INFOCHEMICAL USE
BY INSECT PARASITOIDS
IN A TRITROPHIC CONTEXT:
comparison of a generalist and a specialist.

Jacqueline B.F. Geervliet

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INFOCHEMICAL USE BY INSECT PARASITOIDS IN A TRITROPHIC CONTEXT:

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Proefschrift
ter verkrijging van de graad van doctor
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Cover photograph by Jacques Brodeur; *Cotesia glomerata* wasp emerging from cocoon

STELLINGEN

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1. Om goed inzicht in het gedrag van sluipwespen te krijgen dient dit bestudeerd te worden in een tritrofe context, met inachtneming van de ecologische factoren die het functioneren van die sluipwespen bepalen.

   Price et al. 1980. Annu. Rev. Ecol. Syst. 11: 41-65.; Dit proefschrift.

2. Specificiteit van stimuli is een rekbaar begrip; zodra ook algemene stimuli de gebruiker naar zijn doel leiden, hebben ze een specifieke functie.

   Dit proefschrift

3. Het feit dat *Cotesia glomerata* generalist is op soortsniveau wil niet zeggen dat dat ook zo is op populatieniveau.

   Dit proefschrift

4. Discriminatie op huidskleur kan geaccepteerd worden wanneer het sluipwespen betreft, die op zoek zijn naar ongeparasiteerde gastheren.

   (met dank aan **Machiel van Steenis**)

5. Het feit dat olifanten zich, wat hun gebruik van sexferomonen betreft, nauwelijks onderscheiden van sommige insectensoorten wijst op convergente evolutie.

   (**Rasmussen, L.E.L., Lee, T.D., Roelofs, W.L., Zhang, A. & Doyle Daves, G.** 1996. Insect pheromone in elephants. Nature 379: 684)

6. De naamkeuze van producten van genetische modificatie, manipulatie of experimenten doet vermoeden dat wetenschappers niets menselijks vreemd is.

7. Wetenschappers gaan dikwijls te gemakkelijk voorbij aan de gevolgen van de toepassing van hun verworvenheden.

8. De gevolgen van verkeerd gebruik van antibiotica in de humane geneeskunde vertoont sterke overeenkomsten met die van het overdadig gebruik van chemische gewasbeschermingsmiddelen: in beide gevallen ontstaat onverantwoord snel resistentie.

9. De wens om tot een CAO voor het moederschap te komen lijkt meer op het 'plannen' van de komst van een kind voor het eigen gemak dan dat er sprake is van een natuurlijke drijfveer tot reproductie.

10. Referenda over wie het aanbod op radio en televisie moet bepalen zouden een opkomst teweeg brengen waarvan de Tweede Kamer na het afschaffen van de stemplicht alleen maar heeft kunnen dromen.
    (Ger Groot, NRC Handelsblad, 7 april 1997)

11. 'Kennis is macht' geldt helaas te weinig voor vrouwen op de arbeidsmarkt.

13. De NS-slogan "Met de trein de natuur in" is wat al te letterlijk genomen, nu het traject van de Hoge Snelheids Trein door het groene hart gepland is.

14. Beter tien verre vrienden dan één nare buur.

15. Mieren tuinieren.

16. Siervis is het einde.

Stellingen behorend bij het proefschrift **'Infochemical use by insect parasitoids in a tritrophic context: comparison of a generalist and a specialist'**. Jacqueline Geervliet, Wageningen 21 mei 1997.

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1

GENERAL INTRODUCTION.

I. THEORETICAL BACKGROUND

Behavioural ecology

Parasitoids, whose larvae develop by feeding on the bodies of other arthropods (usually insects), leading to the death of their host, are of immense importance in natural and agricultural ecosystems, where they influence or regulate population densities of their hosts. Many species are successfully used in biological control programs to combat agricultural pests, numerous other species are supposed to keep numbers of potential pest species at low levels (natural control). Research on parasitoids of these pests has generated a huge amount of information on behaviour and ecology of many different species. In addition, more and more attention is given to the fundamental biology of parasitoids both for testing foraging theories or hypotheses in the field of evolution and natural selection, and because fundamental knowledge on parasitoid biology is important for applied entomologists (Godfray, 1994).

Behavioural ecology is concerned with evolutionary questions on the interaction of animal behaviour with the environment (Krebs and Davies, 1981; Godfray, 1994). It investigates the fitness consequences of the options animals have to adopt different behavioural strategies, so following an optimization approach. Parasitoids are excellent candidates for asking evolutionary questions on behaviour, since reproductive strategies of parasitoids are directly linked to their fitness, i.e the production of viable offspring. The fitness consequences of failing to find a suitable host or making incorrect ovipostion decisions are obvious and relatively easy to measure (Godfray, 1994).

Host-searching behaviour

Very generally, insect parasitoids have similar life histories and face similar evolutionary challenges (Godfray, 1994), although evolution of host-searching strategies in insect parasitoids has led to a high diversity of specialized life styles (Price, 1981; van Alphen & Vet, 1986; Lewis et al., 1990). Parasitoid females spend a great deal of their life time searching for hosts, in, on or close to which they lay their eggs. Considering their small size and the small size of their hosts, they are faced with a complex and heterogeneous environment in which to search for hosts. On finding a host they must make a series of reproductive decisions, such as whether to accept the host for oviposition and, in case of gregarious parasitoid species, how many eggs to lay. Females of hymenopterous parasitoids, that have proximate sex control, can decide whether to produce male or female eggs, and also this decision can have great fitness consequences. Survival of the parasitoid eggs and larvae is determined by the host's defenses against parasitism, and, thus, the fate and fitness of the developing parasitoid is influenced by host quality.

Several models describing host-searching behaviour have been developed in the last five decades. A static concept has been the division of successful parasitism into the hierarchical process of host-habitat location, host location, host acceptance and host suitability, some authors adding host regulation (Doutt, 1959; Vinson, 1976, 1984; 1985; Nordlund et al., 1988; van Alphen & Vet, 1986). A more dynamic concept has been proposed by Lewis et al. (1990) and Vet et al., (1990), in which parasitoids are hypothesized to show differential responses to a set of stimuli with the highest and most predictable, least variable responses to those stimuli that are most closely associated with their hosts. Furthermore, for parasitoids using strategies that involve movement in species, such as systematic search, Godfray (1994) states that such parsitoids need to be able to orient themselves by using stimuli that carry directional information.

Infochemicals and host-searching behaviour

Parasitoids have evolved and operate in a multitrophic system (or foodweb) in which natural enemies (primary parasitoids and predators) are classified as the third, their hosts or prey as the second and the food of the host as the first trophic level. To understand interactions between plants, herbivores and natural enemies, Price et al., (1980) stated, that theory and research on any of these levels should be considered within this tri-trophic context. The previously underestimated influence of plants on the interactions between parasitoids and herbivores has shown to be very important. Parasitoids use stimuli from the plant (olfactory stimuli, visual stimuli) during host-habitat location (Vinson, 1981; Nordlund et al., 1988; Vet & Dicke, 1992), and plant characteristics are likely to influence the risk of parasitism of a host (Vinson, 1981; 1985; Price et al., 1980; Hawkins & Lawton, 1987) and hence the population dynamics of parasitoid-host interactions (van Emden, 1966; Lawton & McNeill, 1979). Finally, plants other than the host's food plant may influence parasitism by masking stimuli produced by the attacked plants or providing food for parasitoids such as honeydew or nectar (Price et al., 1980; Godfray, 1994; Wäckers, 1994).

