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Olfactory responses of the parasitic wasp, *Trybliographa rapae* (Hymenoptera: Figitidae)

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Front picture: Parasitic wasp female. Photographer: Jonas Eriksson (family).

1. Summary

Delia flies (Diptera: Anthomyiidae) are economically important pests in several horticultural crops. The control is commonly relying on chemical insecticides, though there are possibilities with biological control from the natural enemies in the field. An important natural enemy is the parasitic wasp, *Trybliographa rapae* Westwood (Hymenoptera: Figitidae) that lays eggs in the *Delia* fly larvae. The parasitoid larva and the host larva have a parallel development until the host dies within its puparium and an adult parasitoid emerges.

The aim of this thesis was to understand the attraction to host- and food-associated plant volatiles of *T. rapae*, in order to enhance the effectiveness of the parasitic wasp as a biological control agent. The hypothesis was that female *T. rapae* would be attracted to the volatiles from cabbage plants infested by cabbage root fly larvae (*Delia radicum* L.) and to the food-associated volatiles from buckwheat flowers (*Fagopyrum esculentum* Moench).

Two-choice experiments were made in an olfactometer, testing root-infested cabbage against non-infested cabbage or against blank odor sources. There was a significant attraction to the infested cabbage plants. Both the below- and aboveground parts of the root-infested cabbage plants were tested against the below- and aboveground parts of non-infested plants. The females significantly chose the belowground parts of infested plants and there was a tendency for attraction to the aboveground parts of root-infested plants.

The volatiles collected from infested and non-infested cabbage plants showed both quantitative and qualitative differences. Two compounds from infested plants repeatedly elicited responses from the antennae of female *T. rapae* in a gas chromatograph coupled with an electroantennograph (GC-EAD).

In a semi-field experiment, there was a tendency that the parasitism rate increased due to nectar from buckwheat flowers. A strong attraction to buckwheat of female wasps was shown in two-choice bioassays, whereas the males showed no interest for either buckwheat or infested cabbage. The differences suggested that males would be guided by sex pheromones rather than female-associated plant volatiles when locating mating sites.

2. Sammanfattning

Delia-flugor (Diptera: Anthomyiidae) är ekonomiskt viktiga skadedjur i ett flertal hortikulturella grödor. Vanligen kontrolleras flugorna med kemiska bekämpningsmedel, trots att det finns möjligheter för biologisk kontroll med hjälp av naturliga fiender. Parasitstekeln *Trybliographa rapae* Westwood (Hymenoptera: Figitidae) är en naturlig fiende till *Delia*-flugor, eftersom de lägger sina ägg i fluglarverna. Parasitstekellarven utvecklas parallellt med värdlarven och vid förpuppning dör värden. Istället kläcks en vuxen stekel fram.

Syftet med det här examensarbetet var att studera parasitstekelns attraktion till doftämnen från en värd- och från en nektar- (energi) planta, för att förstå stekelns sökande efter värdlarven och därmed öka stekelns effektivitet som naturlig fiende. Hypotesen var att *T. rapae* har utvecklat en medfödd attraktion till inducerade doftstimuli från kålplantor angripna av kålflugan (*Delia radicum* L.) och till blommor från bovete *Fagopyrum esculentum* Moench). I beteende-experiment med en olfaktometer testades parasitstekelns val mellan angripna och icke angripna plantor. Parasitstekelhonorna var starkt attraherade av de infesterade kålplantorna. För att undersöka ifall både de underjordiska och ovanjordiska delarna utsöndrade attraktiva dofter, utfördes tvåvals-experiment från angripna och icke angripna plantor. Det var en stark attraktion till de infesterade kålplantornas dofter när den underjordiska delen testades, och det fanns en tendens för attraktion till de infesterade plantornas ovanjordiska del.

Doftuppsamlingar från angripna och icke angripna kålplantor visade både kvantitativa och kvalitativa skillnader. Två doftämnen från angripna plantor gav elektrofysiologisk respons från stekelhonans antenn i en gaskromatograf kopplad till en elektroantennograf (GC-EAD).

I ett semifält-experiment tenderade parasiteringsgraden att öka för parasitstekelhonorna när nektar från bovete fanns tillgängligt. Honorna visade en stark attraktion till dofter från bovetets blommor i tvåvalsexperiment, medan hanarna inte gjorde något val mellan bovete och fuktig luft. Ytterligare skillnader mellan hanar och honor visades när hanarna inte visade något intresse för de infesterade kålplantorna. Det spekulerades därför huruvida hanarna lokaliserar honorna med hjälp av feromoner än med hon-associerade växtdofter.

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4. Introduction

4.1. Plant-insect interactions

Parasitoid females are insect natural enemies, which lay their eggs in or on another insect (host) and that feed on the host tissues as larvae or adults (reviewed in Harvey, 2005). As opposed to parasites, the parasitoid development always causes the host to die, which makes them useful biological control agents of many pests. The largest group of parasitoids belongs to the order Hymenoptera, which attack the host egg, larva, pupa or adult. Some parasitoids are specialized in few hosts and others are generalists with a broader host range.

The behavior and development of parasitoids as third trophic level organisms, is influenced by the host plant (first trophic level) and by the herbivore host (second trophic level) (Turlings *et al*., 1990; Vet & Dicke, 1992). Natural signals between these organisms are referred to as semiochemicals (Schoonhoven *et al*., 1998). The chemical information that the parasitoid receives about the host consists of volatiles directly or indirectly associated with the host (Vinson, 1976). Host-associated volatiles are often released from plants and may be part of an inducible plant defense (Vet & Dicke, 1992).

4.1.1. Plant defenses

Plant defense mechanisms have evolved in response to insects that damage the plant by feeding or laying eggs (Karban, 1989; Dicke *et al*., 2003; Zangerl, 2003). Some defense mechanisms are constitutive, which means that the plants have chemical compounds or mechanical defense structures that are always present. Other plant defenses are inducible and are therefore active only after an insect attack, specifically elicited by the insect damage. The inducible chemical defenses comprise productions of volatile (e.g. Dicke *et al*., 1990) and/or non-volatile (e.g. Stout *et al*., 1994) compounds. The inducible defenses are local if released from the site of damage and systemic when released from undamaged sites of the plant (Karban, 1989). As the defense mechanisms are metabolically costly for a plant, a local response would be considered advantageous if it causes the herbivore to change plant

(Zangerl, 2003). Systemic inducible defenses are advantageous for the plant to protect the most valuable parts from further damage.

The defense-related plant volatiles consist of blends with different degrees of specificity (Dicke *et al*., 2003). There may be a specific ratio of the volatile components released from the infested plants (Visser, 1986). Some induced compounds are produced only from certain plant families, other volatiles are emitted specifically according to the species of the herbivore and the degree of herbivore's damage. Host plants vary in reliability since the plants are dependent on growth factors in different habitus and not always guarantee that there are hosts present. Diverse habitats with non-host plant volatiles could mask the odor of the host plants (Randlkofer *et al*., 2007).

4.1.2. Induced indirect plant defenses

Specialized herbivores have evolved different degrees of adaptation to the plant defenses (Renwick, 2002). Plant compounds which may be deterrent or toxic to a generalist herbivore are used as stimulants or attractants for a specialist. Plants may defend themselves indirectly against such specialists by releasing volatiles that attract the natural enemies of the herbivore (Dicke, 1999). These volatiles are referred to as 'herbivore-induced plant volatiles', 'infochemicals' (Vet & Dicke, 1992; Dicke *et al*., 2003) or 'herbivore-induced carnivore attractants' (Dicke *et al*., 1990). Since the induced volatiles are released only from attacked plants, it may be considered as an honest signaling of the plants (Zangerl, 2003).

The herbivore-induced carnivore attractants may be released systemically from infested plants (Dicke *et al*., 1990). This means that a root herbivore could induce an emission of volatiles from the leaves, which then helps the parasitoid's detectability of the hosts (Brown & Anderson, 1999). When a plant systemically releases volatiles, the odor source becomes larger than if only the damaged part had emitted odors (Dicke, 1999).

4. 1.3. Host finding in parasitoids

Parasitoids can be attracted by general or specific plant- or host volatiles (Dicke, 1999). A specific information can tell the parasitoid which herbivore species that is attacking the plant or which instars of larvae (Vet *et al*., 1995). Both the amount of volatile compounds and the composition of the blends are used by the natural enemy to distinguish the information.

For the long-distance searching, parasitoids use plant volatiles or plant colors to find their habitat or host (Vet *et al*., 1995). At a short distance from the plant, host-derived cues such as host frass or mandibular secretions may be important factors in addition to olfactory, visual, tactile and taste stimuli from the host plant.

Apart from interactions with sensory cues from plants and hosts, parasitoids' host finding is determined by genetic factors (Geervliet *et al*., 1997). Parasitoids can have innate attractions host-derived plant cues (Vet *et al*., 1995; Storeck *et al*., 2000) or learn to discriminate between different blends of plant odors (Dicke *et al*., 1990; Dicke *et al*., 1993, Smith *et al*., 1994) and associate these with specific hosts (Vet & Dicke, 1992).

4.2.2. Food needs of adult parasitoids

While the immature stages of larval parasitoids are provided food from the host organs (Slansky Jr, 1986), adult parasitoid females have short-term needs of energy for flying, host location and for the production and maturation of eggs (Lewis *et al*., 1998). The food may be provided from host feeding, from host products (honeydew) (Wäckers, 1999), or from substrates associated with the host (Wäckers, 1994). Many parasitoid species are known to visit flowers to increase their longevity (Baggen & Gurr, 1998; Vattala *et al*., 2006), fecundity (Jervis *et al*., 1993; Baggen & Gurr, 1998), to get protection (Wäckers, 1994) or to locate mates (Jervis *et al*., 1993). Flowers may be visited for the purpose of feeding on nectar, from which the parasitoids obtain mainly carbohydrates such as sucrose, fructose and glucose (Wäckers, 1999), as well as smaller amounts of proteins, lipids, amino acids, vitamins and secondary metabolites (Wäckers, 2005).

