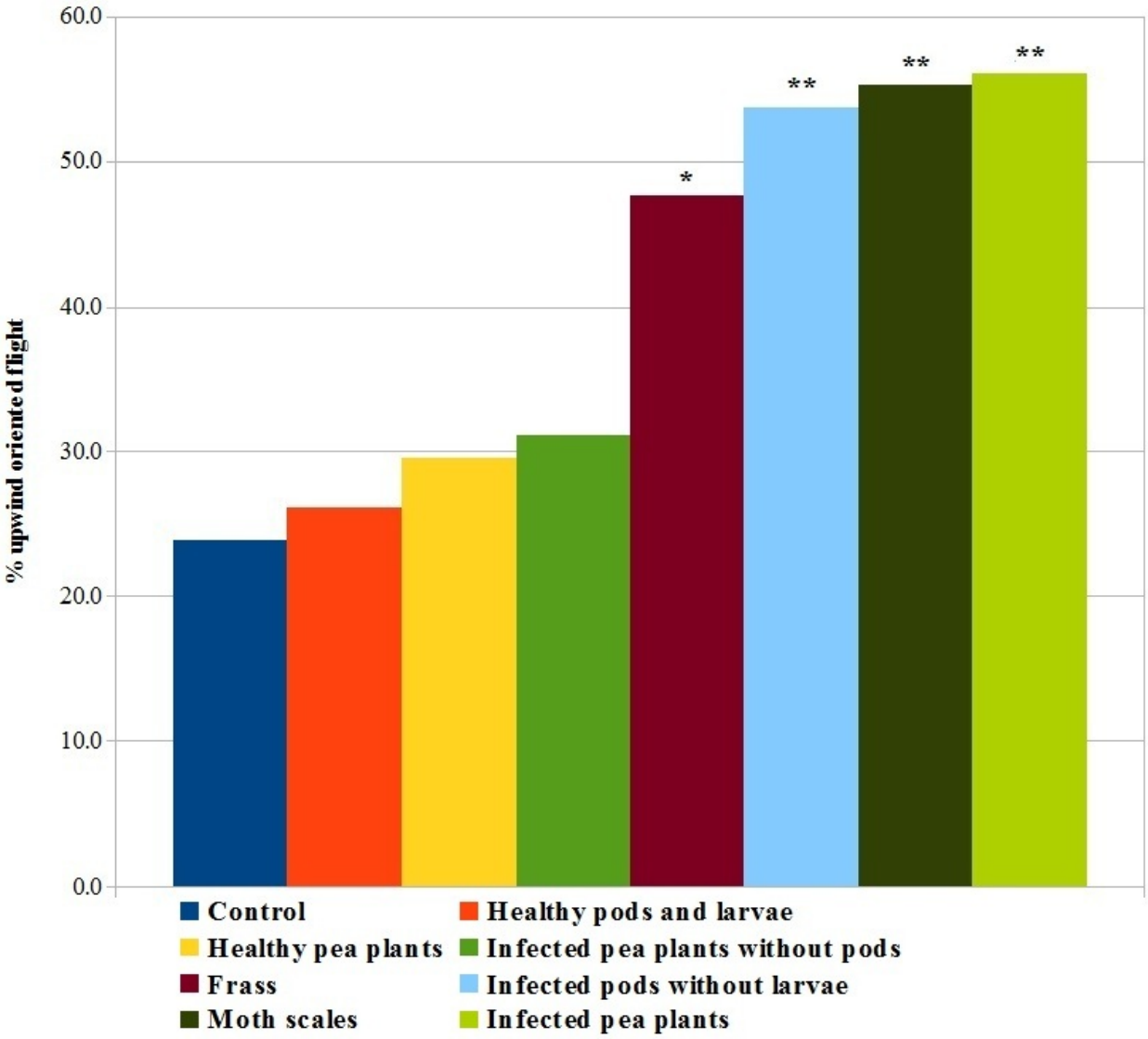

Figure 1. Percent initiated female G. haesitator *showing upwind oriented flight when exposed to 8 different stimuli. Asterisks indicate a significant preference, based on a binary logistic regression model, compared to a cotton wad (control).* $P < 0.05$; ** $P < 0.01$.*