While searching for hosts, female parasitoids use stimuli from their environment and chemical information plays an important role (Price et al., 1980; Vet & Dicke, 1992). A sequence of responses to different information sources brings the searching parasitoid into close vicinity of its host. Cues and stimuli used by parasitoids in host-searching behaviour can be distinguished in stimuli from the host microhabitat or food plant, stimuli indirectly associated with the presence of the host and stimuli from the host itself, this order reflecting the increase in importance as indicator of host presence (Godfray, 1994). Plants can attract parasitoids in the absence of hosts, parasitoids can respond to cues that are derived from activities by the herbivore (feeding damage leads to the emission of herbivore-induced synomones (Vet & Dicke 1992)) and some parasitoids use stimuli produced by host adults (such as sex pheromones (Noldus et al., 1991), oviposition deterring pheromones (Prokopy & Webster, 1978), aggregation pheromones (Wiskerke et al., 1993), honeydew (van Roermund & van Lenteren, 1996) and body scales (Noldus & van Lenteren, 1985)). Host-derived stimuli, from e.g. faeces, silk, cuticle, secretions of mandibular or accessory glands, honeydew and hemolymph) are the most reliable indicators of host presence (Vet & Dicke, 1992). Plant volatiles mediate searching behaviour at longer distances (Vinson, 1981; Vet & Dicke, 1992), while the importance of host-derived stimuli increases when the distance to the host gets smaller (Weseloh, 1981; Vet & Dicke, 1992).

Dietary specialization and infochemical use

The efficiency of a parasitoid's host searching strategy is important for its success. Behaviour is not fixed, but shows inter and intraspecific variation. A large variation in searching behaviour between parasitoid species has been observed (e.g. van Alphen & Vet, 1986), which can be based on genotypic and phenotypic factors. The question arises which differences in parasitoid host-searching strategies are adaptive.

Variability in host-searching behaviour is hypothesized to be partly affected by the degree of dietary specialization of parasitoids (Vet & Dicke, 1992). The degree of specialization sets the degree of specificity of information needed during searching for hosts. Specific information on the identity of hosts present in a certain habitat is supposed to be not very useful for parasitoids that attack a wide variety of hosts, but will be extremely important for parasitoids that are more specialized at the herbivore level. Information on the exact identity of the food plant is supposed to be important for parasitoids that attack one or more host species that occur on a single plant species (Vet & Dicke, 1992).

Furthermore, variability in host-searching behaviour finds its cause in the degree of phenotypic plasticity of the parasitoid. To a certain degree, all animals are able to modify their phenotype in response to different environmental conditions (Via, 1987). In insect parasitoids, behavioural variability can result from variation in the physiological state of the parasitoid (such as mating, egg load, hunger or disease) or from variation in experience with the environment.

Natural selection determines the level of the innate response potentials in parasitoids to stimuli and upon initial perception of a stimulus, the physiological state of the inexperienced individual will further determine the actual response to that stimulus. Variability in the response to a stimulus is found to be negatively correlated with the strength of the response potential to that stimulus (Vet et al., 1990b; 1995). When response potentials are high, the individual has low variability in its response (Fig. 1).
Behavioural responses of parasitoids can change after experience at various stages of their life cycle.

Learning is a common phenomenon in insect parasitoids (Vet & Groenewold, 1990). Differences in the degree to which behaviour is fixed or can be altered through experience are important to explain variability in behaviour between parasitoid species. Different types of learning can be distinguished.
Pre-adult learning may influence responses to stimuli as a result of development in a certain host or on a certain food substrate (Hopkins, 1917; Hérard et al., 1988; Vet & Dicke, 1992; Turlings et al., 1993; Cortesero & Monge, 1994).
More commonly, learning occurs during host search in the *adult* stage. Experience with olfactory, visual or mechanical stimuli from the environment, as well as experience with parasitized hosts, host densities or the presence of competitors may change behaviour through habituation or sensitization (priming) and associative learning. Habituation is the waning of the response to a stimulus with repeated exposure to that stimulus, which can lead to dispersal from a patch when searching for hosts is no longer profitable (Waage, 1978; Vet & Dicke, 1992). Sensitization is a general increase in responsiveness as a result of exposure to an environmental stimulus. In associative learning parasitoids associate innately recognized stimuli (unconditioned stimuli) with new, surrounding stimuli (conditioned stimuli), to which they become responsive in subsequent searching bouts (Turlings et al., 1993). Through learning of a stimulus an increase of the response to that stimulus is expected, accompanied by a decrease in its variability (Vet et al., 1990b; Vet & Papaj, 1992; Papaj, 1993). This is supposed to occur when initially weak responses are involved, for example environmental cues such as plant volatiles, that give only indirect information on host presence. Responses to stimuli with a high response potential are expected to be little variable and hardly influenced by learning, like host-derived products that are reliably linked with the presence of the host (Vet et al., 1990b; Vet & Dicke, 1992; Vet et al., 1995).

In general, learning by insect parasitoids is expected to be adaptive in situations where the individual encounters a variable environment. As stated earlier, the need for learning by insect parasitoids is assumed to depend on the dietary specialization of the species. Parasitoids that are specialized on one host species are supposed to rely heavily on host-derived stimuli that will elicit strong invariable responses, little influenced by learning. Therefore, in specialists learning is expected to be of minor importance. In contrast, learning is expected in generalist species that have to deal with greater host and environmental variation. The variable response model of Vet et al. (1990b) (Fig. 1) predicts that the response potential curve of generalists has a different shape with a higher fraction of intermediate, relatively low response potentials that are greatly modified by learning. By modifying their responses to host and environmental characteristics upon encountering hosts through a process of associative learning, they may enhance their efficiency in subsequent searches. For parasitoids there is evidence that both generalists (e.g. Arthur, 1966; Vet & Schoonman, 1988; Turlings et al., 1989) and specialists (e.g. Arthur, 1971; Vet, 1983; Vet & van Opzeeland, 1984; Sheehan and Shelton, 1989) can learn, but not enough data exist to make a meaningful comparison of the relative learning ability of specialists and generalists. Present data include work on only distantly related species (Vet et al., 1990b). Comparative studies, wherein phylogenetically close species are used to search for correlations between species characteristics and ecological factors, are valuable in delineating the adaptive value of learning traits and answering questions such as: how does learning enhance foraging efficiency in different species?, how much learning is involved? and do the findings support the hypothesis, that learning in specialists is of minor importance than in generalists? However, to design ecologically relevant studies with highly comparable stimuli we would need to use species with highly comparable or even overlapping niches. This sort of comparison enhances the chance of drawing unambiguous conclusions that observed differences in learning are indeed correlated to diet breadth and not to some other ecological or phylogenetic factor (Vet et al., 1995).