Parasitoids have innate responses to a wide range of food plants and respond according to their motivation and hunger state (Wäckers, 1994, Lewis *et al*., 1998). Important parameters of suitability of a food source are the availability, apparency and accessibility (Wäckers, 2005). For example, the availability of floral nectar is restricted to the period of flowering and parasitoids fail to use the nectar if the flowering occurs during the parasitoids immature stages. Only extrafloral nectar is available both during the flowering and the vegetative periods of the plant. The apparency of the floral nectar influences the sensory system of the parasitoid. The parasitoid detects a food source by olfactory, gustatory, tactile or visual cues (Kevan & Baker, 1998). Accessibility is important since many parasitoids have short

mouthparts and thus are restricted to certain flower morphologies to use the nectar (Jervis *et al*., 2003; Wäckers, 2005).

4.2. Habitat management

Habitat management is a form of conservation biological control, which aims to manipulate with the agro-ecosystem in order to increase the effectiveness of natural enemies (Landis *et al*., 2000). The performance of natural enemies may be enhanced by more alternatives of host/prey, creation of favorable microclimates and extra energy sources provided from nectar and pollen plants. In biological control programs, flowering plants may serve as 'selective food plants' to promote the performance of a natural enemy while not the herbivore (Baggen & Gurr, 1998). Examples of such plant species are those belonging to the family Apiaceae, because of their exposed nectars (Jervis *et al*., 1993). Another example is buckwheat, *Fagopyrum esculentum* Moench (Polygonaceae), which grows fast and has the blooming period during the emergence of some adult parasitoids (Lee & Heimpel, 2005). Lee *et al*. (2006) showed that the wasp parasitoid *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) had an improved fitness due to buckwheat nectar feeding, which resulted in higher parasitism rates and consequently decreased densities of the cabbage pest *Plutella xylostella* (L.) (Lepidoptera: Plutellidae).

There are many factors that influence the parasitism rate: the contact chemical stimuli of the host (Heinz & Parella, 1994), the host size (Vinson, 1976), the chemical composition of the plant (Kainoh & Tatsuki, 1988) or the host plant species (Kacem-Haddjel-Mrabet & Nenon, 2003). Because parasitism rates may decline if the distance to the nectar plant increases, the selective food plants should be planted in field edges or in floral strips in-between crop fields (Baggen & Gurr, 1998).

4.3. The parasitic wasp; *Trybliographa rapae*

4.3.1. Biology

The parasitic wasp, *Trybliographa rapae* Westwood (Hymenoptera: Figitidae) is an oligophagous, solitary parasitoid (Wishart & Mounteith, 1954) and an important natural

enemy to insect species within the genus *Delia* (Diptera: Anthomyiidae) (Jones, 1988). *T. rapae* is a koinobiont parasitoid (Wishart & Mounteith, 1954), which means that the parasitoid and host larva have a parallel development until pupation when the adult parasitoid emerges and the host dies (Harvey, 2005). *T. rapae* is a world-wide distributed parasitoid in fields abundant in their insect hosts (Jones, 1988). The documented hosts of *T. rapae* are the cabbage root fly, *Delia radicum* (L.), the onion fly, *Delia antiqua* (Meig.), the bean seed fly, *Delia platura* (Meig.) or *Delia florilega* (Zetterstedt) and the turnip root fly, *Delia floralis* (Fall.) (Wishart & Mounteith, 1954).

T. rapae overwinters in the host puparium under the soil surface. The adult emerges by gnawing a hole in the puparium. Female adult wasps may reach their host larvae by following the burrows made by the larvae (Jones, 1988). The females are pro-ovigenic parasitoids (Wishart & Mounteith, 1954), which means that they can lay eggs immediately after emergence, without the need of host feeding (as synovigenic parasitoid females need for their egg production) (Jervis *et al*., 2001). The female lays eggs by penetrating the host larval skin with a long ovipositor (Wishart & Mountetih, 1954).

Unmated females lay haploid eggs, from which only males emerge (Jones, 1988). Therefore, it is only after mating with males that female progenies are assured. Most eggs are laid between the second and the sixth day after emergence when the female is able to lay seven eggs in 48 hours. The total fecundity is on average 37.7 eggs per female. The eggs are of an ovoid shape and have long pedicels when newly hatched. The hatching of eggs may occur 96 hours after oviposition at 20ºC. Parasitic wasps do not perform superparasitism.

T. rapae has four larval instars and lives as endoparasitoid (inside the host larvae) in its' first, second and third larval instars (Wishart & Mounteith, 1954). The larvae of the first instar have a distinct head and eucoliform shape with leg-like appendages. The second larval instar develops at pupation of the host and has a cylindrical shape with fewer appendages than the first instar. The third larval instar has a slightly flattened shape. Shortly after the third instar, the parasitoid leaves the host pupa by gnawing a hole from inside the puparium (Jones, 1988). In the fourth stage, *T. rapae* lives as an ectoparasitoid larva when feeding on the outside of the host body, embedded in the host puparium. The complete developmental time from egg to adult during laboratory conditions (20 ± 1 ºC, 60 ± 10% r.h., 16L:8D photoperiod) varies from 50 to 62 days (Neveu *et al*., 1996). It was shown that the developmental time was shorter when the parasitoid eggs were laid in third instar *D. radicum* larvae, compared to when laid in the first instar larvae. Koinobiont parasitoids are dependent on the nutrition value

of the host and larger hosts (third instars) are assumed to contain more resources than small hosts (first instars) (Harvey, 2005).

T. rapae has only two generations in northern climates (e.g. southern England) (Jones *et al*., 1993), however, it is able to parasitize up to three generations of *D. radicum* due to the long life-span of the females and because the life cycle of the parasitic wasp is well synchronized with the life-span of *D. radicum* (Jones, 1988). It was shown in laboratory that adult parasitic wasps emerged 9 days after the adult cabbage root flies.

Female *T. rapae* may parasitize *D. radicum* larvae at approximately 4 cm depths, while not at 6 cm depths, indicating that *D. radicum* can have an 'enemy free space' from *T. rapae* (Hemachandra *et al*., 2007).

4.3.2. *T. rapae* parasitation

To orientate towards a larva or an infested plant, the wasp uses both visual (Brown *et al.*, 1998) and olfactory cues (Vet *et al*., 1985).

The initiation of probing is dependent on threshold concentrations of host-emitted compounds or host-related compounds (Brown & Anderson, 1999). Once the host has been located and accepted, oviposition does not take more than one minute (Wishart & Mounteith, 1954).

For many larval parasitoids the main searching modes are vibrotaxis, ovipositor probing and antennal searching (Vet & van Alphen, 1985) and for *T. rapae* the latter two modes are suggested to be the most important (Brown *et al*, 1998). In general, *T. rapae* adults prefer to walk rather than fly (Jones, 1988) but commonly the females mainly stand still while intensively probing the substrate with the ovipositor (Vet & van Alphen, 1985). The antennae are used in a non-rhythmic way for detecting irregularities in the substrate caused by the larval feeding. The searching time on the substrate depends on the response from the sensilla located on the ovipositor (Brown & Anderson, 1999).

4.3.3. Olfaction

At a distance from the host, the volatiles emitted from infested plants are likely to be more readily available for *T. rapae* than those emanating from the host itself (Brown & Anderson,

1999; Finch & Collier, 2000). Although the host larvae are feeding under ground, the olfactory cues have been suggested to originate from both below- and aboveground parts of the plant (Neveu *et al*., 2002). At the moment of oviposition, the females respond to olfactory cues from the larval frass (Jones *et al*., 1993), apart from physical stimuli from the larval movement (Vet & van Alpen, 1985).

The antennal sensilla are likely to be the mediators of the volatile host stimuli (Brown & Anderson, 1999). On the antenna of the parasitic wasp, the olfactory sensilla are described as placoid sensilla, recognized as pore plates and distributed from the third until the eleventh antennal segment. The female antenna consists of 13 segments: a base segment, a pedicel and a flagellum consisting of eleven segments. Most abundant are the olfactory sensilla on the proximal part of the flagellum. The antenna of male *T. rapae* consists of 15 segments.

4.4. The root-feeding insect host: *Delia radicum*

4.4.1. Biology

The cabbage root fly, *D. radicum* (L.) (Diptera: Anthomyiidae) has been recognized since several decades as a serious, economically important pest of *Brassica* crops (Traynier, 1967; Coaker & Finch, 1973). *D. radicum* is found in the temperate zones of the Northern continents of the earth; i.e. in the Holarctic region (35-60°N) (Finch, 1989). In temperate climates the cabbage root flies have two or three generations (Finch, 1971) and the timing of the generations depend on the air- and soil temperatures (Collier *et al*., 2008). In Northern Europe the first generation may appear in April-May, the second generation in July and the third generation in August.

The cabbage root fly larva is an oligophagous herbivorous species that feeds on the belowground parts of Brassicaceae; white cabbage, *Brassica oleracea* var. *capitata* (L.), cauliflowers; *B. oleracea* var. *botrytis* (L.), Brussel sprouts; *B. oleracea* var. gemmifera (Zenker), radishes; *Raphanus sativus* (L.), turnips; *Brassica rapae* (L.), swedes; *Brassica napus* var. *napobrassica* (L.) and cruciferous weeds (e.g. wild radish; *Raphanus raphinistrum* L.) (Finch & Ackley, 1977).

The females lay the first batch of 40-60 eggs in the soil clefts around or on the root of the host plant (Finch & Coaker, 1969). The hatching occurs within three to seven days and the larvae move to feed within the roots and on the stem base. *D. radicum* larvae have three

instars and *in situ* the second instar may be found six days after hatching of eggs, while the third instar larvae are found after 14 days (Neveu *et al*., 1996). However, the time-spans of the different instars vary with environmental factors (Finch, 1971). The larvae move away from the roots to pupate and the pupae remain in the soil for two weeks or for the winter dormancy (Finch & Coaker, 1969b). Pupae diapauses are induced when the length of daylight is less than 15 hours.