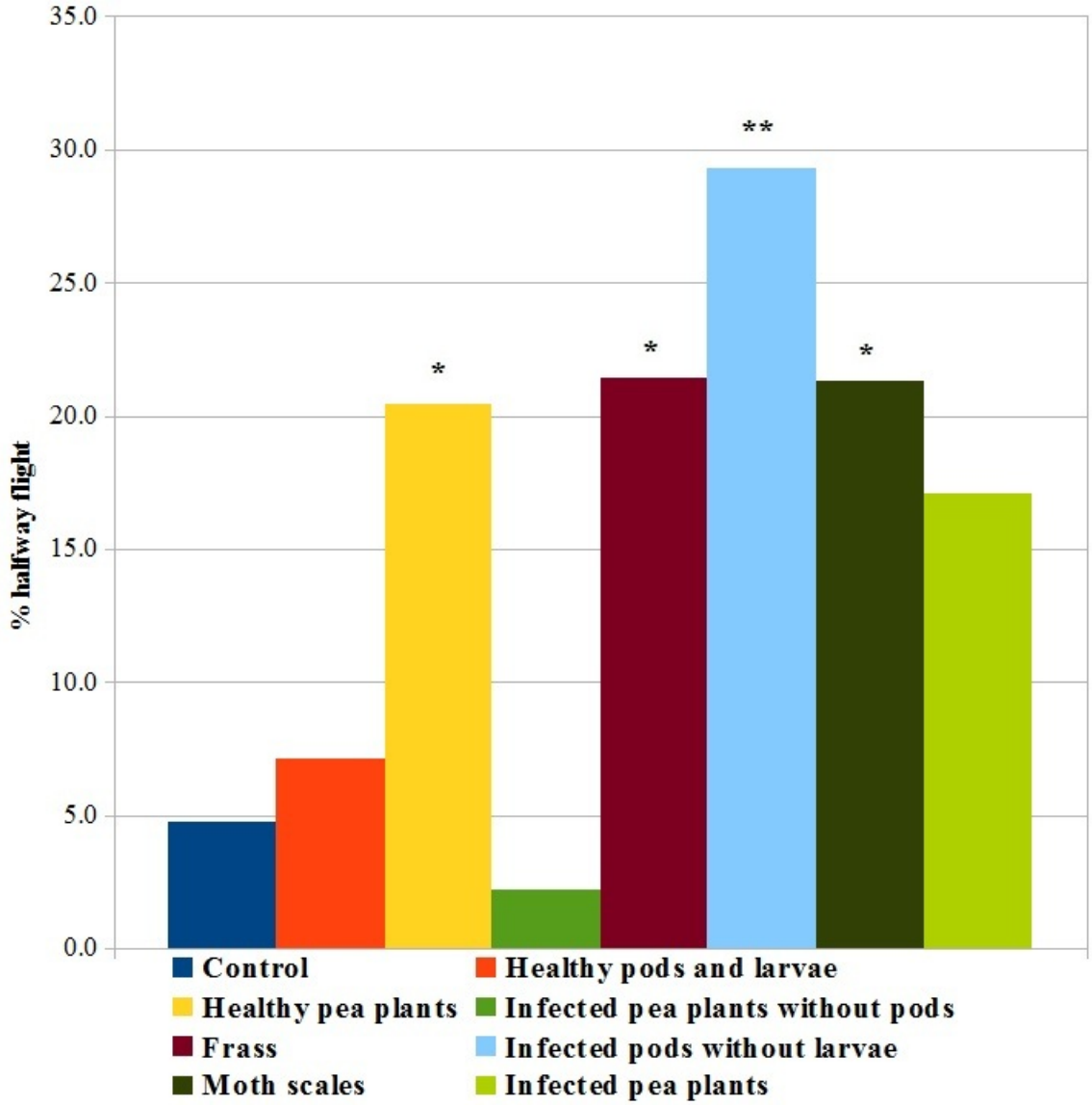

Figure 2. Percent female G. haesitator sustaining flight for half the distance to the stimuli. Data from initiated female G. haesitator *exposed to 8 different stimuli. Asterisks indicate a significant preference, based on a binary logistic regression model, compared to a cotton wad (control).* P < 0.05; ** P < 0.01.*

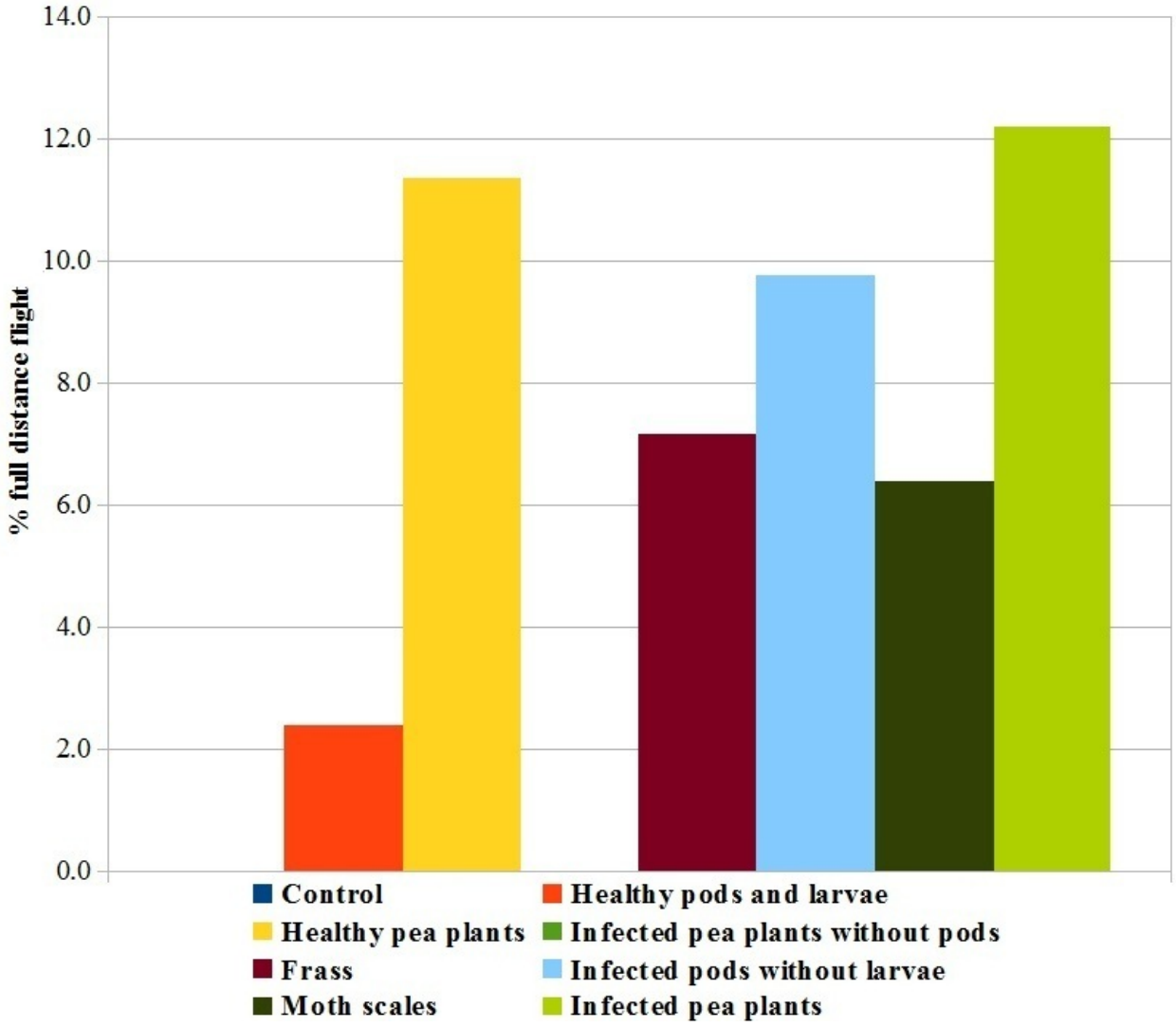

Figure 3. Percent female G. haesitator sustaining flight the full distance to the stimuli. Data from initiated female G. haesitator *exposed to 8 different stimuli. A binary logistic regression model shows no significant differences compared to a control of 1 observed response.*