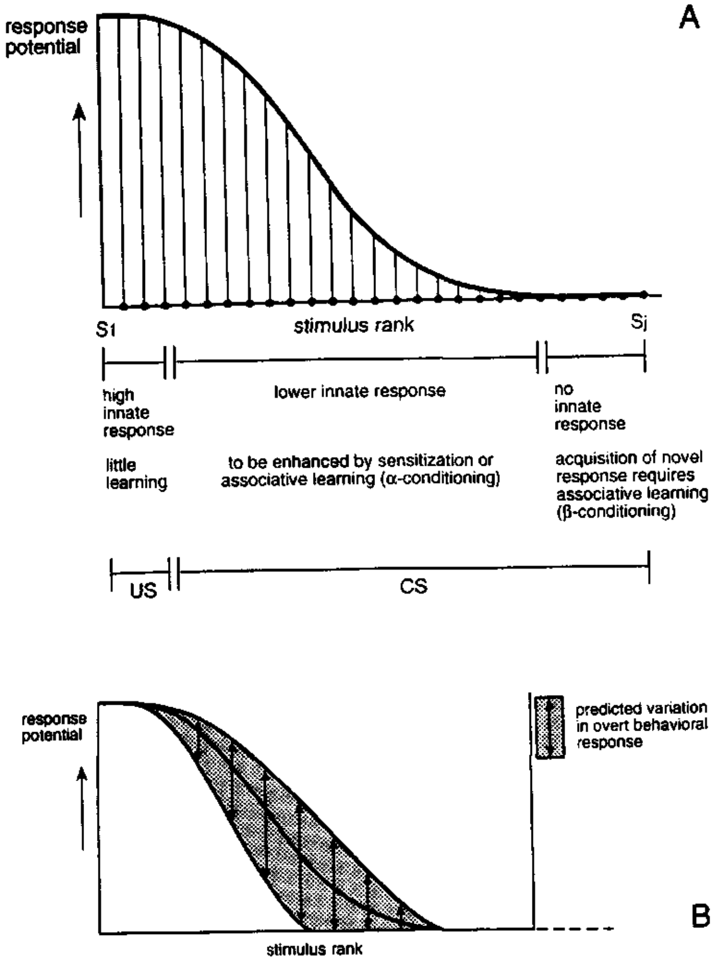

Figure 1. Diagram of a female parasitoid's potential behavioral response to a variety of environmental stimuli and the relationship with learning. A. All stimuli perceived by the insect are ranked according to their response potential in the naive insect. Stimuli beyond S_j are outside the range of sensory perception of the animal and can not be learned; B. Relationship between response potential level and variation in *overt* behavioural response (from: Vet et al., 1995).

Dietary specialization of parasitoids can be determined with respect to the first trophic level and the second trophic level, although extensive field records of plant-host-parasitoid associations are scarce and host and food plant ranges are not always reliable in literature (Askew & Shaw, 1986). This complicates correlating diet breadth with infochemical use. At the herbivore level, not all hosts of a certain parasitoid species will be known, and at the plant level, not all plants in a herbivore's food range are included in the parasitoid 'diet' (Vinson, 1981; Ohsaki & Sato, 1990; 1994; Vet & Dicke, 1992). Furthermore, specialization may be an attribute of populations, and populations in a certain region may be adapted to utilize specific resources. A generalist species can actually consist of specialist local populations (Fox & Morrow, 1981; Futuyma & Peterson, 1985; Vet & Dicke, 1992).

A recent hypothesis on the evolution of infochemical use in a tritrophic context using dietary specialization as a factor determining the use of infochemicals was proposed by Vet & Dicke (1992) as a guidance for future research. The investigations described in this thesis were based on this hypothesis.

Selection of the experimental system

The comparative method is supposed to be an important tool to determine correlations between behavioural traits and ecological factors indicative of adaptation (Vet & Dicke, 1992). Comparison of phylogenetically related species that differ in some ecological characteristics has been applied successfully by ecological entomologists to interpret functional differences in host-searching behaviour of parasitoids (Vet & van Alphen, 1985; Poolman-Simons et al., 1992; Wiskerke & Vet, 1994). The study described in this thesis was carried out with two congeneric and sympatric parasitoid species that differ in their diet breadth, the braconid endoparasitoids *Cotesia glomerata* and *C. rubecula*. The herbivorous host - the second trophic level - consists of several *Pieris* species (Lepidoptera: Pieridae), and the food plant of the hosts, the first trophic level, are plants belonging to the families Cruciferae, Tropaeolaceae and Rosaceae.

Cotesia glomerata is a generalist at the first and the second trophic level, because its potential hosts attack food plants in the above mentioned families, but also Berberidaceae, Capparidacae, Papilionaceae and Resedaceae (see below).

The *C. rubecula-P. rapae* model is more specific at both trophic levels. *Cotesia rubecula* is considered a specialist of the small cabbage white, *Pieris rapae*, which exclusively attacks plants that contain glucosinolates (precursors of isothiocyanates): Cruciferae, Capparidaceae and Tropaeolaceae (Richards, 1940; Chew, 1980; Fernandez Garcia, 1988).

Hypothesis

The present study specifically aimed to test the hypothesis that infochemical use during host-searching by parasitoids is determined by dietary specialization. For this purpose, a comparison was made of the host-searching strategies of the generalist *C. glomerata* and the specialist *C. rubecula*.

Considering the differences in diet breadth of the two *Cotesia* species and the theory on dietary specialization and infochemical use as described above, it is hypothesized that for *C. glomerata* general information from the hosts' habitat is more important than for the specialist *C. rubecula*. Furthermore, *C. glomerata* is expected to tune its behaviour via learning processes to a larger extent than *C. rubecula*, for which a more congenitally fixed behaviour is expected, adapted to specific stimuli as a result of the close association with its host.

II. THE SYSTEM STUDIED

Biology

Cotesia glomerata

Cotesia glomerata (L.) is a common gregarious larval endoparasitoid of unconcealed Lepidoptera (Laing & Levin, 1982). Adults are about 2.6 mm long (le Masurier & Waage, 1993). Although discrepancies exist in the literature about its host-range (Thompson, 1953; Shenefelt, 1972), it is clear that *C. glomerata* attacks and develops successfully in several species belonging to a few genera of the *Pieridae* (Feltwell, 1982). In Europe the main host of *C. glomerata* is the large cabbage white, *P. brassicae* (Feltwell, 1982). In Britain, it attacks *P. brassicae* and *P. rapae*, but the percentage of *P. rapae* parasitized is much lower than that of *P. brassicae* (Moss, 1933; Richards, 1940; le Masurier, 1987). A similar pattern was found in Germany (Peters, 1992). In the United States it has adapted to attack *P. rapae*, the only host available there (le Masurier & Waage, 1993) where it has become an important parasitoid of this pest species (Harcourt, 1966; Oatman, 1966; Van Driessche, 1988a,b). In Japan, *C. glomerata* is able to parasitize three solitary *Pieris* host species, *P. napi*, *P. rapae crucivora* and *Pieris melete* (Ohsaki & Sato, 1990; 1994).

Females lay approximately 25 eggs in first or second instar larvae, and a close correlation exists between oviposition time and clutch size (Laing & Levin, 1982). Two larval instars develop inside the host and parasitoid larvae egress simultaneously during the latter half of the host's fifth instar and immediately spin cocoons in clusters, that are firmly attached to the leaves. When exposed to short photoperiods (12 h) and low temperatures (15-17°C) *C. glomerata* enters diapause as a prepupa when reared on larvae of *Pieris brassicae* (L.) but as a first instar larva in the diapausing host when reared on larvae of *Aporia crataegi* (L.) (Maslenikova, 1958). Diapause of *C. glomerata* in *P. rapae* was found to be induced in late autumn, before egression from the host, so that overwintering occurs as a prepupa (Tagawa et al., 1984). In The Netherlands critical daylength for diapause is 15 hours (Veerman et al., 1985).

In the laboratory females can live four to five weeks when fed a solution of sugar or honeywater (Laing & Levin, 1982). In the field, they feed on nectar from flowers, but longevity of free living adults is unknown. *Cotesia glomerata* females provided with water and a honey solution lived four to five days only in a cage that was placed in the field. (J. Geervliet, unpubl. results).

Females are ready to mate upon emergence and fertilized females immediately begin to search for hosts (Laing & Levin, 1982). Oviposition activity of females increased in virgin females on the third day after emergence and the rate of oviposition of mated, three day old females was higher than that of virgin females (Kitano, 1978). Fecundity of *C. glomerata* females ranges from 1000-2200 eggs per female (Moiseeva, 1976; Shapiro, 1976).