Adult cabbage root fly females feed on nectars composed of mainly fructose and sucrose (first generation flies) or fructose and glucose (second generation flies) (Finch, 1974). The carbohydrates are necessary for the maturation of eggs. Also protein, obtained from pollen (and to some extent from nectars), is needed to mature the eggs, however most females have sufficient amounts of absorbed proteins (Finch, 1971). *D. radicum* females are attracted to flowering dill, *Anethum graveolens* (L.) (Apiaceae) and alyssum, *Lobularia maritima* (L.) (Brassicaceae), while less to buckwheat, *F. esculentum* (Rännbäck, 2008).

4.4.2. Host plant stimuli

D. radicum females orientate towards their host plants by olfaction (Traynier, 1967; Kostal, 1992) or by visual cues (Kostal & Finch, 1994). The cabbage root fly is a specialized insect that can use the specific variations of the volatile blends emitted by the Brassicaceae plants to discriminate between host- and non-host plants (Ferry *et al*., 1997). A specific group of volatile compounds found in Brassicaceae are the isothiocyanates, which derive from sulfur-containing glucosinolates (Brown & Morra, 1997). Glucosinolates (contact cues) and isothiocyanates (volatile metabolites) are recognized as the major oviposition stimulants for the cabbage root flies (Traynier, 1967; Roessingh *et al*., 1997).

Although the larvae of *D. radicum* only need to find a suitable feeding site on the roots (Kostal, 1992), the larvae are able to orient themselves by both general plant odors (e.g. CO_2) and more specific plant metabolites (Jones & Coaker, 1978; Roessingh *et al*., 1997). For example, when turnip plants were attacked by the closely related turnip root fly larvae (*D. floralis*); the glucosinolate composition was altered in the roots (Birch *et al*., 1990) and in the leaves (Birch *et al*., 1992).

4.4.3. Related species

The species belonging to the genus *Delia* (Diptera: Anthomyiidae) are all pests of economic importance (reviewed in: Finch, 1989). For the control of *Delia* species, there are no resistant cultivars and chemical insecticides are used at conventional farms. However, it would be possible to control *Delia* pests by alternative strategies, such as soil cover crops, crop rotation and intercropping. Also sowing earlier in the season could minimize the degree of crop damage since the plants would have more established roots.

In all species it is the larva that causes damage (Finch, 1989). The turnip root fly larva, *D. floralis*, is similar to *D. radicum* as it attacks the roots of cruciferous crops (Brassicaceae). The bean seed fly larvae, *D. platura* or *D. florilega* primarily attack bean crops (Fabaceae) and secondarily onion crops (Alliaceae). *D. platura* oviposits on decaying plant material, e.g. on onions damaged by *D. antiqua* or on seeds that have failed to germinate (Finch, 1989). Subsequently the bean seed fly females orientate towards odors associated with microbial decomposition of the seed coat (Gouinguené & Städler, 2006).

The onion fly, *D. antiqua*, is a pest of alliaceous crops. The first generation onion fly larvae causes young onion plants to wilt and the later generations attack the bulbs (Ellis & Scatcherd, 2007). *D. antiqua* and *D. floralis* larvae are phytophagous herbivores, while *D. platura* is saprophagous.

4.5. Aims and hypotheses

The main aim of this thesis was to study the attraction of *T. rapae* to cues emitted from cabbage plants infested by cabbage root fly larvae. The hypothesis was that the female wasps would associate the plant cues with their root living hosts and therefore choose the infested plants in olfactometer experiments. Males were hypothesized to be attracted to infested cabbage plants because of the probability to find females and consequently have best possibilities for offspring. Moreover, the attractive cues were thought to be emitted from both above- and belowground parts of infested cabbage plants, due to a systemic production of herbivore-induced compounds.

Volatile collections from infested and non-infested plants were thought to be quantitatively different and the antennae of female wasps would respond to the active compounds in electrophysiological (GC-EAD) experiments.

Buckwheat flowers were hypothesized to increase the wasps' parasitism rate in a semi-field experiment, since the floral nectar would be an extra energy source. Both male and female wasps were thought to be attracted to buckwheat in two-choice experiments. Two different age classes were tested in the olfactometer experiments, where the hypothesis was that 3-5 day old wasps would be hungrier than 1-2 day old wasps.

Taken together, the long-term goal of this thesis was to contribute knowledge about the attraction of *T. rapae* to host- and food plant cues, in order to enhance the effectiveness of the parasitic wasp in a vegetable crop system.

5. Materials and methods

5.1. Insects

Parasitic wasp adults (*T. rapae*) originated from pupae provided by the University of Rennes (France) and from KVL (Denmark). Additionally adults were collected at different cabbage fields at Torslunda Experimental Station (Öland, Sweden).

The field sampling of adult wasps was made during clear days, from 11:00 h to 14:00 h, with temperatures of 15 ± 5ºC. Since *T. rapae* previously had been described as "walkers" rather than flying wasps (Jones, 1986), and had been observed on lower plant leaves next to the stem base (Pers. obs., A. Eriksson), the method of sampling was dependent on the plants and on the field conditions. In cabbage fields where the plants (swedes; *B. napus* var. *napobrassica*) were of smaller size (5 true leaves), the adults were caught by carefully hitting the leaves with a net. In fields exposed to strong winds or with larger plants (8-10 leaves), (white cabbage; *B. oleracea* var. *capitata f. alba*, cv. 'Castello'), it was not possible to use the net; instead the leaves close to the stem base were examined for walking adults. All wasps were blown with a Teflon pipe (6 mm i.d.) into test tubes (37 × 12 mm), protected by lids. Due to the low number of field collected wasps, these were however not used in experiments.

Cabbage root fly adults (*D. radicum*) originated from field collections of larval infested cabbage plants at Torslunda Experimental station. The larvae were reared into lab colonies (see: Rearing of *D. radicum*), from which eggs were taken to the wasp experiments.

5.2. Rearing of *D. radicum*

A modified rearing technique, first described by Finch & Coaker (1969b) was used for the rearing of the cabbage root fly. The flies were kept in a rearing cage (33 × 33 × 33 cm) placed in a controlled temperature cabinet (19°C, 16L:8D photoperiod).

As an oviposition site, a Petri dish (92 × 16 mm) was half-filled with moist sand (0.1-2.0 mm) and with a small piece (4 × 5 cm) of cabbage root (swede, commercial cultivar). The oviposition site was sprayed daily with water to avoid the eggs from drying out. Collection of eggs was made by carefully adding water until the eggs were floating. The eggs were rinsed into a 2-cm deep cavity of moist sand (0.1 – 2.0 mm), kept within 3-L pots. Half a root of swede (approximately 700 g) had been scored on the surface with a knife and subsequently put into the cavity. Each pot was protected by a polyester net (holes; 1.5 × 1 mm i.d.), then placed in room temperature for 4-5 weeks until pupation. The collection of pupae was made by floatation in water, and the pupae were stored at 9°C within plastic beakers (250 ml or 850 ml) containing moist vermiculate, until further rearing.

D. radicum adults were provided food twice every week. The food consisted of honey, smoothed out thinly on Petri dishes and with a teaspoon of milk powder and dry yeast on top. As an additional energy source for the flies, two small plastic cups with cotton rolls were filled with tap water and a lump of sugar. Another two small plastic cups with cotton rolls were filled with tap water only.

5.3. Rearing of *T. rapae*

For the rearing of the parasitic wasp, a modified method initially described by Neveu *et al*. (1996) was used. The rearing was based on larvae of the host *D. radicum*, which were fed on swede.

The eggs of *D. radicum* were collected by a careful floatation with water poured into a cavity of moist sand (0.1-2.0 mm, Fogsand), kept in pots of various sizes. On the cavity, a scored swede half was pushed down slightly. The pot was kept in room temperature for 10-13 days to assure an adequate amount of eggs hatched and to have *D. radicum* larvae of at least the 2nd instar for *T. rapae*. The swede half was placed with the scored side up to facilitate for the female wasps.

After one week in the rearing cage, the swede was put on moist sand (1.2 - 2 mm) in a plastic box (19 × 19 × 11 cm) covered by a lid. This was left for 20 days in room temperature (20 ± 2°C) for the flies to complete the larval development and pupate.

The parasitized and unparasitized pupae were collected by flotation in water and carefully taken up with a pair of tweezers. All pupae were counted and examined under a binocular microscope and then put in moist vermiculate within glass beakers (8 × 6 cm) or in Petri dishes (9 × 15 × 1.6 cm), placed on moistened kitchen paper within plastic boxes (19 × 19 × 11 cm). The boxes were kept in a temperature controlled cabinet (19°C, 16L:8D photoperiod) until the emergence of *T. rapae*.

In the rearing cage the adult wasps were provided water and food twice a week. Similar to the cabbage root flies, the diet consisted of honey, dried milk powder and yeast. Two plastic lids with small dabs of honey served as an additional energy source for the wasps and one plastic cup protected by lid with a cotton roll was filled with tap water and a lump of sugar.

5.4. Plants

White cabbage plants (*B. oleracea* var. *alba*, cv. 'Castello') and buckwheat plants (*F. esculentum*) were grown individually from seeds (cabbage: Lindbloms Frö, Kivik, and buckwheat: Olssons Frö, Helsingborg, Sweden) in 1.5–L pots, kept in a controlled glasshouse chamber (22 ± 2°C, RH 75%, 16L:8D photoperiod) until start of the experiments. The substrate for the plants consisted of a peat and sand mixture (NPK PG Mix 14-7-15). At the start of an experiment, the white cabbage plants were approximately one month old, grown in 3-L pots and with 8-10 true leaves. The buckwheat plants were grown in 5-L pots when used for the semi- field experiments and in 1.5-L pots for the laboratorial olfactometer experiments. The buckwheat plants were used when having 14 open flowers present. Swede roots (*B. napus* var. *napobrassica*), used for the rearing of the insects, were bought from commercial stores and were cut in halves to each with a weight of approximately 700 gram.