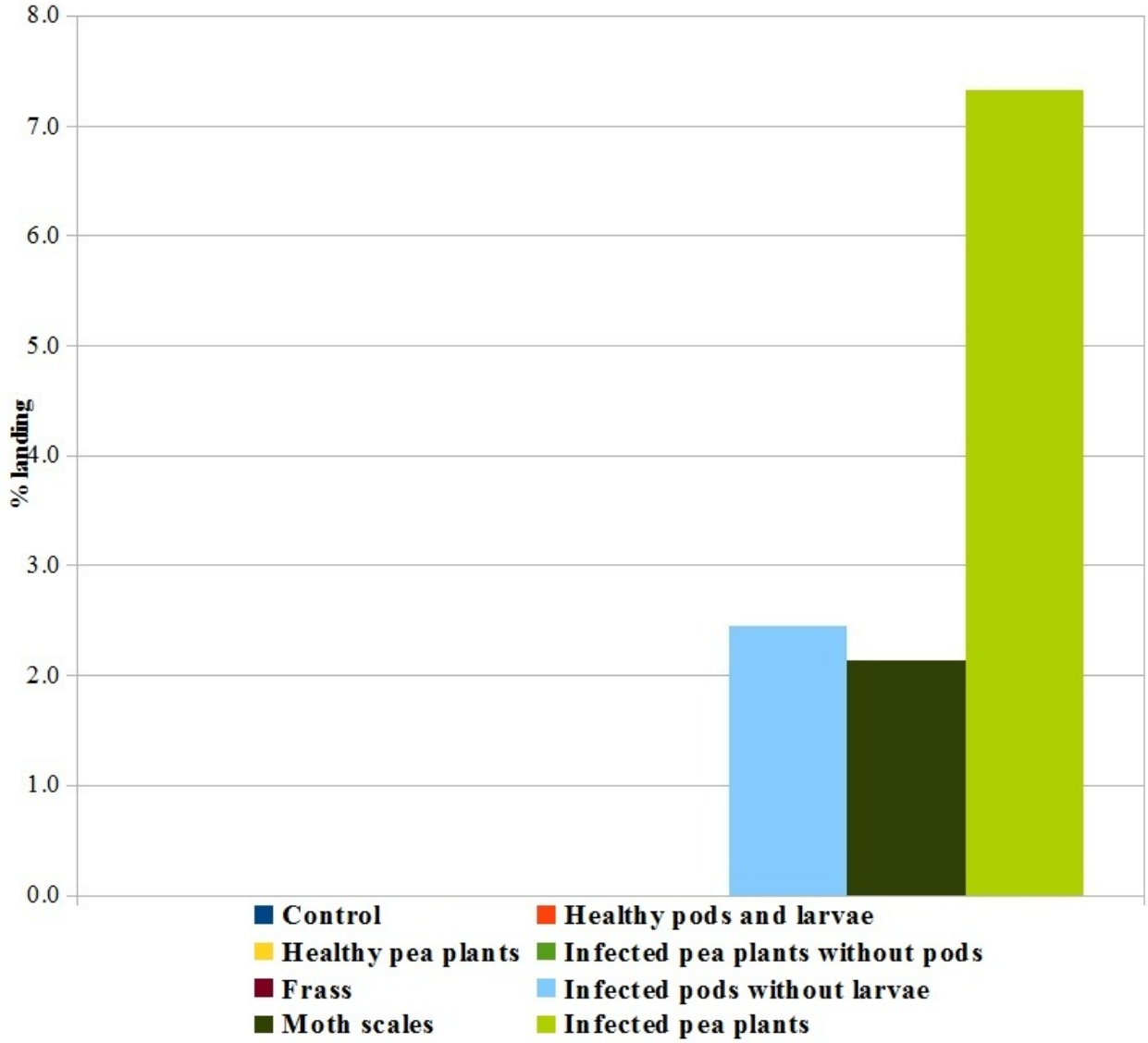

Figure 4. Percent female G. haesitator landing on a predefined area. Data from initiated female G. haesitator *exposed to 8 different stimuli. A binary logistic regression model shows no significant differences compared to a control of 1 observed response.*

Identification of volatile compounds

Volatiles identified by SPME and headspace collection are listed in Table 3. In the samples of male and female moth wing volatiles six ketones and two alcohols were identified, namely 2-hexanone, 2-heptanone, 2-octanone, 2-pentanone, 2-nonanone, 3-hydroxy-2-butanone, 1-butanol and 2-ethyl-1-hexanol. In samples of frass five ketones and two alcohols were identified, namely 2-hexanone, 2-pentanone, 2-octanone, 3-octanone, 2-heptanone, 1-octen-3-ol and 1-hexanol. In samples from infected and healthy pea plants the ester Z-3-hexenyl acetate, the alcohol Z-3-

hexenol, the bicyclic monoterpenes 3-carene and camphene, the hydrocarbon pinene and the aldehydes nonanal and decanal were identified. Also identified in the samples of infected pea plant volatiles were the alcohols 1-hexanol-2-ethyl and 1-hexanol, the hydrocarbon (E)-β-ocimene and the aldehyde octanal.

Table 3. Compounds tentatively identified by GC-MS, and sampled by SPME or headspace collection from pea moth male and female wings, pea moth larval frass, infected pea plants and healthy pea plants. Relative amounts were calculated for different SPME samples, taken during similar sampling conditions.

Compounds identified		Solid phase micro extraction					Headspace collection	
Name	Cas nr.	Female wings[1]	Male wings[1]	Female wings[2]	Male wings[2]	Frass	Infected pea plant	Pea plant
2-hexanone	591786	100%	81%	38%		86%		
2-heptanone	110430	100%	70%	50%	21%	29%		
2-octanone	111137	100%	85%	54%	23%	6%		
1-hexanol 2-ethyl	104767	100%	287%	281%	294%		X	
1-butanol	71363	100%	157%		X			
1-octen-3-ol	3391864					X		
1-hexanol	111273					X	X	
2-pentanone	107879	100%	59%	23%	19%	18%		
2-nonanone	821556	100%	83%	78%	26%			
2-butanone 3-hydroxy	513860	100%	2%	X	X			
3-octanone	106683					X		
Z-3-hexenyl acetate	3681718						X	X
Z-3-hexenol	928961						X	X
3-carene	13466789						X	X
Camphene	79925						X	X
Nonanal	124196						X	X
Decanal	112312						X	X
(E)-b-ocimene	3779611						X	
Octanal	124130						X	
Pinene	80566						X	X

1= Moths stayed minimum 24h inside the infection chamber. 2=One to seven days old moths.

Discussion

Parasitization rates

Parasitization of pea moths by parasitic wasps in an agricultural system removed 70% of the overwintered pea moth population (Table 1). Pea moth management methods reducing parasitization rates are thus likely to facilitate a net increase in the pest population. Cocoons from organically managed fields in Germany had a higher combined parasitization rate, and a higher parasitization rate for *G. haesitator*, than observed in England (Cameron, 1938; Wright and Geering, 1948). Applications of pesticides (Hardin et al., 1995; Ruberson et al., 1998), different pea varieties and different landscapes surrounding the pea fields (Altieri and Letourneau, 1982; Barbosa and Benrey, 1998; Mills and Wajnberg, 2008) probably account for the observed differences. Hence, pea production systems infested by pea moths may benefit substantially from conservation biological control.

Parasitoid specialization

Godfray (1994b) states that "the main selection pressure influencing female parasitoid emergence is the availability of hosts". According to Table 1 *G. haesitator* and *A. quadridentata* life cycles are synchronized to the life cycle of the pea moth. The observations are in accordance with those of Wright and Geering (1948) and DeLury et al.(1999), indicating that *G. haesitator* and *A. quadridentata* are specialists on parasitizing species of the Tortricidae, like the pea moth. However, timing of parasitoid diapause can be determined by the host (Godfray, 1994b). If *G. haesitator* or *A. quadridentata* diapause takes place in another host, *G. haesitator* and *A. quadridentata* may require a different amount of day degrees before emergence.