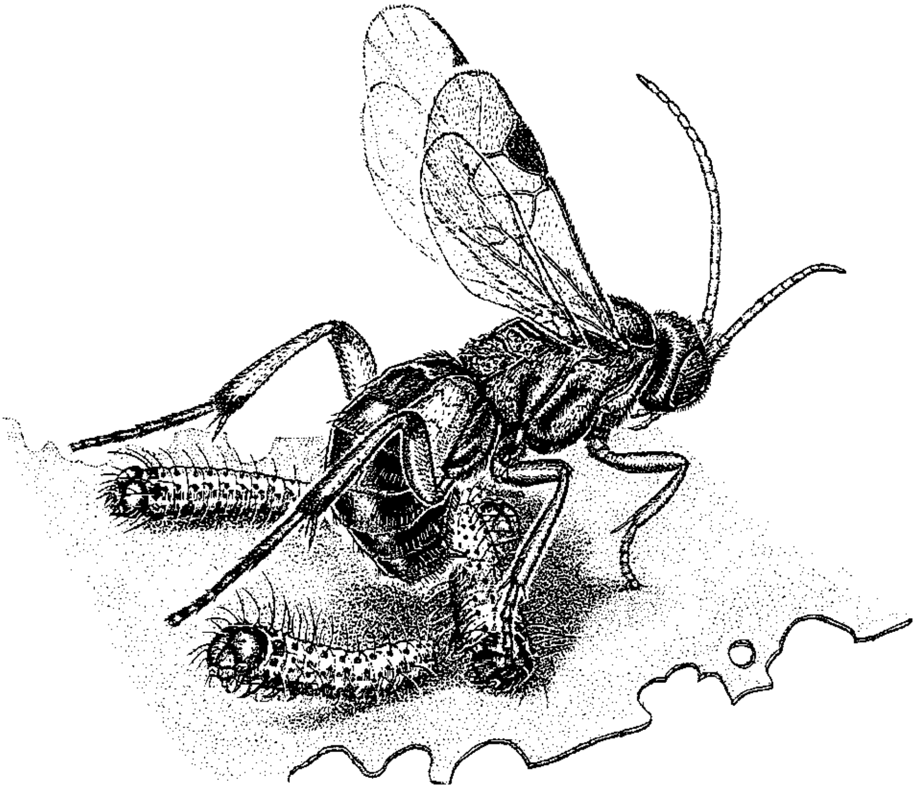

Fig. 2. Cotesia glomerata parasitizing *Pieris brassicae* larvae. Drawing by Piet Kostense.

Cotesia rubecula

Cotesia rubecula (Marshall) is a solitary larval endoparasitoid, native to the Paleartic region. Adults are about 3 mm long (le Masurier & Waage, 1993). It is reported to be host-specific to *Pieris rapae*. The species was originally described by Marshall in 1885 as a solitary parasitoid of *P. rapae* under the name *Apanteles rubecula* (Shenefelt, 1972). According to reports from other parts of Europe, Wilkinson (1945) redescribed the species, adding two more host species, *P. napi* (L.) (Lepidoptera: Pieridae) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Shenefelt (1972) reported eight hosts of *C. rubecula* based on a review of literature. Although rarely, it has been recovered from *P. brassicae* caterpillars in cabbage fields (Richards, 1940) and from *Plutella xylostella* (Peters, 1992). However, despite the great abundance of *Plutella xylostella* in Australia, a South Australian population of *C. rubecula* was only recovered from *P. rapae* (Agelopoulos & Keller, 1994a).

Field studies on *C. rubecula* revealed that parasitoids aggregate in patches with the highest densities of host larvae, but without increase in the rate of attack (Nealis, 1986). The rate of attack was highest where feeding damage by *P. rapae* was highest, irrespective of current host density (Nealis, 1990). Wasps do no readily leave plants that have host feeding damage (Nealis, 1986).

Developmental time from egg to adult ranges from 17 days at 25°C to 47 days at 15°C (Peters, 1992). In the laboratory average longevity of female wasps was 17 days at 20 ± 1°C, with a minimum of 3 days and a maximum of 26 days, and of male wasps 6 days, with a minimum of 2 days and a maximum of 12 days (Peters, 1992). In field cages female wasps lived on average 14 days (J. Geervliet unpublished results).

Newly emerged *C. rubecula* females are able to successfully parasitize *P. rapae* larvae, since they have a load of 30 mature eggs upon emergence and total fecundity increases to 70-110 eggs in 20 days old females (Nealis, 1990).

Parasitization of hosts is restricted to younger larval instars (L1, L2 and L3), since from fourth and fifth instars no wasps could be reared (Parker & Pinnell, 1973; Nealis et al., 1984).

The solitary parasitoid larva egresses from the host larva when it reaches the 4th-instar, when parasitized in L1 and L2 (Puttler et al., 1970), upon which it immediately spins a white cocoon. When parasitized in L3, it can also emerge from the fifth instar (J. Harvey, pers. comm.).

In response to light conditions, *C. rubecula* enters diapause in the eonymph. Critical daylength is 14 hours in Germany (Peters, 1992), less than 13 hours in Canberra, Australia (Nealis, 1985), and 15-16 hours in Vancouver, Canada (Nealis, 1985). There is evidence that the parasitoid's photoperiodic response is largely independent of the host's response. Once diapause is induced, there is an obligatory dormant period of at least two months, after which diapause terminates when specific heat requirements are fulfilled. Parasitoid activity begins almost 6 weeks after that of its host (Nealis, 1985).

In tests of intrinsic competition in the host *Pieris rapae*, the solitary *C. rubecula* was superior to the gregarious *C. glomerata* (Laing & Corrigan, 1987).

Table 1. Main characteristics of the parasitoids *Cotesia glomerata* and *C. rubecula*

Cotesia glomerata	*Cotesia rubecula*
larval endoparasitoid of *Pieris* caterpillars	larval endoparasitoid of *Pieris* caterpillars
hosts on cruciferous and non-cruciferous food plants	hosts mainly on cruciferous food plants
gregarious	solitary
generalist	specialist
adult females approx. 2.6 mm.	adult females approx. 3 mm.
synovigenic	synovigenic
fecundity 1000-2200 eggs	fecundity approx. 100 eggs
parasitizes young host instars	parasitizes various host instars
egression from 5th host instar	egression from 4th host instar

Pieris *hosts and their food plants*

Of the pierid hosts (Lepidoptera: Pieridae) studied in this thesis, *Pieris brassicae* and *P. rapae* are common species in the Netherlands. They have overlapping niches in various biotopes consisting of wild vegetation and cultivated crops (Davies & Gilbert, 1985; Tax, 1989). *Pieris napi* is common in The Netherlands too. Habitat selection differs from that of *P. brassicae* and *P. rapae*, since it is mainly found on wild vegetation (Richards, 1940; Tax, 1989; Ohsaki & Sato, 1990). *Aporia crataegi* is a very rare species in The Netherlands, whose host range differs from that of the other three *Pieris* species, and consists of rosaceous species (Tax, 1989).

P. brassicae and *Aporia crataegi* are gregarious. Female butterflies deposit clusters of up to 100 eggs (Davies & Gilbert, 1985; Tax, 1989) and a great deal of their larval developmental time they feed gregariously. Eggs of the two other host species, *P. rapae* and *P. napi* are laid singly, and larvae feed solitary on the leaves (Richards, 1940). Larvae of the gregarious species are aposomatically coloured, whereas the solitary species are more cryptic in their environment. Caterpillars have four molts and five larval stadia, after which they pupate.

for *P. napi*. Peaks in oviposition flights have a duration of 20-30 days per generation (Smith & Smilowitz, 1976; Tax, 1989). *Aporia crataegi* flies in one generation per year, with an oviposition peak of 15 days (Tax, 1989).