5.5. Infesting plants

Twenty-five *D. radicum* eggs moistened in water were deposited on the stem base of white cabbage, using a small paintbrush. For the semi-field experiment, the white cabbage plants

with *D. radicum* eggs were kept outdoors (day: 19 ± 2°C, night: 14 ± 2°C), protected from surrounding insects by using a fiber cloth until start of experiment. For the laboratorial experiments, the infested white cabbage plants were kept in a controlled glasshouse chamber (22 ± 2°C, RH 75%, 16L: 8D photoperiod) until start of experiments.

5.6. Two-choice bioassays

5.6.1. Set up

T. rapae females or males were presented two different odors in a mobile two-choice olfactometer. The set-up and procedure for the two-choice bioassays were according to Jönsson *et al*. (2005). The experiments were performed either outdoors during days of sunlight, between 10.00 h and 17.00 h at 15-20°C, or in a bioassay room (25°C, 60% RH, 12L:12D photoperiod). In the bioassay room, a mercury-vapor lamp provided illumination.

The olfactometer consisted of a box (50 × 50 × 50 cm) with three of the sides covered by white fabric and an observation side covered by a dark blue fabric. Within the box, a Y-shaped glass tube (arm length 220 mm, 155 mm i.d.) was placed. The air was pumped with a battery (Micropump, 7 Ah, 12 V, KNF Neuberger, Germany) into the odor source kept in polyethylene cooking bags (control, buckwheat flowers, cabbage roots: 25 × 38 cm; cabbage plants, cabbage leaves, buckwheat plants: 45 × 55 cm, Toppits, Melitta). From these odor sources, Teflon tubings were connected to the arm of the Y-tube.

The initial air was first pumped through a bottle (250 ml) with activated charcoal to eliminate surrounding odors and secondarily through a bottle with water to provide humidity. The airflow within the cooking bag was adjusted by a flow meter, at an airflow rate set at 1.1 L/min. Moreover, a piece of black plastic tube (2 cm) was placed one cm from the Y-tube junction, in order to calm down the insect before choosing direction. For equal light on the Y-tube arms, the box was placed towards the light.

Before an olfactometer experiment, the Teflon tubes and the cork plugs were sterilized with ethanol (70 %) and the Y-shaped glass-tube was heated in an oven at 350°C for 10 h. To use *T. rapae* of identified ages; females and males, newly emerged within the pupae boxes, were daily separated and transferred into plastic beakers (250 ml), kept in a controlled climate

chamber (19°C, 16L:8D photoperiod) with only water provided. Thus, the wasps could be defined as unfed and inexperienced females/males when used for the experiments.

Table 1. Odor sources that were tested in two-choice bioassays for 1-2 or 3-5 days old *T. rapae* males (M) and females (F).

Exp.	Sex	Age (days)	Odor sources
1a, b[1]	F	1-2, 3-5	Buckwheat flowers[2] vs. Blank (air)
2	F	1-2	Non-infested Cabbage[3] vs. Infested Cabbage[4]
3	F	1-2	Infested Cabbage vs. Blank (soil)
4	F	1-2	Infested Cabbage vs. Non-infested Cabbage
5a	F	1-2	Aboveground inf.[5] vs. Aboveground non-inf.[6]
5b	F	1-2	Aboveground inf. vs. Blank (air)
6a	F	1-2	Belowground inf. [7] vs. Belowground non-inf.[8]
6b	F	1-2	Belowground inf. vs. Blank (soil)
7	F	1-2	Belowground inf. vs. Aboveground inf.
8a, b[1]	M	1-2, 3-5	Buckwheat flowers vs. Blank (air)
9	M	1-2	Buckwheat plant vs. Inf. Cabbage
10	M	1-2	Infested Cabbage vs. Blank (soil)
11	M	1-2	Infested Cabbage vs. non-infested Cabbage

[1] Experiments performed outdoors

[2]Three bunches of buckwheat flowers cut off with a razor blade. The first three cm of the flower stalks were wrapped with moist tissue paper and secured by parafilm

[3] Intact white cabbage plant (8-10 true leaves), including roots and soil

[4] White cabbage plant (8-10 true leaves) infested with *D. radicum* eggs 10-13 days prior to experiment

[5] The leaves and stem of a *D. radicum* root-infested plant

[6] The leaves and stem of an intact plant

[7] The roots and stem base of a *D. radicum* root-infested plant, including larvae and soil

[8] The roots and stem base of an intact plant, including soil

5.6.2. Experimental procedure

The insects were introduced to the Y-tube 21 cm from the beginning of the bifurcation. An observation was recorded with a timekeeper from when the insect passed the first 5-cm-limit. The insect was considered to have made a choice when it had entered 5 cm into one of the two branches and remained there for 30 seconds. The n-number was calculated as the number of *T. rapae* that had made a choice. If the insect not made a choice during 5 minutes, it was recorded as a "No choice". The percentage of these no-responders was calculated from the total number (N) of responding and not responding *T. rapae* tested in the bioassay.

5.7. Semi-field experiment

5.7.1. Set up

Eight cages (1.2 × 1.2 × 1.5 m) were placed at 1.5-meter distances on a clay soil field. The cages consisted of transparent polyester fabric (4 × 3 m, 'Sarita', Ikea, Sweden), placed on top of three strings, fastened on two curved bows (2.5 m) made of elastic plastic tubes (16 mm i.d.). These plastic tubes were standing on iron rods (8 mm i.d.) stationed in the ground, 40 cm above the soil surface. On the sides of the cage, the polyester fabric was dug down and covered by stones and soil, to prevent any insects from escaping or entering.

Four white cabbage plants with 7-8 true leaves, 13 days previously infested with *D. radicum* eggs were placed in the cage corners with 45 cm distances in-between. One buckwheat plant, either with 14 flowers (Flower treatment) or with no flowers present (control) was placed in the center of the cage.

5.7.2. Experimental procedure

Four 2-5 days old starved *T. rapae* females, kept in open test tubes were put on the buckwheat plant in a cage. After 72 hours, the wasps were removed and counted. In addition, the *D. radicum* larval mortality within the cabbage roots was calculated. If all wasps were not found, the cabbage roots were washed and carefully searched through for *T. rapae* females that had dug down. To let parasitized and unparasitized *D. radicum* larvae develop, all cabbage plants

were cut off approximately 4 cm from the stem base and put into plastic boxes (19 × 19 × 5.5 cm) containing moist sand (0.1 - 2.0 mm). Next, two small pieces of a scored root of swede (3 × 4 × 1 cm), were put on the moist sand close to the white cabbage roots to provide additional food for the larvae.

The plastic boxes were kept in room temperature for three weeks. Then, the sand and cabbage roots were carefully rinsed by floatation for collecting the *D. radicum* pupae. The pupae were studied under a binocular microscope in order to identify pupae filled to three fourths, which thus were parasitized with 4th instars *T. rapae*. In case of difficulties in differentiating the parasitized pupae from the unparasitized, the pupae were left in the temperature controlled cabinet (19°C, 16L:8D photoperiod) until emergence of adult parasitoids. The parasitism rate was defined as the number of parasitized *D. radicum* pupae per cages in a treatment.

5.8. Electrophysiological experiments

5.8.1. Volatile collections

The headspace collections from infested and non-infested cabbage plants were modified according to previous methods described for crucifers (Brussel sprouts: Mattiacci e*t al*., 1994; Oilseed rape: Jönsson *et al*., 2005), as well as from discussions with an experienced chemist (Birgersson, G. pers comm). The plants infested by *D. radicum* or the non-infested plants were kept with both the roots and substrate (NPK PG Mix 14-7-15), since the sites of odor emission were unknown. One Blank, consisting of only the growth substrate, was made as control to a sample. The adsorptions were performed in room temperature (20-22ºC) either during night for 17 hours or during 8 day hours.

The volatiles were absorbed into aeration columns made of 25 mm Teflon-tubes (3 mm i.d), filled by 15 mm adsorbent; Porapack Q, 80/100 mesh (Supelco Inc., Bellefonte, PA, USA), held by two stoppers of Teflon-tube (2 mm i.d.) and glass wool. Shortly before collections the columns were rinsed with 1 ml methanol, 1 ml dichloromethane, 1 ml pentane and dried with nitrogen gas.

The sample and the blank odor sources were enclosed in polyethylene cooking bags (sample: 45 × 55 cm, blank: 25 × 38 cm, Toppits), sealed with scotch 10 cm from the top of

the bag. In order to have the same pressure within the bags, the airflow through the Teflon tubing (3 mm i.d.) was adjusted by a flow meter to 50-100 ml/min. A glass tube with activated charcoal was inserted into the bottom of the cooking bag through a hole, sealed with scotch, to have charcoal filtered air pumped through the system. The sample and the blank adsorbent columns were each connected to one reversed aquarium pump Rena 300 (Mars Fishcare, Chalfont, PA, USA). Within the cooking bag containing the cabbage plant, one pair of aeration columns (Teflon-tubes, 3 mm i.d.) were placed close to the plant, while in the blank cooking bag, one single aeration column was placed next to the soil. The air pushed through the Teflon tubing and the inlet air filtered by the activated charcoal were arranged diagonally to get airflow across the plant.

The adsorbents were extracted by adding 500 µl pentane (Purix p.a., Fluka, Buchs, Switzerland) with a 500 µl syringe into 1.5 ml vials enclosed with polyethylene lids. The extracts were stored at - 22 ºC until usage.