Behavioural responses to larval frass, moth scales and healthy pea plants

Additional indications on *G. haesitator* being a specialist on parasitizing pea moths, are evidence suggesting that pea moth scales, frass from the pea moth larvae and healthy pea plants are sources of kairomones/synomones for female *G. haesitator*. Wind tunnel bioassay is a well established technique for studying insect responsiveness to visual and olfactory stimuli (Schoonhoven et al., 2005d). The wind tunnel bioassay reported here therefore tested if pea moth scales, frass from the pea moth larva and healthy pea plants are sources of attractive stimuli for naive female *G. haesitator*. Volatiles from host food are known to orient the parasitoid to the habitat of the host (Vinson, 1976; Godfray, 1994a), and to increase the parasitoids behavioural response to host derived cues (Mattiacci et al., 2000). Also, when the herbivore life stage follows the plant life stage, a parasitoid may benefit from perceiving differences between plant

developmental stages. Moths deposit scales during oviposition (DeLury et al. 1999), and pea moth larvae deposit frass when entering the pea pod. Studies by Leong and Oatman (1968), Eller et al. (1988) and Turlings et al. (1991) suggest that larval frass emit volatiles attractive to parasitoids. A study by DeLury et al. (1999), using female *A. quadridentata* with an oviposition experience, detected scales of *Cydia pomonella* L. (Lepidoptera: Tortricidae) as a source of kairomones for the pea moth parasitoid *A. quadridentata*. Moth scales are a source of kairomones for other parasitoids as well (Lewis et al., 1972; Chiri and Legner, 1986).

A significantly higher amount of naive female *G. haesitator* showed upwind oriented flight (Figure 1) when exposed to cues from moth scales or frass compared to the control. Analyses of halfway flight observations showed that a significantly high percentage *G. haesitator* relative to the control was attracted to larval frass, moth scales and healthy pea plants (Figure 2). Lepidopteran scales and larval frass are both indirect, host derived cues, and thus have a relatively high degree of consistency and predictability when used in host location (Lewis et al., 1990; Vet et al., 1990; Vet and Dicke, 1992). Female *G. haesitator* responses to volatiles from healthy pea plants, frass and scales are probably adapted to maximize host location efficiency. The responses are likely to intensify searching behaviour in areas where host food is abundant, host eggs have been laid and hosts have entered pods.

Interactions with background odours

The wind tunnel is an artificial environment where VOC from the background have been removed by a charcoal filter. The importance of background odours for insect attraction to stimuli has been shown by Knudsen et al. (2008) and Webster et al. (2010). A combination of host frass and host food, and a combination of scales and host food plant without host food, were therefore tested to investigate how altering the background odours affected attraction to moth scales and larval frass.

Analyses of observed halfway flight show that infected pods without larvae were the only stimuli having a P-value lower than 0.01 relative to the control (Figure 2). Possibly, the green leaf volatiles from the pods enhanced the parasitoids responses to the frass inside the infected pods without larvae. Also, the infected pod without larvae probably released a higher amount of frass derived volatiles compared to the infected pea plants.

Healthy pods with larvae and infected pea plants without pods were not attractive (Figures 1, 2, 3 and 4). However, healthy pea plants were significantly attractive (Figure 2). The observations are in accordance with Webster et al. (2010), who found that the same compounds could be repellents individually, and attractants if mixed together.

The infected pea plants without pods were exposed to pea moths for two weeks, and thus likely contained residues of scales after ovipositions and/or landings. Nevertheless, infected pea plants without pods were no more attractive than the control (Figures 1, 2, 3 and 4). Possibly, the exclusion of pea pods from infected pea plants made the background odours a non-host cue for female *G. haesitator*. Knudsen et al. (2008) detected that interactions with the background odours contribute to the behavioural effect of plant volatile stimuli on *Argyresthia conjugella* (Lepidoptera: Argyresthiidae). In their study, anethole and 2-phenyl ethanol showed discrepant behavioural effects in wind tunnel bioassays compared to field traps. The discrepancy was due to interactions with background odours. Webster et al. (2010) found that "similar VOC can function as both host and non host cues, depending upon the context in which they are perceived". Hence, moth scale derived VOC could repel *G. haesitator* when emitted together with the background odour from a pea plant without pods. Absence of female *G. haesitator* response to moth scales with an off-blend host habitat background odour would likely prevent energy consuming searches in areas where hosts are no longer present. Responses to frass and pea pod interactions are likely to intensify searching behaviour in areas where larvae have entered pods.

Ratio-specific odour recognition

Twenty volatile compounds (Table 3) emitted by significantly attractive sources were identified. All compounds have previously been identified in volatile emissions from plants, and neither of them are taxonomically specific compounds (Knudsen et al., 1993; Jakobsen and Olsen, 1994; Baraldi et al.,1999; Rochat et al., 2000; Knudsen et al., 2006). In addition, *G. haesitator* have been observed on other hosts in the family of Tortricidae. The observations suggest that *G. haesitator* use ratio-specific instead of species-specific odour recognition, as described by Bruce et al. (2005). Utilization of ratio-specific odours provides an insect with the ability to evaluate a greater range of potential hosts, and enables adaptation to a new abundant host by altering the processing in the central nervous system (Bruce et al., 2005).

Possible limitations

A relatively high percent of the animals tested on the control stimulus showed upwind oriented flight (24%) and halfway flight (4.8%). The observer's definition of upwind oriented flight and halfway flight was too wide, and as a result many observations are probably take offs and flights outside the odour plume. The inclusion of take offs into upwind oriented flight could be the reason why healthy pea plants were significantly attractive in the analyses of halfway flight and not in the analyses of upwind oriented flight. However, the inclusion of take offs into upwind

oriented flights and inclusion of flights outside the odour plume into halfway flights, have been consistent in all treatments. The significant differences between the control and the treatments in observations of upwind oriented flights and halfway flights are therefore relevant.
No observations on full distance flights and landings, in spite of having no errors from take offs, showed significant attraction to healthy pea plants, larval frass or moth scales relative to the control. The highest response for halfway flight (29.3%), full distance flight (12.2%) and landing (7.3%) were also relatively low. The main reason is probably the insensitivity of the experimental method. An effective bioassay should result in high responsiveness and minimum variability. Barometric flux, photoperiod, light and temperature modulate insect responses to stimuli (Steinberg et al., 1992; Gullan and Cranston, 2010). Performing the bioassays on days with increasing barometric flux would probably enhance responsiveness (Steinberg et al., 1992). *G. haesitator* show positive phototropism in the laboratory. For some parasitoids, high light intensity is necessary for searching behaviour (Vinson, 1975), and a low light intensity can significantly reduce parasitization (Schirmer et al., 2008). Maximum photosynthetic photon flux density in the wind tunnel was 10 μmol/m^2/s, which is substantially lower than the rearing chamber (25-50 μmol/m^2/s) and daytime during summer (2000 μmol/m^2/s) (Bævre and Gislerød, 1999). Hence, increased light in the wind tunnel would probably have altered the results significantly. The studies by Aak and Knutsen (2011) and Bahlai et al. (2008) points out that adding visual stimulus to olfactory cues can significantly increase the landing rate on an attractive source. Thus, placing a contrast behind treatments with frass and moth scales could increase landing rates in wind tunnel experiments with *G. haesitator.*
Out of 401 tested animals, only 5 landed on a predefined area (Table 2). No animal landed on healthy pea plants nor larval frass, in spite of observed significant attraction relative to the control (Figures 1 and 2). Of the 5 landings, 3 landed on the infected pea plants, 1 landed on the infected pea pods without larvae and 1 landed on the moth scales. Obviously, no statistically relevant conclusion can be made based on the amount of landings. However, the observations might be biologically relevant, showing that a G. haesitator only lands if exposed to frass and/or scales in combination with a satisfactory background odour.