Larval feeding behaviour of *P. brassicae* indicates that this species is adapted to oviposition on clumped vegetation (Davies & Gilbert, 1985). After defoliating a plant, larvae disperse simultaneously to a neighbouring food plant. Advantages of this clustered oviposition strategy are to be found in advantages for egg survival and larval fitness (reduced predator or parasitoid risk) (Morrison & Strong, 1981) and advantages for the ovipositing female (higher released fecundity) (Courtney, 1984; Parker & Courtney, 1984). Females of *P. brassicae* can assess the egg load on a host and thereby avoid committing eggs to a site where competition is likely to be intense (Rothschild & Schoonhoven, 1977).

Pieris rapae and *P. napi* are solitary species, although compared to *P. rapae*, *P. napi* shows a more clustered distribution. *Pieris rapae* is able to spread quickly (Fahrig & Paloheimo, 1986), since they are effective colonizers, because of a short generation time and production of many small eggs (Ohsaki, 1980, 1982). Caterpillars of *P. rapae* are found to be less abundant on host plants that grow in large dense stands, than on host plants that are scattered sparsely in diverse vegetation (Root & Kareiva, 1984), indicating that they behave contrarily to predictions from the resource concentration hypothesis (Root, 1973; Cromartie, 1975). The very mobile *P. rapae* butterflies tend to follow linear flight paths, passing over many suitable hosts, which leads to a scattered distribution of eggs. This species is supposed to be selected for exploiting isolated food plant-plots (Davies & Gilbert, 1985). Although oviposition behaviour of *P. rapae* butterflies can be influenced by oviposition deterring pheromones in the laboratory (Schoonhoven, 1990), they are reported to oviposit on plants that already contain eggs or larvae, which agrees with the observation that it lays a disproportionate number of eggs on peripheral or isolated plants (Jones, 1977; Root & Kareiva, 1984; Davies & Gilbert, 1985).

Pieris napi selects much more permanent habitats than *P. rapae* (Ohsaki & Sato, 1990; 1994).

For *Pieris* butterflies, only plants which contain glucosinolates are recognized as potential food (Chew, 1980). Individual glucosinolates and their hydrolysing products stimulate feeding *Pieris* caterpillars (Verschaffelt, 1911; Schoonhoven, 1967) and oviposition behaviour in *Pieris* adults (Ma & Schoonhoven, 1973; Lundgren, 1975).

Pieris species show different responses to parasitoids. Three different mechanisms are known by which *Pieris* hosts can reduce mortality by *Cotesia* species. Hosts may be able to encapsulate the parasitoid eggs by its haemocytes (Ohsaki & Sato, 1990; Brodeur & Vet, 1995). They can escape parasitoid attack in space and time by dispersing to newly-opened temporary habitats in which parasitoid populations have not yet become established (*P. rapae*) (Ohsaki & Sato, 1990). Thirdly, hosts can use food plants that are less likely to be visited by their parasitiods. For example, *P. napi* uses *Arabis* food plants that are concealed by other plants, this way escaping parasitism by *C. glomerata* (Sato & Ohsaki, 1987).

Host-searching behaviour of C. glomerata *and* C. rubecula.

From a distance *C. glomerata* and *C. rubecula* are strongly attracted to plants with larval feeding damage (Sato, 1979; Laing & Levin, 1982; Nealis, 1990; Kaiser & Cardé, 1992; Steinberg et al. 1992; Wiskerke & Vet, 1994; Mattiacci et al., 1994; Agelopoulos & Keller, 1994a,b). Females of both parasitoid species are able to distinguish from a distance between plants damaged by lepidopterous larvae and those damaged by mechanical means (Steinberg et al., 1993; Mattiacci et al., 1994; Agelopoulos & Keller, 1994a). Mattiacci et al. (1995) demonstrated that the enzyme ß-glucosidase in *P. brassicae* regurgitant is the elicitor of the defense response in cabbage plants to insect herbivory, inducing the emission of volatiles that are used by the parasitoids during host-habitat location.

Cotesia glomerata shows equal motivation to search for and parasitize larvae of different host species (Sato, 1976, 1979; Wiskerke & Vet, 1994). Although *C. rubecula* did not always demonstrate preferences in landing behaviour after flight initiation, females searched cabbage damaged by *P. rapae* more intensely than cabbage damaged by other host or herbivore species (Agelopoulos & Keller, 1994b; Wiskerke & Vet, 1994). Analysis of headspace samples from *Pieris*-infested cabbage plants revealed that differences in volatile profiles between different types of damage were mainly of quantitative nature, rather than qualitative (Blaakmeer et al., 1994; Mattiacci et al., 1994).

Cotesia glomerata and *C. rubecula* are not attracted from a distance to host larvae (Kaiser & Cardé, 1992; Steinberg et al., 1993; Agelopoulos & Keller, 1994a). Chemical analysis showed evidence of *Pieris* specificity related to volatile chemicals emitted by feces of larvae (Agelopoulos & Keller, 1994c; Agelopoulos et al., 1995), although plant damage was more important to the searching females than host feces in the long-distance searching phase. The blend of chemicals emitted by feces of different instars of *P. rapae* and different *Pieris* species exhibited an instar and species

Table 2. Host plants of three *Pieris* species recorded from literature (*) and field observations (f).

	Host plant species	***P. brassicae***	***P. rapae***	***P. napi***	***Aporia crataegi***
*	**Berberidaceae**				+
	Capparidaceae				
*	*Capparis* sp.	+			
*	*C. spinosa*	+			
*	*Cleome spinosa*	+	+		
*	*C. lutea*		+		
*	*Polonisia trachysperma*	+			
*	*Steriphana paradoxum*	+			
	Cruciferae				
*	*Alliaria* sp.			+	
*	*A. officinalis*		+		
*f	*A. petiolata*	+	+	+	
*	*Alyssum* sp.		+		
*	*A. saxatile*	+			
*f	*Arabis* sp.		+	+	
*f	*A. alpina*	+		+	
*	*A. drummondii*		+	+	
*	*A. flagellosa*			+	
*	*A. gemmifera*			+	
f	*A. glabra*		+		
f	*A. hirsuta*		+	+	
f	*Arabidopsis thaliana*	?	+		
*	*Aubrieta* sp.		+		
*	*A. deltoides*	+			
*f	*Barbarea* sp.		+	+	
*	*B. stricta*	+			
*f	*B. vulgaris*	+	+	+	
*	*Biscutella* sp.			+	
*	*B. auriculata*	+			
*	*B. laevigata*	+			
*f	*Brassica* sp.	+	+	+	
*f	*B. juncea*	+	+	+	
*f	*B. napus*	+	+		
*f	*B. nigra*	+	+	+	
*f	*B. oleracea* var.	+	+	+	
*f	*B. rapa*	+	+	+	
*	*Bunias orientalis*	+			
*	*Cakile* sp.	+	+		
*	*C. maritima*	+			
*	*Capsella bursa-pastoris*	+			
*f	*Cardamine* sp.			+	
f	*C. amara*			+	
*	*C. cordiofolia*			+	
*f	*C. hirsuta*	+			
*f	*C. pratensis*		+	+	
*	*Cardaria (Lepidium) draba*	+			
*	*Cheiranthus* sp.	+			
*f	*C. cheiri*	+			
*f	*Cochlearia* sp.	+	+		
*f	*C. armoracia* (= *Armoracia rusticana*)	+	+		
f	*C. officinalis*		+		
*	*Crambe cordifolia*	+			
*f	*C. maritima*	+			
*	*Descurainia richardsonii*			+	
*	*D. sophia*		+		
*f	*Diplotaxis* sp.		+	+	

Table 2. (continued) Host plants of three *Pieris* species recorded from literature (*) and field observations (f).