5.8.2. GC-EAD recordings

The electrophysiolgical activity of the volatiles collected from infested or non-infested cabbage plants on the antenna of female *T. rapae* were analyzed using coupled gas chromatography electroantenna detection (GC-EAD), following the method described by Jönsson & Anderson (1999). The volatile collections extracted with pentane were evaporated until approximately 6 µl remained. From this evaporated extract, 2 µl were injected into a Hewlett Packard (HP 6890) gas chromatograph (GC) with an HP-INNOWax column (30 m × 0.25 mm i.d.). The programmed velocity of the N_2 carrier gas within the GC was 45 cm/s and an injector temperature was set at 225ºC.

The female wasps were 1-5 days old and inexperienced to white cabbage plants when used for the experiment. For the recordings, the antenna was cut off from the insect head using a pair of scissors. Approximately half the antennal base segment was carefully inserted into a glass capillary (1.5 cm i.d.), filled with a Ringer solution. This was then brought in contact with a silver wire of a recording electrode connected to a DC amplifier (Syntech, Hilversum, The Netherlands). Next, the tip of the antenna (i.e. the 13^{th} segment) was cut off with a razor blade to minimize disturbances during the recordings. The cut end of the antenna was brought in contact with a glass capillary filled with Ringer solution, thereafter connected

to a grounded silver wire. By carefully adjusting the antenna through the two glass capillaries, a closed electric circuit (measured in µV) was obtained.

Before start of an injection, the condition of the antenna was tested by odor puffs from a Pasteur pipette containing a small piece of infested plant leaf (1 cm × 1 cm). The plant volatile extract was injected into the GC, in order to make the antenna exposed to an air stream carrying the eluting compounds during 32 minutes. Voltage responses of the parasitoid antenna were shown on a computer with EAG software (Syntech). A compound was considered electrophysiologically active when it elicited an antennal response greater than the background noise (Faccoli *et al*., 2008), which was repeated in at least three different recordings.

5.9. Statistical analysis

The percentage parasitized or non-parasitized pupae in the semi-field experiments, as well as the responses by the wasps in two-choice Y-tube experiments were analyzed with a G-test after Williams' correction, against an expected ratio of 50:50 (Sokal & Rohlf, 1981).

6. Results

6.1. T. rapae female responses to cabbage volatiles

When odors from non-infested cabbage plants were compared with soil (Blank), female *T. rapae* did not show any preferences (Figure 1a) ($G = 0.11$; $df = 1$; $P = 0.74$). Instead, when infested cabbage plants were tested against soil (Blank), the females were significantly attracted to the infested cabbage odors (Figure 1b) ($G = 15.42$; $df = 1$; $P < 0.001$) and the females showed strong preference for infested cabbage when tested against non-infested cabbage (Figure 1c) ($G = 34.14$; $df = 1$; $P < 0.001$).

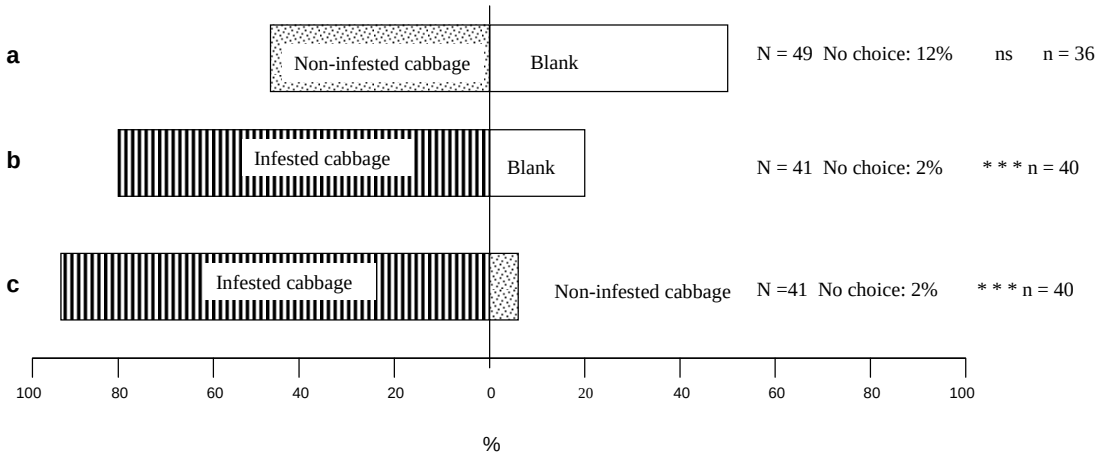

Fig. 1. *Trybliographa rapae* female (1-2 days old) response in Y-tube experiments. N, the total number of responding and non-responding parasitoids. The percentages to the right represent the individuals not making a choice. The graphs show the proportions of the females' responses. The results from the statistical tests (G-test); ns, not significant; significant differences *** $P < 0.001$ are based on n, the number of females making a choice.

The females which were tested odors from the belowground parts of infested and non-infested cabbage plants significantly chose the odors from infested plants (Figure 2a) ($G = 8.40$; $df = 1$; $P < 0.01$). Also when the odors from belowground parts of infested cabbage were tested against soil (Blank), the females showed strong preference for the belowground parts of infested cabbage (Figure 2b) ($G = 39.57$, $df = 1$, $P < 0.001$).

There was a non-statistically significant difference between the choice of odors from aboveground parts of root-infested plants and from non-infested plants, however a tendency was shown for the choice of the root-infested plant odors (Figure 2c) ($G = 3.66$, $df = 1$, $P = 0.057$). Instead there was a significant preference for the odors from aboveground parts of root-infested plants when these were tested against humid air (Blank) (Figure 2d) ($G = 8.40$, $df = 1$, $P < 0.05$).

The female wasps showed no statistical differences between the choice of odors from aboveground parts of root-infested plants and from belowground parts of the infested plants (Figure 2e) ($G = 1.61$, $df = 1$, $P = 0.21$).

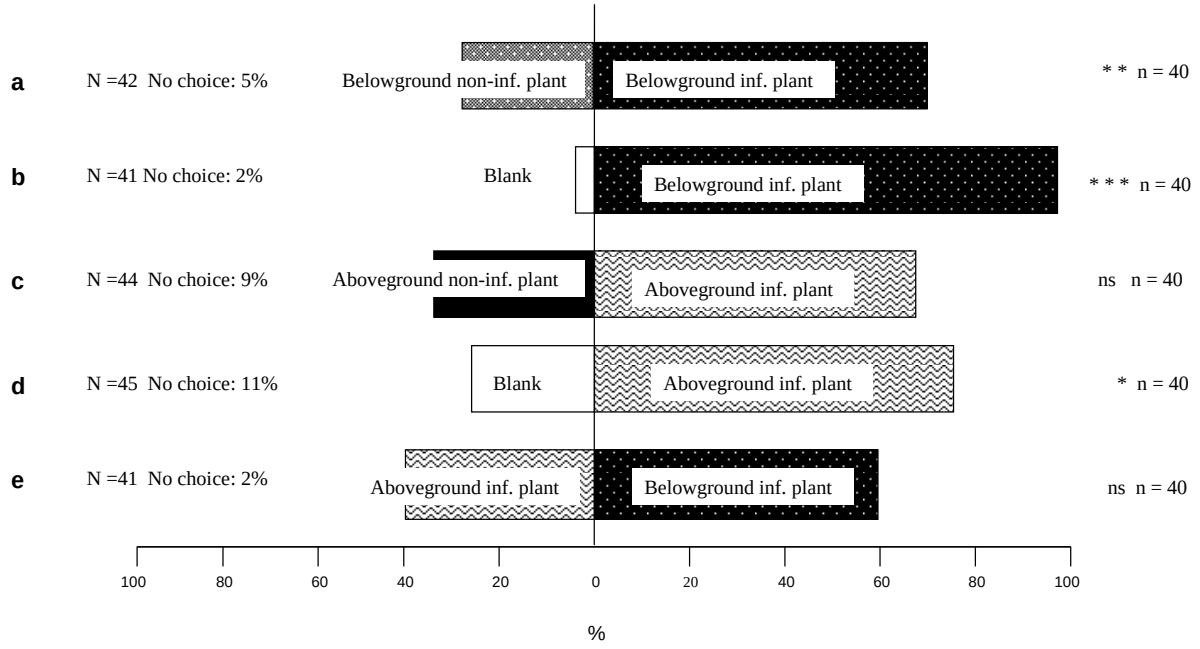

Fig. 2. *Trybliographa rapae* female (1-2 days old) response in Y-tube experiments. N, the total number of responding and non-responding parasitoids. The percentages to the left represent the individuals not making a choice. The graphs show the proportions of the females' responses. The results from the statistical tests (G-test); ns, not significant; significant differences *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ are based on n, the number of females making a choice.

6.1.1. Volatile collections from cabbage plants

The GC-chromatograms showed a tendency for quantitative and qualitative differences between infested and non-infested cabbage plant volatiles, collected during 17 night hours (Figure 3). The GC-chromatogram of an infested cabbage plant showed 165 compounds with an area larger than 1 pAs (Figure 3a) and the GC chromatogram of a non-infested cabbage plant extract showed 30 compounds with an area larger than 1 pAs (Figure 3b).