Future prospects

In order to identify the key compounds of *G. haesitator* host location, I suggest the following course of action: Detect retention times of authentic standards sampled under similar conditions as samples of VOC from attractive stimuli. Then, measure antennal responses to the authentic standards as well as the samples made by SPME and headspace collection, by an

electroantennographic detector coupled with a gas chromatograph (GC-EAD). Finally, observe female *G. haesitator* behavioural responses in a multi-arm olfactometer and/or a wind tunnel to blends of authentic standards eliciting antennal responses.

It would also be interesting to investigate if female *G. haesitator* respond the same to female and male pea moth scales, and detect how the age of pea moth scales and frass affect female *G. haesitator* attraction.

In order to improve *G. haesitator* responsiveness and lower variability in a wind tunnel bioassay, using lighting similar to daytime in July is recommended. Also, assays should within practical limits be done on days with increasing barometric flux. I hypothesize that female *G. haesitator* locate host habitats by exploiting VOC emitted by pea plants at the pod development stage. I further hypothesize that additional stimulus emanating from frass and/or moth scales needs to be present in order to initiate landing. Hence, the optimal sources of attraction are likely to be heavily infected pea plants with several opened pods and artificially applied moth wing scales.

Conclusion

This study detected that female *G. haesitator* are attracted to volatiles emanating from pea moth larval frass, pea moth scales, and pea plants, and that the recognition of these volatiles probably is ratio-specific. In addition, observations indicate that odours from pea pods enhance female *G. haesitator* attraction to frass, and that an off-blend background odour can act as a non-host cue.

Practical implications

Both female *G. haesitator* and female pea moths are attracted to pea plants at the pod development stage (Figure 2; G. Thöming, personal communication). Further research is needed to investigate if the same compounds in the same ratio are responsible for the attraction.

However, if pea plant derived kairomones attractive to both female *G. haesitator* and female pea moths are applied in fields, female *G. haesitator* could be aggregated in areas without hosts, refuge or food, leading to a reduced parasitization rate. Further research is needed to detect how long *G. haesitator* resides in a host habitat without finding hosts.

Pea plant derived synomones are probably, on their own, not sufficient to elicit *G. haesitator* landing behaviour. However, if the synomones are emitted from a trap together with volatiles from captured female pea moths, female *G. haesitator* might be prone to land on the trap, thereby reducing *G. haesitator* population and parasitization rates.

References

Aak, A. and Knudsen, G. K. 2011. Sex differences in olfaction-mediated visual acuity in blowflies and its consequences for gender-specific trapping. Entomologia Experimentalis et Applicata. 25-34.

Aak, A. Knudsen, G. K. and Soleng, A. 2010. Wind tunnel behavioural response and field trapping of the blowfly Calliphora vicina. Medical and Veterinary Entomology. 250-257.

Aarvik, L. Corley, M. Huemer, P. Jensen, J. Kullberg, J. Olafsson, E. Ronkay, L. Sammut, P.M. Tokar, Z. and de Prins, W. 2011. Fauna Europaea: Cydia nigricana. In: Karsholt, O. and van Nieukerken, E.J. (eds.) 2011. Fauna Europaea: Lepidoptera, Moths. Fauna Europaea version 2.4, http://www.faunaeur.org

van Alphen, J.J.M. and Vet, L.E.M. 1986. Chapter 2. An evolutionary approach to host finding and selection. In: J.K. Waage and D. Greathead (eds). Insect Parasitoids. Academic Press, London. 389 pp.

Altieri, M.A. and Letourneau, D.K. 1982. Vegetation management and biological control in agroecosystems. Crop Protection. 405-430.

Bahlai, C.A.Welsman, J.A. Macleod, E.C. Schaafsma, A.V. Hallett, R.H. and Sears, M.K. 2008. Role of visual and olfactory cues from agricultural hedgerows in the orientation behavior of multicolored Asian lady beetle (Coleoptera: Coccinellidae). Environmental Entomology. 973–979.

Baker T.C., Fadamiro H.Y. and Cosse A.A. 1998. Moth uses fine tuning for odour resolution. Nature. 530.

Balasus, A. Kratt, A. and Saucke, H. 2008. Field experiments on mating disruption of pea moth Cydia nigricana with sex-pheromone. Mitteilungen der deutschen Gesellschaft fur allgemeine und angewandte entomologie. p.253-256.

Baraldi, R., Rapparini, F. Rossi, F., Latella, A., and Ciccioli, P. 1999. Volatile organic compound emission from flowers of the most occurring and economically important species of fruit trees.

Physics and Chemistry of the Earth. 729-732.

Barbosa, P. and Benrey, B. 1998. Chapter 4. Plant influences on parasitoids. In: Conservation biological control. Academic press, San Diego. 396 pp.

Bell, W.J. Kipp, L.R. and Collins, R.D. 1995. Chapter 4. The role of chemo-orientation in search behaviour. In: Cardé, R.T and Bell, W.J. (eds.) Chemical ecology of insects 2. Chapman & Hall, New York. 433 pp.

Bernays, E.A. and Chapman, R.F. 1994a. Chapter 4. Behaviour: Process of host plant selection. In: Host plant selection by phytophagous insects. Chapman & Hall, New York. 312 pp.

Bernays, E.A. and Chapman, R.F. 1994b. Chapter 7. Genetic variation in host selection. In: Host plant selection by phytophagous insects. Chapman & Hall, New York. 312 pp.

Brandsæther, L.O. and Birkenes, S.M. 2006. Chapter 4. Direkte tiltak. In: Plantevern og plantehelse I økologisk landbruk. Bind 1. Bakgrunn, biologi og tiltak. Gan forlag, Oslo. 304 pp.

Brandsæther, L.O. Mangerud, K. Birkenes, S.M. Brodal, G. and Andersen, A. 2009. Chapter 3. Skadedyr I korn, oljevekster og kjernebelgvekster. In: Plantevern og plantehelse I økologisk landbruk. Bind 3. Korn, oljevekster og kjernebelgvekster. Gan forlag, Oslo. 198 pp.

Bævre, O.A. and Gislerød, H.R. 1999. Chapter: Lys. In: Plantedyrking i regulert klima. Landbruksforlaget, Valdres. 220 pp.

Cameron, E. 1938. A study of the natural control of the pea moth, Cydia nigricana, Steph. Bulletin of entomological research. 277-313.