	Host plant species	***P. brassicae***	***P. rapae***	***P. napi***	***Aporia crataegi***
*	*D. erucoides*	+			
*f	*D. muralis*	+	+		
*f	*D. tenuifolia*	+	+		
*	*D. virgata*		+		
*	*Draba aurea*		+	+	
*	*D. incana*	+			
*	*Eruca sativa*	+			
*	*Erucastrum nasturtifolium*	+			
*f	*Erysimum* sp.		+	+	
f	*E. cheiranthoides*			+	
*	*E. hieraciifolium*	+			
*	*E. perofskianum*	+			
*f	*Hesperis matronalis*	+	+	+	
*	*Hirschfeldia (Sinapis) incana*	+	+		
*	*Hornungia petraea* (= *Hutchinsia alpina*)	+			
*f	*Iberis amara*	+			
*	*I. gibraltaria*	+			
*	*I. umbellata*	+			
*f	*Isatis tinctoria*	+			
*	*Kernera saxtilis*	+			
*	*Lepidium* sp.	+	+	+	
*	*L. latifolium*	+	+		
*	*L. densiflorum*			+	
*	*L. campestre*		+		
*	*L. sativum*	+			
*	*L. virginicum*		+		
*	*Lobularia (Clypeola) maritima*	+			
*f	*Lunaria* sp.	+	+		
*f	*L. annua*	+	+		
*	*Matthiola* sp.	+	+		
*	*M. annua*	+			
*	*M. incana*	+			
*f	*Nasturtium officinale*	+			
*	*Peltaria turkmeria*	+			
*f	*Raphanus* sp.	+	+	+	
*f	*R. sativus*	+	+	+	
*f	*R. raphanistrum*	+	+		
*	*Rorippa* sp.				
f	*R. amphibia*	+	+	+	
*	*R. indica*		+		
*	*R. islandica*			+	
*	*R. nasturtium-aquaticum*	+			
*f	*R. sylvestris*	+	+	+	
*f	*Sinapis* sp.		+	+	
*f	*S. alba*	+	+	+	
*f	*S. arvensis*	+	+	+	
*f	*Sisymbrium* sp.	+	+	+	
*f	*S. officinale*	+	+	+	
*	*S. sophia*	+			
*	*S. strictissimum*	+			
f	*Thlaspi alpestre*		+	+	
*f	*T. arvense*	+	+	+	
*	*T. montanum*		+	+	
*	*T. rotundifolium*	+			
	Adv. Cruciferae				
*	*Thelypodium affine*		+		
	Papilionaceae				
*	*Genista alba*	+			
*	*G. tinctoria*	+			
*	*Glycine soja*	+			
*	*Lathyrus latifolius*	+			
*	*L. sylvestris*	+			

Table 2. (continued) Host plants of three *Pieris* species recorded from literature (*) and field observations (f).

	Host plant species	***P. brassicae***	***P. rapae***	***P. napi***	***Aporia crataegi***
*	*L. tuberosus*	+			
*	*Medicago sativa*	+			
*	*Pisum* sp.	+			
*	*Vicia* sp.	+			
*	*V. cracca*	+			
	Resedaceae				
*	*Caylusea abyssinica*	+			
*	*Reseda* sp.	+	+	+	
*	*R. alba*	+			
*	*R. lutea*	+			
*	*R. luteola*	+			
*	*R. odorata*	+			
*	*R. virgata*	+			
	Rosaceae				
*	*Crataegus* sp.				+
*	*Prunus spinosa*				+
*	*Pyrus communis*				+
*	*Sorbus aucuparia*				+
	Tropaeolaceae				
*f	*Tropaeolum* sp.	+	+	+	
*	*T. brasiliense*	+			
*f	*T. majus*	+	+	+	
*	*T. peregrinum*	+			

References: Bowden, 1971; Chew, 1980; Feltwell, 1982; Fernandez Garcia, 1988; Forsberg, 1987; Hovanitz, 1969; Renwick & Radke, 1985; Sato & Ohsaki, 1987; Tax, 1989; Terofal, 1965.
Field data were obtained by Dr. J.J.A. van Loon in 1995 in the surroundings of Wageningen.

specificity in both quantity and quality, which is reflected in preferences of *C. rubecula* in behavioural studies (Agelopoulos et al., 1995).

Olfactory stimuli emitted by the host itself or by host by-products such as frass or silk stimulate searching behaviour of *C. glomerata* and *C. rubecula* after arrival on the plant (Sato, 1979; Nealis, 1986; Mattiacci & Dicke, 1995a). To *C. glomerata* wasps volatile chemicals emitted by plants infested by the more suitable first instar *P. brassicae* larvae were equally attractive as volatiles from plants infested by fifth instar larvae (Mattiacci & Dicke, 1995a). However, wasps can discriminate between stimuli from first and fifth instar *P. brassicae* larvae by exploiting instar-related cues such as cues on the margin of feeding damage, silk and frass (Mattiacci & Dicke, 1995b). Under laboratory conditions *C. glomerata* can distinguish previously parasitized hosts from healthy ones. Parasitization in parasitized larvae may occur, but the size of the second clutch is reduced by approximately 50% (Kusano & Kitano, 1974).

Visual stimuli play a role in host-searching behaviour of *C. glomerata* and *C. rubecula* females. Laboratory studies showed that both species use visible leaf damage, such as holes in the leaves, during their search for hosts (Wäckers, 1994; Verdegaal, Wäckers & Vet, in prep).

III. RESEARCH OBJECTIVES

The three main objectives of this research were to determine 1) the specificity of the information used by the generalist *C. glomerata* and the specialist *C. rubecula*, 2) the variability in behavioural responses of both parasitoid species as a result of learning, and 3) the fitness consequences of the host-searching decisions of the *Cotesia* species through a correlation of host-searching and host suitability.

Specificity of stimuli

The first objective was to determine the specificity of the information used by females of *C. glomerata* and *C. rubecula*. When parasitoids are able to respond to specific stimuli related to preferred hosts on a suitable food plant, and thus to discriminate between habitats that differ in profitability, we expect this to enhance their host-searching effectiveness. For *C. glomerata* the relative importance of stimuli from different trophic levels was already studied (Steinberg et al., 1993). For *C. glomerata* the rank in preference for stimuli was: plant-host complex with feeding larvae > herbivore-damaged plants = artificially damaged plants > host faeces = host larvae > uninfested cabbage. To elucidate the specificity of stimuli used by *C. rubecula* a comparable study was carried out (chapter 2). For *C. glomerata* and *C. rubecula* odor preferences for certain plant-host combinations were determined (chapter 3). Naive parasitoids were given the choice between different herbivore species and between different plant species and cultivars in wind tunnel experiments. Part of the experiments described in chapter 7 determined whether naive females of *C. glomerata* and *C. rubecula* are able to distinguish different host species or plant species after landing, upon contacting herbivore-damaged leaf material. A comparison was made of search times on leaves damaged by different herbivore species and of search times on leaves of different food plants infested by a certain herbivore species.