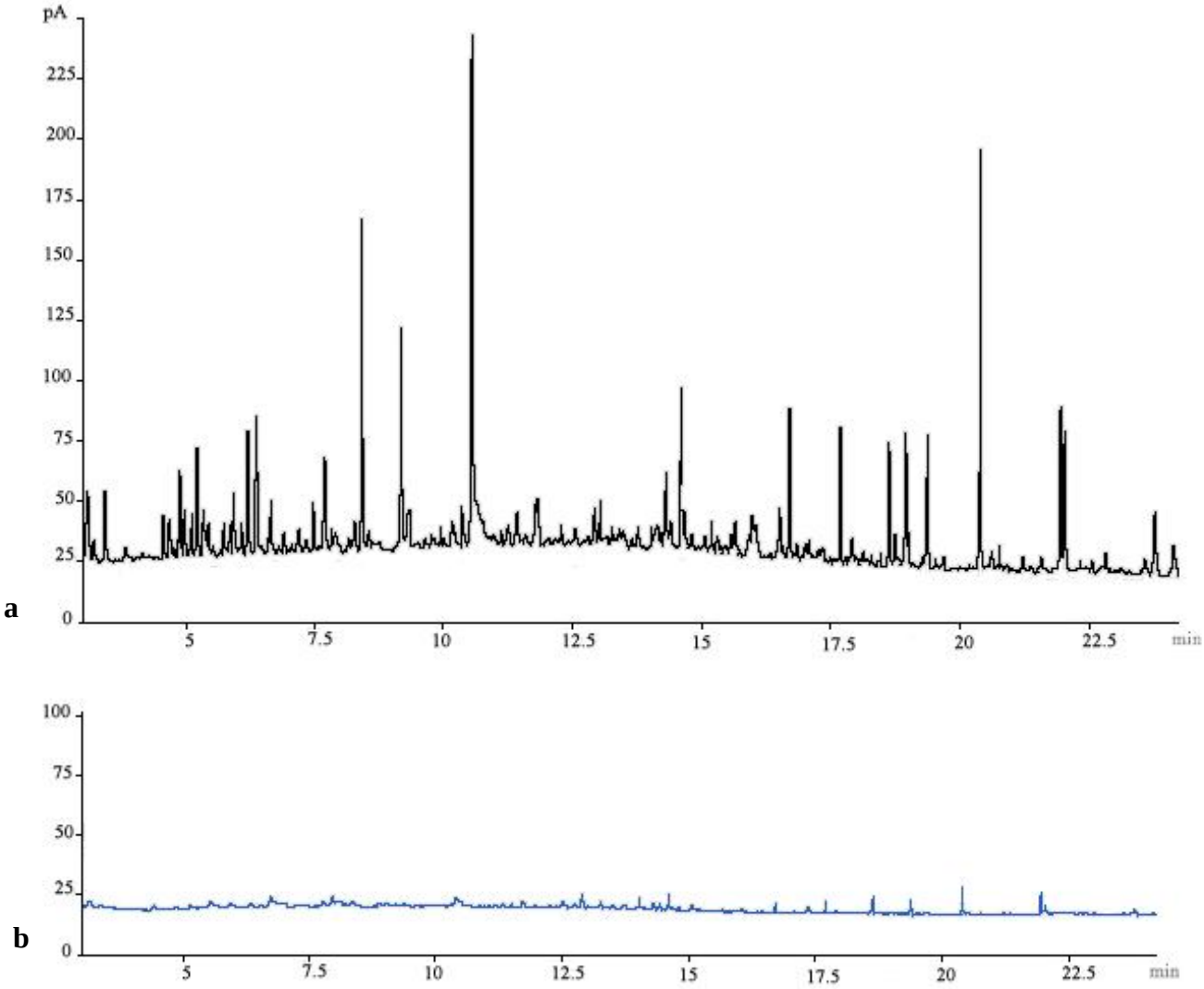

Fig. 3. GC chromatograms (FID responses) of volatiles collected during 17 night hours from a) infested cabbage plants (black colored FID response) and b) non-infested cabbage plants (blue colored FID response).

6.1.2. GC-EAD

From a simultaneously recorded gas chromatograph-electroantennodetection (GC-EAD) of volatiles collected from infested white cabbage plants, two compounds eluting at retention times 6.650 minutes and 8.414 minutes elicited responses from three *T. rapae* female antennae (Figure 4). Repeated disturbances on the antennae were shown from the retention time 8,414 minutes.

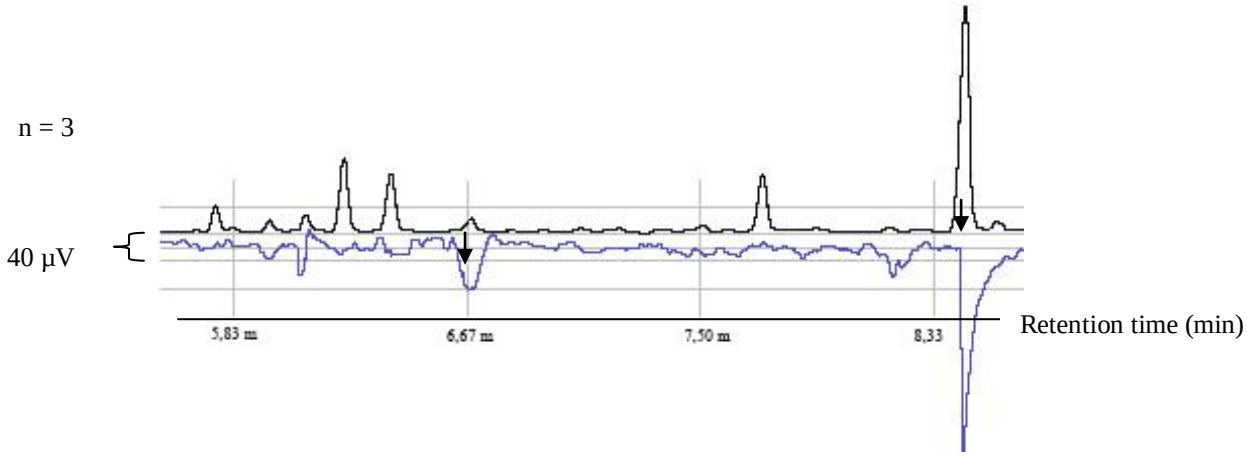

Fig. 4. Selected time section (5 min - 9 min) of a gas chromatography-electroantennadetection analysis (GC-EAD) of volatiles from infested cabbage plants collected from 8 day hours. Possible antennal responses from *Trybliographa rapae* females are indicated with the arrows. The upper black colored trace represents the compounds from the GC (FID response) and the lower blue colored trace represents antennal responses (EAD) of the parasitic wasp females. n, number of recordings.

6.2. The effect of buckwheat flowers on *T. rapae* females

From the initial number of 400 *D. radicum* eggs per flower treatment cages and control cages, the natural larval mortality in the semi-field was 62% in the cages with flower treatment and 61% in the control cages (Table 2). Two out of sixteen female wasps in the flower treatment and in the control were found within roots next to the *D. radicum* larvae (Table 2).

Table 2. Larval mortality of *Delia radicum* and the number of *Trybliographa rapae* females found during semi-field experiment. The numbers represent the total sum of eggs, mortal larvae (also shown in percentage), *T. rapae* females found after 72 hours in four cages. The total number of pupae and the number of parasitized pupae were calculated from four cages after three weeks. n, number of cages per treatment.

	Flower treatment cages (n=4)	Control cages (n=4)
Number of eggs	400	400
Larval mortality	247 (62%)	244 (61%)
Number of *T. rapae* in cabbage roots	2	2
Total number of pupae	153	156
Number of parasitized pupae	51	33

The total parasitism of *D. radicum* larvae was shown by the number of parasitized pupae three weeks after the removal of female wasps from the cages. No statistically significant difference in parasitation rate was found between cages with flowering buckwheat (parasitism 33%) and control cages (parasitism 21%) (G = 2.69; df = 1; P = 0.10) (Figure 5).

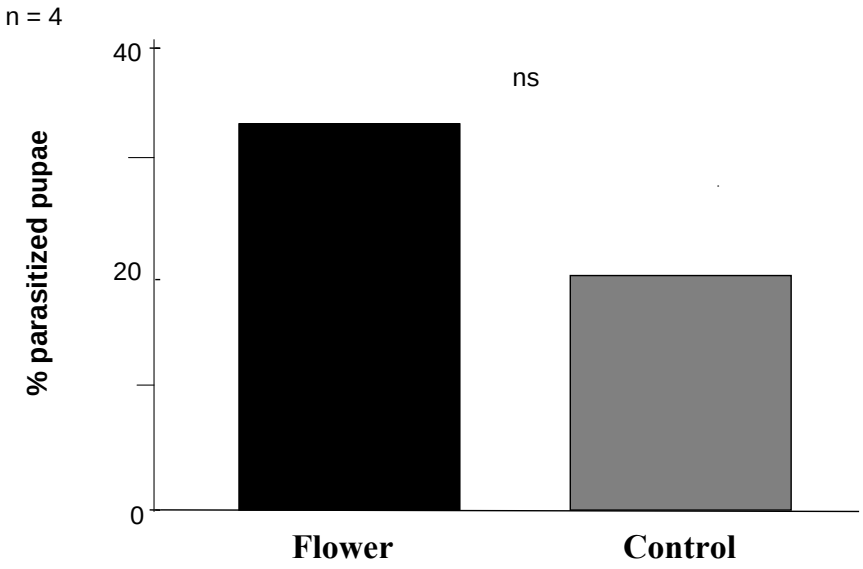

Fig. 5. The percentage of *Delia radicum* pupae parasitized by *Trybliographa rapae* in cages with a flowering buckwheat plant (Flower) or a non-flowering buckwheat plant (Control). The results of the statistical tests (G-test); ns, not significant. n, number of cages per treatment.

6.3. *T. rapae* female attraction to buckwheat volatiles

Both 1-2 days old and 3-5 days old female *T. rapae* showed significant preferences for flowering buckwheat when tested against air (Figure 6a) (G= 17.68; df = 1; P < 0.001), (Figure 6b) (G = 18.03; df = 1; P < 0.001).