Camors, F. B. and Payne, T.L. 1972. Response of Heydenia unica (Hymenoptera: Pteromalidae) to Dendroctonus frontalis (Coleoptera: Scolytidae) Pheromones and a Host-Tree Terpene. Annals of the Entomological Society of America. 31-33.

Capinera, J.L. 2001. Chapter: Family Tortricidae – Leafroller moths. In: Handbook of vegetable pests. Academic press, San Diego. 800 pp.

Chiri, A.A. and Legner, E.F. 1986. Response of three Chelonus (Hymenoptera: Braconidae) species to kairomones in scales of six Lepidoptera. The Canadian Entomologist. 329-333.

Cortesoro, A.M. De Moraes, C.M. Stapel, J.O. Tumlinson, J.H. and Lewis, W.J. 1997. Comparisons and contrasts in host-foraging strategies of two larval parasitoids with different degrees of host specificity. Journal of Chemical Ecology. 1589-1606.

Cortesero, A. M. and Monge, J. P. 1994. Influence of pre-emergence experience on response to host and host plant odours in the larval parasitoid Eupelmus vuilleti. Entomologia Experimentalis et Applicata. 281–288.

D'Arcy P. and Mallard W.G. 2004. Automated Mass Spectral Deconvolution & Identification System – User Guide. U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg. 62 pp.

DeLury, N.C. Gries, R. Gries, G. Judd, G.J.R. and Khaskin, G. 1999. Moth scale-derived kairomones used by egg-larval parasitoid Ascogaster quadridentata to locate eggs of its host Cydia pomonella. Journal of Chemical Ecology. 2419-2431.

Dicke M. and Sabelis M.W. 1988. How plants obtain predatory mites as bodyguards. Netherlands Journal of Zoology. 148-165.

Doutt, R.L. 1959. The biology of parasitic Hymenoptera. Annual Review of Entomology. 161-182.

Eller, F.J. Tumlinson, J.H. and Lewis, W.J. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals: Source of volatiles mediating the host-location flight behavior of Microplitis croceipes (Cresson) (Hymenoptera: Braconidae), a parasitoid of Heliothis zea (Boddie) (Lepidoptera: Noctuidae). Environmental Entomology. 745-753.

Godfray H.C.J. 1994a. Chapter 2. Host location. In: Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, Princeton, New Jersey. 473 pp.

Godfray H.C.J. 1994b. Chapter 7. The adult parasitoid. In: Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, Princeton, New Jersey. 473 pp.

Greenway, A.R. 1984. Sex pheromone of the pea moth, Cydia nigricana (F.) (Lepidoptera: Olethreutidae). Journal of Chemical Ecology. 973-982.

Gross, H.R.JR. 1981. Chapter 8. Employment of kairomones in the management of parasitoids. In: Semiochemicals. Their role in pest control. John Wiley & Sons, New York. 306 pp.

Gullan, P.J. and Cranston, P.S. 2010. Chapter 4. Sensory systems and behaviour. In: The insects. An outline of entomology. Wiley-Blackwell, Malaysia. 565 pp.

Hanson A.J. and Webster B.L. 1936. The pea moth, Laspeyresia nigricana, Steph. Washington Agricultural Experiment Station Bulletin. 327.

Hardin, M.R. Benrey, B. Coll, M. Lamp, W.O. Roderick, G.K. and Barbosa, P. 1995. Arthropod pest resurgence: an overview of potential mechanisms. Crop protection. 3-18.

Hare J.D. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annual Review of Entomology. 161–180.

Ruberson, J.R. Nemoto, H. and Hirose, Y. 1998. Chapter 11. Pesticides and conservation of natural enemies in pest management. In: Conservation biological control. Academic Press, San Diego. 396 pp.

Huusela-Veistola, E. and Jauhiainen, L. 2006. Expansion of pea cropping increases the risk of pea moth (Cydia nigricana; Lep., Tortricidae) infestation. Journal of Applied Entomology. 142–149.

Jakobsen, H.B. and Olsen, C.E. 1994. Influence of climatic factors on emission of flower volatiles in situ. Planta. 365-371.

Kaiser, L. Pham-Delegue, M.H. Bakchine, E. and Masson, C. 1989. Olfactory responses of Trichogramma maidis Pint, et Voeg.: Effects of chemical cues and behavioral plasticity. Journal

of Insect Behavior. 701-712.

Khan, Z.R. Ampong-Nyarko, K. Chiliswa, P. Hassanali, A. Kimani, S. Lwande, W. Overholt, W.A. Pickett, J.A. Smart, L.E. Wadhams, L.J. and Woodcock, W.A. 1997. Intercropping increases parasitism of pests. Nature. 631–632.

Knudsen, G.K. Bengtsson, M. Kobro, S. Jaastad, G. Hofsvang, T. and Witzgall, P. 2008. Discrepancy in laboratory and field attraction of apple fruit moth Argyresthia conjugella to host plant volatiles. Physiological Entomology.1–6.

Knudsen, J.T. Eriksson, R. Gershenzon, J. and Ståhl, B. 2006. Diversity and distribution of floral scent. The Botanical Review. 1-120.

Knudsen, J. T. Tollsten, L. and Bergstrøm, G. 1993. Floral scents – a checklist of volatile compounds isolated by head-space techniques. Phytochemistry. 253-280.

Landis, D.A. Wratten, S.D. and Gurr, G.M. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of entomology. 175 – 201.

Leal W.S. 2005. Pheromone reception. Topics in current chemistry. 1-36.

van Lenteren, J.C. Roskam, M.M. and Timmer, R.1997. Commercial mass production and pricing of organisms for biological control of pests in Europe. Biological Control. 143-149.

Leong, J.K.L. and Oatman, E.R. 1968. The biology of Campoplex haywardi (Hymenoptera: Ichneumonidae), a primary parasite of the potato tuberworm. Annals of the Entomological Society of America. 26-36.

Lewis, W. J. Jones, R. L. and Sparks A. N. 1972. A host-seeking stimulant for the egg parasite Trichogramma evanescens. Its source and a demonstration of its laboratory and field activity. Annals of the Entomological Society of America. 1087-1089.

Lewis, T. and Sturgeon, D. M.1978. Early warning of egg hatching in pea moth (Cydia nigricana). Annals of Applied Biology. 199–210.

Lewis, W. J. Oscar Stapel, J. Cortesero, A.M. and Takasu, K. 1998. Understanding how parasitoids balance food and host needs: Importance to biological control. Biological control. 175–183.

Lewis, W. J. Vet, L.E.M. Tumlinson, J. H. van Lenteren, J. C. and Papaj, D. R. 1990. Variations in parasitoid foraging behavior: Essential element of a sound biological control theory. Environmental Entomology. 1183-1193.

Light, D.M., Knight, A.L., Henrick, C.A., Rajapaska, D. and Lingren, B. 2001. A pearderived kairomone with pheromonal potency that attracts male and female codling moth, Cydia pomonella (L.). Naturwissenschaften. 333–338.