Volatiles from herbivore-damaged plants are an important source of information for parasitoids searching for these herbivores. Variation in headspace composition that is affected by herbivore species is relevant to the parasitoid female, since it provides information on the herbivore's identity (Vet & Dicke, 1992). This may also be true for variation due to plant species or cultivar, because the plant may affect the herbivore's suitability for parasitoid development (Godfray, 1994). To elucidate the type and degree of variation that natural enemies face, it is important to study the variation in headspace composition among plant species and plant cultivars infested by the same herbivore species, as well as among plants of one cultivar that are infested by different herbivore species (Dicke et al., 1990b; Turlings et al., 1993; Takabayashi et al., 1991a, 1994b; Takabayashi & Dicke, 1996). To understand the mechanism underlying the results of behavioural studies in chapter 2 and 3, the headspace profiles of three food plants of *Pieris* hosts, either undamaged or damaged by *P. rapae* or *P. brassicae*, were compared and discussed in chapter 4.

Chapter 5 describes the host acceptance behaviour of *C. glomerata* and *C. rubecula*. The study intends to determine the extent to which host-acceptance behaviour, as related to host species, age and defensive behaviour might explain the differences in host use that exist between the two *Cotesia* species. Host specificity of parasitoids is generally thought to reflect phylogenetic differences and to be determined by both the physiological capacity of the immatures to survive and exploit the host and the behavioural ability of the adults to find the resources. Parasitoids are strongly constrained by the physiological suitability and nutritional value of the host, as reflected by immature survival and overall adult fitness components (Slansky, 1986). Hosts also differ in spatial and temporal availability as well as in detectability for parasitoids (Vet & Dicke, 1992). The expression of host use by parasitoids can furthermore be affected by a variety of other determinants, such as the effectiveness of behavioural defenses of the host and the presence of the parasitoid's natural enemies or competitors.

Variability in infochemical use as a result of learning

Animals can adapt to varying circumstances through behavioural plasticity. To maximize their host-finding success, parasitoids adjust their behaviour to changing availability and quality of hosts. The second objective of this thesis was to compare this capacity for behavioural adjustment through learning between the two parasitoid species. In chapter 6 the role of preadult learning was studied by investigating the effect of the host's diet on the parasitoid's innate preference for odours of plant-host complexes. Furthermore, the importance of adult preference learning was investigated. The first part of chapter 7 discusses the influence of a period of habitat-sampling on the ability of *C. glomerata* and *C. rubecula* to distinguish plants infested with different host species.

The experiments described in chapter 8 determined how naive and experienced females of *C. glomerata* and *C. rubecula* deal with volatile cues of patches that differ in profitability, i.e. host encounter rate.

Host suitability

A better insight in the host-searching strategies of parasitoids should include the suitability and availability of the hosts for the development of the parasitoid larvae. The third objective of this study was to determine the correlation between the host-searching decisions of parasitoid females and the suitability of a potential host, i.e. the parasitoid's fitness. In chapter 9 the question was put forward whether the female's host-searching decisions and oviposition preferences are reflected in the expected fitness of her offspring. The capacity of *C. glomerata* and *C. rubecula* to parasitize three *Pieris* species was determined in the laboratory by measuring several important life-history components such as survival, development, sex ratio and size of parasitoid progeny.

Field populations

Field observations are essential to validate the conclusions from laboratory studies and to determine whether the parameters measured in the laboratory are the most relevant ones.

In the Netherlands, *C. glomerata* and *C. rubecula* occur together in the same habitats (J. Geervliet, J. Brodeur, unpublished results), where they share host resources, since their *Pieris* hosts have overlapping niches (Terofal, 1965). Furthermore, both species attack *P. rapae* larvae and a study by Laing & Corrigan (1987) suggests that there may be fitness penalties for *C. glomerata* to parasitize *P. rapae*, due to competition with *C. rubecula*. In case of multiparasitism of *P. rapae*, *C. glomerata* is outcompeted by *C. rubecula* in most of the cases. Hence, field experiments are needed to investigate the ultimate performance of the two *Cotesia* species under more natural circumstances. In chapter 10, field studies are described that determine the outcome of parasitization of three *Pieris* host species by natural populations of the two parasitoid species. This study investigated whether parasitization reflects diet breadth and varies with host species and with the food plant of the caterpillars. Furthermore, suitability of different food plants for parasitoid development was investigated and compared to host-searching decisions by *C. glomerata* and *C. rubecula*.

This study intends to increase ecological and evolutionary insight into a complex sub-ecosystem, i.e. the community of plants, pierids and parasitoids.

I. VOLATILES AND HOST-SEARCHING BEHAVIOUR

2

VOLATILES FROM DAMAGED PLANTS AS MAJOR CUES IN LONG-RANGE HOST-SEARCHING BY THE SPECIALIST PARASITOID *Cotesia rubecula*.

ABSTRACT

The role of volatile stimuli in the long-range host-searching behaviour of the specialist parasitoid *Cotesia rubecula* Marshall (Hymenoptera: Braconidae) was studied. Components from the plant-host-complex Brussels sprouts (Brassica oleracea L. var. gemmifera (DC.) Schulz. cv. 'Titurel') - *Pieris rapae* L. (Lepidoptera: Pieridae) were compared for their attractiveness in dual choice tests in a wind tunnel. Stimuli from cabbage plants that were mechanically damaged or damaged by *P. rapae* caterpillars were more attractive to this parasitoid species than stimuli emitted by the host larvae or their faeces. Parasitoids preferred leaves from the plant-host-complex over artificially damaged leaves. Undamaged cabbage plants were the least attractive to the foraging females. These results indicate that in-flight searching behaviour of *C. rubecula* is guided by plant-derived information and that for this specialist species more reliable and specific host-derived cues play a minor role at longer distances.

INTRODUCTION

Successful parasitism by parasitoids of herbivorous insect hosts is preceded by several phases of host searching that lead females into the close vicinity of their potential hosts (Vinson, 1975; 1976). In each of these phases, host-searching behaviour is often characterized by responses to various types of stimuli (Price, 1981; Wäckers & Lewis, 1994). Chemical stimuli emitted by members of the different trophic levels in the parasitoid's environment, such as the herbivorous host or the host's foodplant, are often used in the host-searching process (Price, 1981). Host-derived stimuli are the most reliable indicators of the presence of hosts, but they may be hard to detect at long distances, whereas plant-derived stimuli are assumed to be more detectable (Vet et al., 1991; Vet & Dicke, 1992).

Since successful foraging is directly linked with reproductive success, natural selection will favour animals that make optimal use of foraging cues. This means that parasitoids should respond at various distances to the most reliable cues that can be detected. For the long-range searching phase an efficient strategy may be to respond to herbivore-induced synomones, chemicals that are produced by the plant upon feeding damage caused by the herbivores. In addition to being highly detectable, these herbivore-induced synomones may also be reliable, through the specific interaction of the plant with the herbivore (Dicke et al., 1990a,1990b; Turlings et al., 1990; Vet & Dicke, 1992).