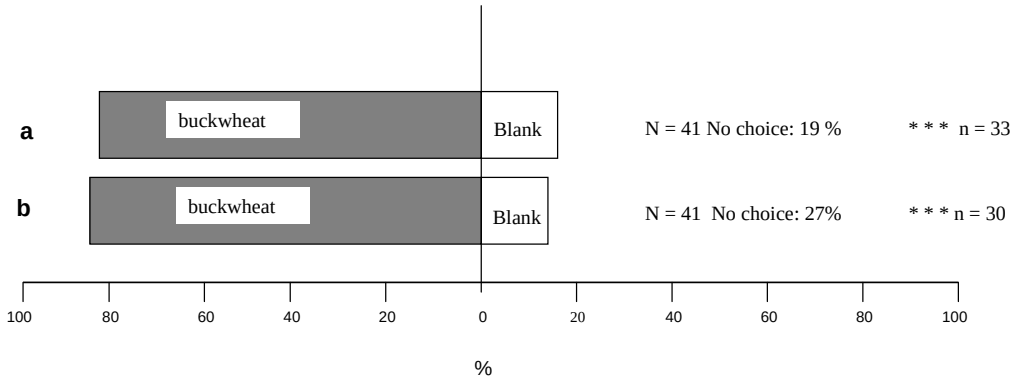

Fig. 6. *Trybliographa rapae* female response in Y-tube experiments to volatiles from flowering buckwheat and air (Blank); a) 1-2 days old females; b) 3-5 days old females. N, the total number of responding and non-responding parasitoids. The percentages to the right represent the individuals not making a choice. The graphs show the proportions of the females' responses. The results from the statistical tests (G-test); significant differences *** P<0.001 are based on n, the number of females making a choice.

6.4. T. rapae male responses

The newly emerged 1-2 days old males showed a tendency to avoid buckwheat when tested against air (Blank) (Figure 7a) (G = 1.33; df = 1; P = 0.16) however there was a significant number of no-responders (Figure 8a) (G = 12.80; df = 1; P <0.001). During the experiments, the males were observed to either stand still or move backwards in the direction away from the odor sources.

Of the 3-5 days old males, 55% did not make a choice between buckwheat and air, which was a non-statistically significant behavior (Figure 8b), (G = 0.40; df = 1; P = 0.53). The 3-5 days old males that made a choice showed non-significant avoidance of buckwheat (Figure 7b) (G = 2.04; df = 1; P = 0.16).

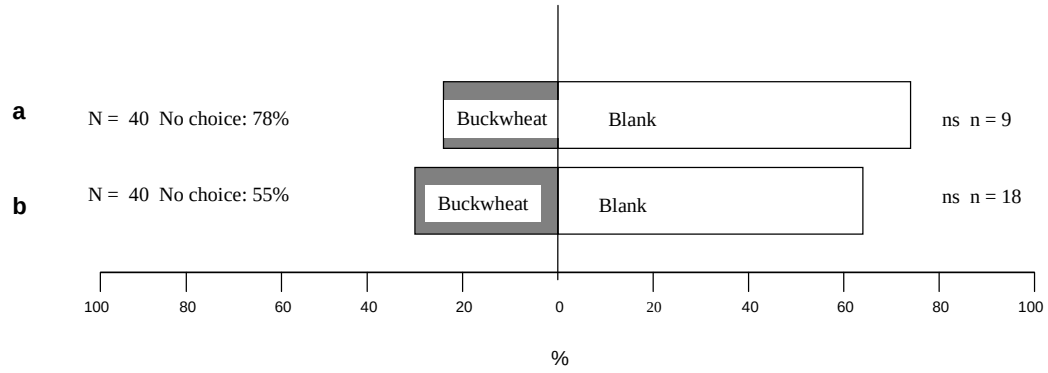

Fig. 7. *Trybliographa rapae* male response in Y-tube experiments to volatiles from buckwheat and air (Blank); a) 1-2 days old males; b) 3-5 days old males. N, the total number of responding and non-responding parasitoids. The percentages to the left represent the individuals not making a choice. The graphs show the proportions of the males' responses. The results from the statistical tests (G-test); ns, not significant are based on n, the number of males making a choice.

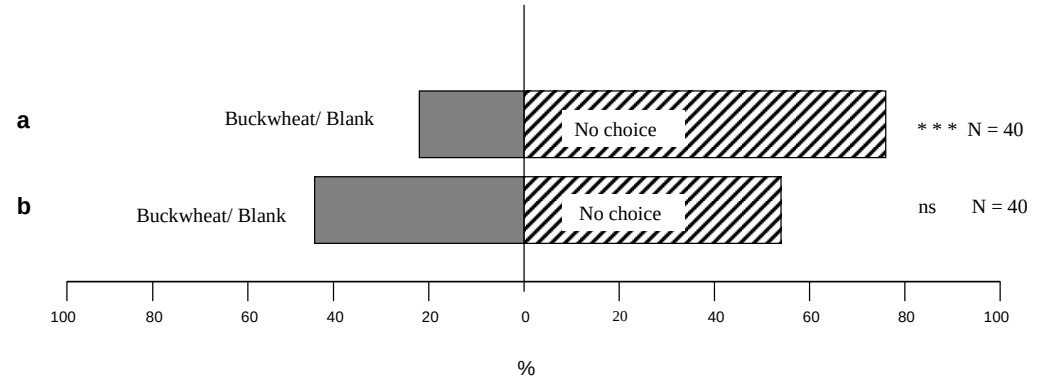

Fig. 8. *Trybliographa rapae* males that made a choice or that not made a choice in Y-tube experiments with volatiles from buckwheat / air (Blank). a) 1-2 days old males; b) 3-5 days old males. The results of the statistical tests (G-test); ns, not significant; significant differences *** $P<0.001$ are based on N, the total number of responding and no-responding males.

The males showed no significant differences in the choices between buckwheat and infested cabbage plants (Figure 9a) ($G = 0.03$; $df = 1$; $P = 0.86$). Similarly, when the infested cabbage plant was tested against soil (Blank), no significant differences were found (Figure 9b) ($G = 0.40$; $df = 1$; $P = 0.53$). When odors from infested cabbage plants were tested against non-infested cabbage plants, the males showed no differences between the odor sources (Figure 9c) ($G = 0.48$; $df = 1$; $P = 0.50$).

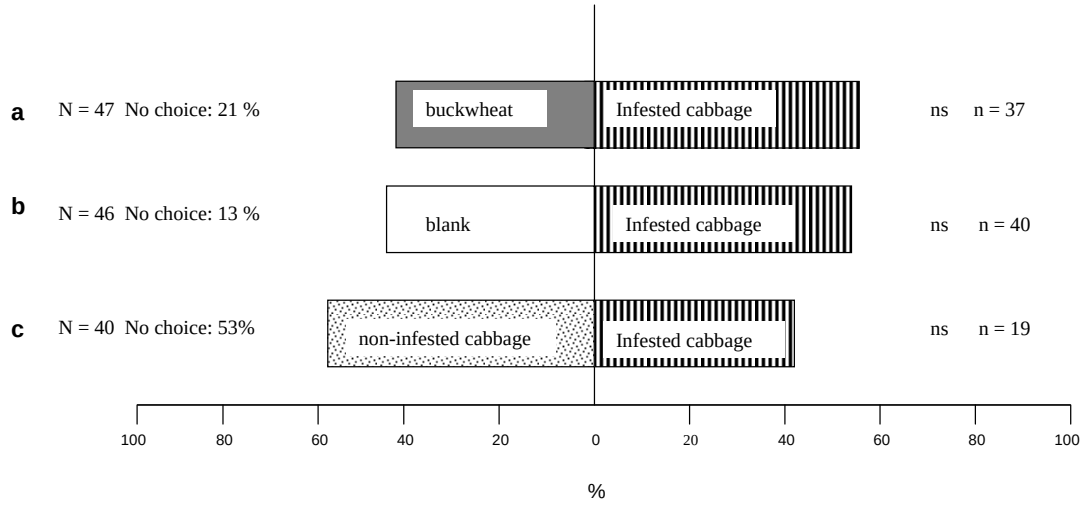

Fig. 9. *Trybliographa rapae* male (1-2 days old) response in Y-tube experiments; N, the total number of responding and non-responding parasitoids. The percentages to the left represent the individuals not making a choice. The graphs show the proportions of the males' responses. The results from the statistical tests (G-test); ns, not significant are based on n, the number of males making a choice.

7. Discussion

Female parasitic wasps were strongly attracted to odors emitted by infested cabbage plants when these were tested against odors from non-infested cabbage plants or against blank odor sources. These results indicate that *T. rapae* females are guided by herbivore-induced plant volatiles when searching for *D. radicum*. Previous studies have also shown the importance of infested plant cues for *T. rapae* to locate their host larvae (Jones, 1988; Brown & Anderson, 1999; Neveu *et al.*, 2002; Hemachandra *et al.*, 2007). The behavioral experiments of this thesis support the previous studies and provide new suggestions about the male and female wasp's relation to floral nectar, both in semi-field and in laboratory conditions.

7.1. Attraction to host plant cues

Contrarily to the attraction for infested cabbage cues, the non-infested plants were not attractive for the female wasps in the two-choice experiments. This shows that parasitoids distinguish between volatiles from infested and non-infested plants. The GC-chromatograms showed that non-infested plants released low quantities of volatiles, which may suggest that

the cabbage plant aims to be invisible, possibly in order to avoid herbivores, and that it is only after the root damage that it releases significant amounts of volatiles. The volatile collections indicated both quantitative and qualitative differences between the infested and non-infested plants. This result was surprising since previous volatile collections on Brassicaceae have revealed mainly quantitative differences (Agelopolous & Keller, 1994; Geervliet *et al*., 1997; Mattiacci *et al*., 1994; 2001). It is however possible that the volatile collections from the non-infested plants should have been performed differently, because of the expected low quantity of volatiles. Further collections may use longer adsorbent columns or modify the previously described for undamaged Brussel sprouts (Mattiacci *et al*., 1994).