Macaulay, E. D. M. Etheridge, P. Garthwaite, D. G. Greenway, A. R. Wall, C. and Goodchild, R. E. 1985. Prediction of optimum spraying dates against pea moth, Cydia nigricana (F.), using pheromone traps and temperature measurements. Crop Protection. 85-98.

Madden, J. 1968. Behavioural responses of parasites to the symbiotic fungus associated with Sirex noctilio F. Nature (London). 189-190.

Mattiacci, L. Hütter, E. Schoch, D. Scascighini, N. and Dorn, S. 2000. Plant-odour mediates parasitoid host handling and oviposition in an endophytic tritrophic environment. Chemoecology. 185-192.

McAuslane, J. H., Vinson, S. B. and Williams, H. J. 1991. Influence of adult experience on host microhabitat location by the generalist parasitoid, Campoletis sonorensis (Hymenoptera: Ichneumonidae). Journal of Insect Behaviour. 101–113.

McCullagh, P. and Nelder, J.A. 1989. Generalized linear models 2 ed. Chapman and Hall, London. 532 pp.

McLeod, J.H. 1962. Biological control of pests of crops, fruit trees, ornamentals and weeds in Canada up to 1959. In: A review of the biological control attempts against insects and weeds in Canada. Commonwealth Agricultural Bureaux, Reading. 216 pp.

Meiners, T. and Hilker, M. 2000. Induction of plant synomones by oviposition of a phytophagous insect. Journal of Chemical Ecology. 221-232.

Mills, N.J. and Wajnberg, E. 2008. Chapter 1. Optimal foraging behaviour and control methods. In: Wajnberg, E. Bernstein, C. and Van Alphen, J. (eds). Behavioral ecology of insect parasitoids. Blackwell Publishing, Eastbourne. 445 pp.

Milonas, P. G. Martinou, A. F. Kontodimas, D. C. Karamaouna and F. Konstantopoulou M.A. 2009. Attraction of different Trichogramma species to Prays oleae sex pheromone. Annals of the Entomological Society of America. 1145-1150.

Montgomery, D. 2009. Chapter 2. Basic statistical methods. In: Design and analysis of experiments. John Wiley & Sons, Asia. 656 pp.

Mudd, A. and Corbet, S.A.1973. Mandibular gland secretion of larvae of the stored products pests Anagasta kuehniella, Ephestia cautella, Plodia interpunctella and Ephestia elutella. Entomologia Experimentalis et Applicata. 291-292.

Murlis, J. Elkinton, J.S. and Cardé, R.T. 1992. Odor plumes and how insects use them. Annual Review of Entomology. 505-532.

Pawliszyn J. 1997. Solid Phase Microextraction: Theory and Practice. Wiley-VCH, New York. 264 pp.

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

Prokopy, R.J. and Owens, E.D. 1983. Visual Detection of Plants by Herbivorous Insects. Annual Review of Entomology. 337-364.

Rochat, D. Nagnan-Le Meillour, P. Esteban-Duran, J.R. Malosse, C. Perthuis, B. Morin, J.P. and Descoins, C. 2000. Identification of pheromone synergists in american palm weevil,

Rhynchophorus palmarum, and attraction of related *Dynamis borassi.* Journal of Chemical Ecology. 155-187.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Sarwar, S.L. 1973. Untersuchungen zur biologie und bekämpfung des erbsenwicklers, laspeyresia nigricana steph. Dissertation, Karl-Marx-Universität Leipzig.

Schirmer, S. Sengonca, C. and Blaeser, P. 2008. Influence of abiotic factors on some biological and ecological characteristics of the aphid parasitoid Aphelinus asychis (Hymenoptera: Aphelinidae) parasitizing Aphis gossypii (Sternorrhyncha: Aphididae). European Journal of Entomology. 121–129.

Schoonhoven, L.M. Loon, J.J.A. and Dicke, M. 2005a. Chapter 3: Plant structure: The solidity of anti-herbivore protection. In: Insect-Plant biology. Oxford University Press, Wiltshire. 421 pp.

Schoonhoven, L.M. Loon, J.J.A. and Dicke, M. 2005b. Chapter 6: Host plant selection; how to find a host plant. In: Insect-Plant biology. Oxford University Press, Wiltshire. 421 pp.

Schoonhoven, L.M. Loon, J.J.A. and Dicke, M. 2005c. Chapter 12: Insects and flowers: Mutualism par excellence. In: Insect-Plant biology. Oxford University Press, Wiltshire. 421 pp.

Schoonhoven, L.M. Loon, J.J.A. and Dicke, M. 2005d. Appendix C: Methodology. In: Insect-Plant biology. Oxford University Press, Wiltshire. 421 pp.

Stein S.E. 1999. An integrated method for spectrum extraction and compound identification from GC/MS data. Journal of the American Society of Mass Spectrometry. 770-781.

Steinberg, S. Dicke, M.Vet, L. E. M. and Wanningen, R. 1992. Response of the braconid parasitoid Cotesia (=Apanteles) glomerata to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. Entomologia Experimentalis et Applicata. 163–175.

Thöming, G. and Saucke, H. 2011. Key factors affecting the spring emergence of pea moth (Cydia nigricana). Bulletin of Entomological Research. 127–133.

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

Turlings, T.C.J. Tumlinson, J.H. Eller, F.J. and Lewis, W.J. 1991. Larval-damaged plants: Source of volatile synomones that guide the parasitoid cotesia-marginiventris to the microhabitat of its hosts. Entomologia Experimentalis Et Applicata. 75-82.

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

Vet L.E.M. and Dicke M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology. 141–172.

Vet, L.E.M and Godfray, H.C.J. 2008. Chapter 11. Multitrophic interactions and parasitoid behavioral ecology. In: Wajnberg, E. Bernstein, C. and Van Alphen, J. (eds). Behavioral ecology of insect parasitoids. Blackwell Publishing, Eastbourne. 445 pp.

Vet, L.E.M, Lewis, W.J. and Carde, R.T. 1995. Chapter 3. Parasitoid foraging and learning. In: R.T. Carde and W.J. Bell (eds.) Chernical Ecology of Insects 2. Chapman and Hall, New York. 433 pp.

Vet, L. E. M. Lewis, W. J. Papaj, D. R. and van Lenteren, J. C. 1990. A variable-response model for parasitoid foraging behavior. Journal of lnsect Behavior. 471-490.

Vinson, B.S. 1975. Biochemical coevolution between parasitoids and their hosts. In: Price, P.W. (ed.) Evolutionary strategies of parasitic insects and mites. Springer, New York. 224 pp.

Vinson, B.S. 1976. Host location by insect parasitoids. Annual Review of Entomology. 109-133

Vinson B.S. 1981. Chapter 4. Habitat location. In: Semiochemicals. Their role in pest control.

John Wiley & Sons, New York. 306 pp.

Vinson, S.B. 1998. The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biological Control. 79–96.