The electrophysiological activity was not statistically tested due to antennal disturbances in most GC-EAD recordings. From the recordings only two compounds from infested plant extracts were found to repeatedly elicit response. Most likely the infested white cabbage extracts contained more than two active compounds, because previous studies showed e.g. 13 antennal responses from a pollen beetle parasitoid to volatile compounds from infested oilseed rape (Jönsson *et al*., 2005). Comparisons may be made from volatile collections of other cruciferous plants (e.g.; Brussel sprouts: Mattiacci *et al*., 1994; oilseed rape: Jönsson *et al*., 2005; Jönsson *et al*., 2008), in order to speculate on which volatile compounds that were released. Mattiacci *et al*. (1994) and Jönsson *et al*. (2008) reported that herbivore damaged Brussel sprouts and oilseed rape plants released enhanced levels of green-leaf volatiles (GLVs) in response to herbivore damage. GLVs are commonly produced in altered quantities from lipid degradations within damaged tissues and are released from the green parts of the plant (Hatanaka, 1993).

Other likely volatile compounds are isothiocynates, since these glucosinolate products constitute important oviposition stimuli for *D. radicum* (Roessingh *et al*., 1997) and ‘glucobrassicin’ has been shown by Gouinguené *et al*. (2006) to stimulate oviposition in both the turnip root fly as well as in the cabbage root fly. The isothiocyanate ‘dimethyl disulfide’ (DMDS) was identified as a major volatile constituent of swede roots infested by cabbage root fly larvae (Ferry *et al*., 2007). DMDS has a sulphur-containing end-product that occurs both in Brassicaceae and in Alliaceae. This is interesting considering *T. rapae*, since the wasps could associate this compound with the alliaceous herbivore *D. antiqua*.

7.2. Below- and aboveground released cues

Further electrophysiological studies from both night and day headspace collections could show if cabbage plants depend on light or time of day for the release of volatiles, as well as it could indicate whether the host finding in parasitic wasps depends on light. It is possible that there are differences between the night and day headspace collections from white cabbage plants since a previous study of turnips showed that the emission of limonene and sabinene was three times higher in light than in darkness (Jakobsen *et al*., 1994). In addition, headspace collections should be made on the belowground parts of cabbage to investigate if there is a different volatile blend released from these parts than from the whole plant.

There was a strong, significant attraction of the female wasps to the belowground parts of infested cabbage plants, both when tested against non-infested cabbage plants and against blank odor sources. It may be discussed whether the volatiles were emitted systemically or locally, since both damaged and undamaged roots, as well as the stem base of the plant were tested. A systemic emission of volatiles from undamaged parts of damaged turnip plants was previously suggested by Neveu *et al*. (2002). A possible hypothesis is therefore that a cabbage plant attracts *T. rapae* with a systemic release of volatiles from both the roots and the leaves. This is likely also because the aboveground part of the plant constitutes a larger odor source than the belowground part (Dicke, 1999).

The aboveground parts from infested plants were significantly attractive when tested against a blank odor source and the parasitoids tended to be attracted when these were tested against the parts from non-infested plants. The belowground parts of the infested plant tended though to be more attractive than the aboveground parts.

Further studies may test the below- and/or aboveground parts of infested plants against an entire infested plant, in order to indicate a synergism between the different plant parts. It is possible that cues emanating from belowground elicit responses according to the wasps' behavior at a short distance from the host and that the long-distance host searching behavior is guided by aboveground cues. Further studies should test this hypothesis in wind tunnel experiments. Although *T. rapae* are known to be walking wasps (Jones, 1988), they most likely have a flying behavior, considering that the parasitoids otherwise only would get offspring with the same individuals of the population.

7.3. Differences between male and female wasps

T. rapae males and females differ in their olfactory responses to host and food (nectar) plants. Both younger and older females significantly chose buckwheat odors against blank odor sources in the olfactometer experiments, which indicated that olfaction is important for their orientation to a food source. Contrarily, both younger and older males tended to avoid the buckwheat odors and the youngest male wasps showed a significant no-response behavior. This no-choice behavior of males may be due to that males are less interested in nectar during their relatively short life span (9.5 ± 1.2 days) (Jones, 1988) and the fact that they do not lay eggs. Yet, further studies are needed to verify the nectar requirements of *T. rapae* males and also consider other flower species. The present results have revealed primarily suggestions about the flower attractiveness for male parasitoids, because most previous studies have investigated the behavior of females rather than males (Lee *et al*., 2006; Winkler *et al*., 2005).

The females were not tested in the olfactometer experiments between buckwheat and infested cabbage, since Wäckers (1994) showed that the physiological state of parasitoids has an influence on their mode of choosing between host and food odors. For example, the female could have chosen flower odors in laboratory where it may be food deprived, while it likely could be attracted to infested plant cues as sugar-fed wasps in the field.

The male wasps were tested with buckwheat and cabbage odors since the males had been suggested by the present results to not associate buckwheat volatiles with food. However, the males did not choose either the infested cabbage or buckwheat. Surprisingly, this result suggests that the males do not respond to plant volatiles which could have given them best possibilities for offspring. Consequently, it may be speculated whether *T. rapae* males use female sex pheromones for mating site orientation. The males have also larger antenna (15 segments) than the females (13 antennal segments), and this could indicate that the males have antenna with pheromone specific receptor cells that do not occur on the female antenna.

Indeed, volatile sex pheromones have been identified in a number of other hymenopteran parasitoid families: Aphelinidae, Chalcididae, Cynipidae, Pteromalidae, Scelionidae, Braconidae, Ichneumonidae (reviewed in Eller *et al*., 1984), Eulophidae (Finidori-Logli *et al*., 1996) and in Trichogrammatidae (Pompanon *et al*., 1997). The sex pheromone may be substrate-borne and have low volatility (Pompanon *et al*., 1997; Sullivan, 2002) or the recognition of the female by the male may occur only at close range (Finidori-Logli *et al*., 1996). Yet, if a possible pheromone in *T. rapae* has these characteristics, the males would find

the females while walking on the cabbage plant. Therefore, the males are likely to first locate the host habitat. Surprisingly the males did not respond to the cabbage plants in the olfactometer experiments, however further studies should be made also on vision and taste.

7.4. Semi-field observations of female wasps

In the semi-field, the female wasps showed a tendency of an increased parasitism rate due to the nectar of buckwheat flowers, although there was a low n-value to show any statistically significant differences. Nectar has been shown in previous studies to either enhance female parasitoids' egg maturation or increase their capacity to fly and find or attack hosts (Jervis *et al*., 1993; Baggen & Gurr, 1998). In addition, Vattala *et al*. (2006) showed that another parasitoid species had an increased mean longevity when feeding on buckwheat flowers.

It is possible that the host plant characteristics (chemical properties or root structure and texture) had an influence on the capacity of *T. rapae* to attack *D. radicum*. A previous study of *T. rapae* showed that cauliflowers resulted in pupae with a higher percentage of parasitism (50.5 %) than swedes (28.5 %) (Kacem-Haddjel-Mrabet & Nenon, 2003). It is also possible that the cabbage root fly larvae were in an 'enemy free space' within the cabbage roots. Hemachandra *et al*. (2007) showed that a *D. radicum* larva feeding at a 6 cm depth was in a physical refuge from parasitism by *T. rapae*.

Another possibility of a low parasitism rate may be due to the high mortality of the *D. radicum* larvae and that the female wasps could have avoided unhealthy or already parasitized larvae. In addition, abiotic conditions in the field (strong winds, heavy rain and low temperatures), could have decreased the potential parasitoid activity. Therefore, further semi-field experiments should be performed during at least two seasons.

In a real field situation there are other biotic factors that influence the parasitism success. Pathogens or predators are risk factors for parasitoids (Rosenheim, 1998) and some parasitoid species constitute competition factors. For example, it is likely that *T. rapae* would compete with the pupa parasitoid or egg and larvae predatory beetle *Aleochara bilineata* (Gyll.) and *A. bipustulata* (L.) (Coleoptera: Staphylinidae). *A. bilineata* larvae make punctures on the pupal cuticle of *D. radicum* and thereafter feed on the semi-fluid content (Jones, 1988). The effect of the *Aleochara* ssp. on the parasitism by *T. rapae* needs to be investigated in further studies.

7.5. Application options

Biological control in cruciferous crops is difficult since these plants are attacked by a wide range of specialist pests (Finch, 1989). Therefore, both selective and broad-spectrum insecticides are used in the field. However, specialist insects often develop resistance to insecticides (Kanga *et al*., 2003). Studies similar to this thesis are needed to minimize the use of insecticides by applying the understandings of the natural enemies into the management strategies. The enhancement of natural enemies in an agro-ecosystem is an approach of habitat management (Landis *et al*., 2000).

The understandings of the host and food cues that attract *T. rapae* enable us to create alternate growing systems. The crop rotation systems have mostly been used to avoid soil pathogens (Finch, 1989); however an alternate growing of host plants and non-host plants may avoid maintenance of *D. radicum*. Alternatively, a rotation of host plants could maintain the populations of *T. rapae*. Potential crops to alternate are bean, onion and cabbage, since *T. rapae* may attack the bean seed flies (*D. platura*, *D. florilega*) or the onion fly (*D. antiqua*), apart from *D. radicum* (Wishart & Mounteith, 1954).

An increased biodiversity with non-host nectar plants is an important tool for a successful conservation biological control strategy (Landis *et al*., 2000). Rännbäck, (2008) suggested that *D. radicum* was less attracted to buckwheat than other nectar plants. Therefore, buckwheat may be used as a 'selective food plant' for enhancing mainly the populations of *T. rapae*.

7.6. Concluding remarks

Further ecological studies are required about the parasitoids, their hosts and host plants, in order to increase the knowledge about the parasitoids long distance- or short distance host searching. This thesis has contributed understandings to the chemical ecology of a cabbage-parasitoid- root herbivore- system, which should help future management strategies to develop practices for reducing cabbage root fly infestation without dependence of insecticides.

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