Vinson S. B. 1999. Parasitoid manipulation as a plant defense strategy. Annals of the Entomological Society of America. 812-828.

Wall, C. Garthwaite, D.G. Blood Smyth, J.A. and Sherwood, A. 1987. The efficacy of sex-attractant monitoring for the pea moth, Cydia nigricana, in England, 1980–1985. Annals of Applied Biology. 223-229.

Webster, B. Bruce, T. Pickett, J. and Hardie, J. 2010. Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. Animal Behaviour. 451–457.

Weseloh, R.M. 1981. Chapter 5. Host location by parasitoids. In: Semiochemicals. Their role in pest control. John Wiley & Sons, New York. 306 pp.

Whitman, D.W. and Eller, F.J. 1990. Parasitic wasps orient to green leaf volatiles. Chemoecology. 69-75.

Witzgall, P. Bengtsson, M. Karg, G. Bäckman, A.C. Streinz, L. Kirsch, P.A. Blum, Z. and Löfqvist, J. 1996. Behavioral observations and measurements of aerial pheromone in a mating disruption trial against pea moth Cydia nigricana F. (Lepidoptera, Tortricidae). Journal of Chemical Ecology. 191-206.

Witzgall, P. Stelinski, L. Gut, L. and Thomason, D. 2008. Codling moth management and chemical ecology. Annual Review of Entomology. 503-522.

Witzgall, P. Kirsch, P. and Cork, A. 2010. Sex pheromones and their impact on pest management. Journal of Chemical Ecology. 80-100.

Woodford, J.A.T. Birch, A.N.E. Gordon, S.C. Griffiths, D.W. McNicol, J.W. and Robertson G.W. 2003. Controlling raspberry beetle without insecticides. Integrated Plant Protection in Orchards – Soft Fruits IOBC/wprs Bulletin. 87-92.

Wright, D.W. and Geering, Q.A. 1948. The biology and control of the pea moth Laspeyresia nigricana Steph. Bulletin for Entomological Research. 57–84.

Zwakhals, K. 2009. Fauna Europaea: Glypta haesitator, In: Achterberg, K.V. (ed.) 2009. Fauna Europaea: Hymenoptera, Ichneumonidae. Fauna Europaea version 2.4, http://www.faunaeur.org

Appendix 1: Results from the logistic regression models

Response: **Initiation**

Variable	Chisq	Df	Pr(>Chisq)
age	1.6238	1	0.2026
stimuli	4.1502	7	0.7623
age:stimuli	8.8594	7	0.2629

Response: **Initiation**

Variable	Chisq	Df	Pr(>Chisq)
age	6.676	1	0.009772
stimuli	4.0585	7	0.773011

Response: **Initiation**

Variable	Chisq	Df	Pr(>Chisq)
age	7.0828	1	0.007783

Response: **Upwind oriented flight**

Variable	Chisq	Df	Pr(>Chisq)
age	2.6694	1	0.1023
stimuli	10.0298	7	0.1869
age:stimuli	5.6404	7	0.5823

Response: **Upwind oriented flight**

Variable	Chisq	Df	Pr(>Chisq)
age	3.3947	1	0.0654082
stimuli	25.8915	7	0.0005267

Response: **Upwind oriented flight**

Variable	β	Std. Error	Z value	Pr(>|z|)
Control	-1.1632	0.3623	-3.211	0.00132
Frass	1.0678	0.4761	2.243	0.02491
Infected pea plants	1.4083	0.4799	2.935	0.00334
Infected pods without larvae	1.3098	0.4789	2.735	0.00624
Larvae in healthy pods	0.1271	0.5044	0.252	0.80112
Infected pea plants without pods	0.3682	0.4847	0.76	0.44744
Healthy pea plants	0.2941	0.4903	0.6	0.54863
Moth scales	1.3767	0.4662	2.953	0.00315

Response: **Halfway flight**

Variable	Chisq	Df	Pr(>Chisq)
stimuli	14.3197	7	0.04578
age	0.1227	1	0.72608
age:stimuli	5.6992	7	0.57528

Response: **Halfway flight**

Variable	Chisq	Df	Pr(>Chisq)
stimuli	24.546	7	0.000913
age	0.1518	1	0.696795

Response:	**Halfway flight**			
Variable	β	Std. Error	Z value	Pr(>|z|)
Control	-2.9957	0.7246	-4.135	3.56e-05
Frass	1.6964	0.8163	2.078	0.0377
Infected pea plants	1.4153	0.835	1.695	0.0901
Infected pods without larvae	2.1133	0.8018	2.636	0.00839
Larvae in healthy pods	0.4308	0.9402	0.458	0.64682
Infected pea plants without pods	-0.7885	1.2441	-0.634	0.52622
Healthy pea plants	1.6376	0.8153	2.009	0.04457
Moth scales	1.6874	0.8075	2.09	0.03664

Response:	**Full distance flight**		
Variable	Chisq	Df	Pr(>Chisq)
stimuli	7.9954	7	0.333
age	0.9741	1	0.3237
age:stimuli	8.931	7	0.2576

Response:	**Full distance flight**		
Variable	Chisq	Df	Pr(>Chisq)
stimuli	13.0323	7	0.07132
age	0.4558	1	0.49961

Response:	**Full distance flight**			
Variable	β	Std. Error	Z value	Pr(>|z|)
Control	-3.714e+00	1.012e+00	-3.669	0.000243
Frass	1.149e+00	1.176e+00	0.977	0.328776
Infected pea plants	1.739e+00	1.119e+00	1.555	0.120065
Infected pods without larvae	1.489e+00	1.141e+00	1.305	0.191831
Larvae in healthy pods	3.318e-14	1.431e+00	0	1
Infected pea plants without pods	-1.485e+01	9.723e+02	-0.015	0.987813
Healthy pea plants	1.659e+00	1.118e+00	1.484	0.137746
Moth scales	1.028e+00	1.175e+00	0.875	0.381604

Response:	**Landing**		
Variable	Chisq	Df	Pr(>Chisq)
stimuli	5.5895	7	0.5884
age	0.9741	1	0.3237
stimuli:age	8.7451	7	0.2715

Response:	**Landing**		
Variable	Chisq	Df	Pr(>Chisq)
stimuli	10.6499	7	0.1546
age	0.3072	1	0.5794

Response:	**Landing**			
Variable	β	Std. Error	Z value	Pr(>|z|)
Control	-3.71357	1.01212	-3.669	0.000243
Frass	-17.8525	4510.66322	-0.004	0.996842
Infected pea plants	1.1746	1.17645	0.998	0.318074
Infected pods without larvae	0.02469	1.43157	0.017	0.986238
Larvae in healthy pods	-17.8525	4510.66322	-0.004	0.996842
Infected pea plants without pods	-17.8525	4357.71468	-0.004	0.996731
Healthy pea plants	-17.8525	4406.95596	-0.004	0.996768
Moth scales	-0.11507	1.43043	-0.08	0.935884