The role of stimuli from different trophic levels in various phases of the host-searching process, is hypothesized to be determined by the parasitoid's degree of specialization on the first and second trophic level (Vet & Dicke, 1992). The behaviour of specialists may be better adapted to specific stimuli from the plant-host complex as a result of the close association with their hosts. This is likely to be expressed in their innate behavioural responses to host-derived cues, such as herbivore products (faeces, silk) and the herbivore itself, or to specific herbivore-induced synomones. For generalists such fixed responses to specific stimuli do not seem to be functional or may be impossible due to more physiological constraints compared to specialists. Instead, they are hypothesized to be guided to their hosts by more general stimuli at first and subsequently learn to respond to more specific stimuli (Vet & Dicke, 1992). Comparative studies of phylogenetically closely related species that differ in their degree of specialization can be used to test the hypothesis that the degree of specialization affects foraging strategies (Vet & Dicke, 1992; Wiskerke & Vet, 1994; Poolman-Simons et al., 1992). Analogous to a previous study on the generalist species *Cotesia glomerata* (Steinberg et al., 1993), females of the closely related specialist parasitoid species *C. rubecula* were subjected to long-range (1 m) host-searching experiments. The gregarious *Cotesia glomerata*, attacking several pierid species on cruciferous and non-cruciferous host plants, is considered to be a generalist, whereas *Cotesia rubecula*, a solitary endoparasitoid, is specific to *Pieris rapae*, that mainly feeds on cruciferous food-plants (Shenefelt, 1972; Laing & Levin, 1982). At long distances *C. rubecula* is attracted to odours emanating from plants infested by host larvae (Kaiser & Cardé, 1992; Wiskerke & Vet, 1994). Olfactory stimuli from host larvae themselves are supposed to have an arrestant effect and stimulate foraging females to search for hosts after landing (Nealis, 1986). However, the exact origin of the stimuli that affect the long-range host-searching behaviour of this parasitoid species is unknown. Responses of individual females to stimuli from components of the first and second trophic level of the cabbage - *Pieris* complex were measured in a wind tunnel. We studied the relative importance for the long-range host-searching process of stimuli from the host larvae and their frass, of stimuli from the plant-host-complex, either with or without feeding caterpillars, and of stimuli from mechanically damaged plants.

MATERIALS & METHODS

Insects - *Cotesia rubecula* was reared on *Pieris rapae* larvae in a climatic room at 20-22^{0}C, 50-70% r.h. and a L16:D8 photoregime. *P. rapae* larvae were obtained from a colony maintained on Brussels sprouts plants (*Brassica oleracea gemmifera* cv. Titurel) under the same climatic conditions. Both parasitoid- and host colonies originated from individuals collected in Brussels sprouts fields in the vicinity of Wageningen. Parasitoids used in the bioassays were 3-7 days old, mated females. As a pre-flight treatment, females had contacted leaf tissue only, recently damaged by first instar larvae, 16-24 h prior to testing to enhance responsiveness. After this treatment, wasps were kept individually in glass vials closed with a cotton-wool plug and supplied with honey, at 15°C overnight and transferred to the wind tunnel room 1 h before testing.

Odour sources - Odour sources were based on 3 months old individually potted Brussels sprouts plants bearing 8-10 leaves, reared under greenhouse conditions (22-26°C, 16L:8D photoregime, 50-60% r.h.). To obtain the different odour sources, which are components of the plant-host complex, the method described by Steinberg et al. (1993) was adapted to the solitary life-style of the *P. rapae* larvae. A plant-host-complex (PHC) consisted of a Brussels sprouts plant with 25-30 neonate *P. rapae* larvae, that had been feeding for 24 hours, and their by-products such as silk and faeces. Larvae were distributed over three leaves, each leaf bearing 8-10 larvae. A herbivore-damaged plant (HD) was a PHC plant from which larvae, faeces and silk had been removed with a paintbrush just prior to testing. An artificially damaged plant (AD) was obtained by punching three groups of three holes per leaf (each hole two mm diameter), in three leaves. This treatment was started 24 h before testing, to initiate volatile production at the same moment as in the PHC- and HD-treatments. During the bioassay, continuous damage was simulated by repeatedly (every 15 min) punching one extra hole per group of holes, to ensure a release of AD-volatiles that was continuously increased relative to undamaged cabbage leaves (see Steinberg et al., 1993). The faeces (F) and larval (L) odour sources were prepared 24 hours before testing by punching a group of three small holes in three seperate leaves of a clean cabbage plant, through which air could pass and to control for visual cues. Testing responses of females to these plants 24 hours later revealed no attractiveness, which was also shown in the study of Steinberg et al. (1993). The faeces odour source (F) was obtained by placing the plant adjacent to a PHC, with the three prepared leaves positioned under three infested leaves of the PHC. Larval faeces dropped on the leaves and the amount of faeces collected was equivalent to what was produced by the larvae used in the other treatments. A plant with non-feeding larvae (L) was acquired by transferring 25-30 first instar larvae from the PHC to the three prepared leaves just prior to testing. Per leaf 8-10 larvae were placed inside of a ring of tanglefoot glue, on a piece of fine mesh gauze, that was attached to the underside of the leaf with insect pins. The gauze and the glue had been applied to the leaves 24 h before testing. For tests with the odours of the non-feeding larvae also F, CC and PHC leaves were equipped with the gauze, the insect pins and the glue to control for the damage and possible odours emitted by the glue. The undamaged cabbage (CC) control odour source was taken from the greenhouse. Prior to testing, of each plant the three treated leaves were cut from the plants with a surgical knife and immediately transferred to Erlenmeyer flasks that were filled with fresh tap water. When necessary, a few days before use, plants were treated with DDVP against aphids.

Bioassay - Dual choice tests were carried out in a wind tunnel that was constructed for daytime-active, flying insects (fig. 1). Outside air was filtered over glasswool and led over a humidity controlled air humidifier. Subsequently the air was pressurised, filtered for a second time over an active charcoal filter and passed through a temperature controlled heating system. Finally the laminar air-flow was led into the wind tunnel. The polyacrylate flight compartment of the set-up measured 200x60x60 cm. Both ends of this compartment were covered with fine mesh copper gauze and in the sidewall at the observer's side three gates could be opened for handling purposes.

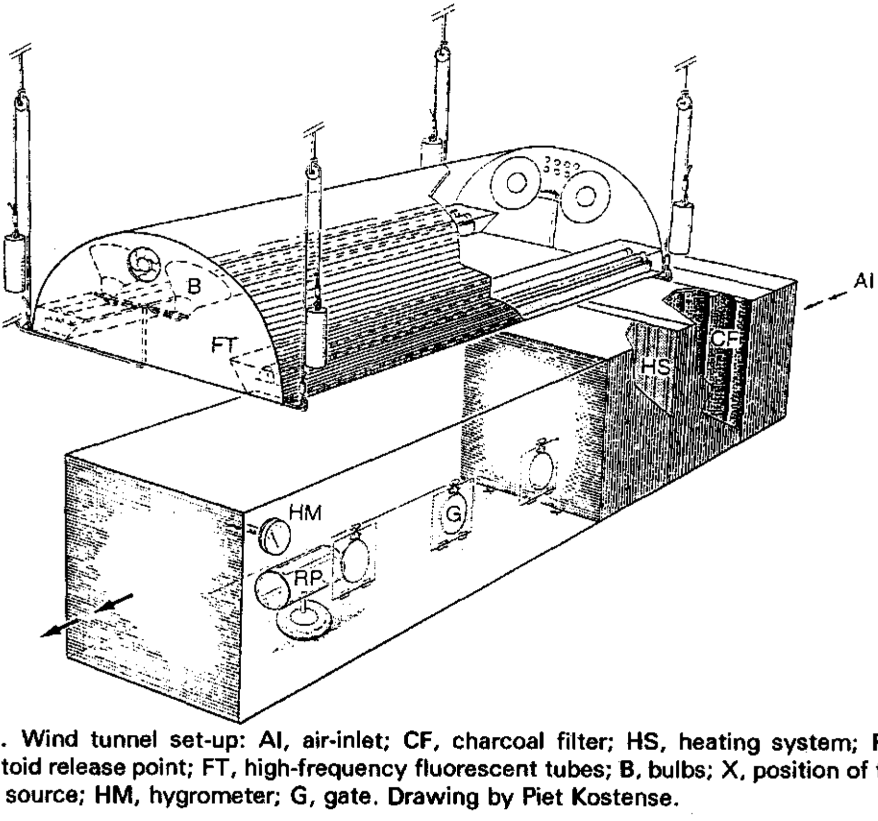

Fig. 1. Wind tunnel set-up: AI, air-inlet; CF, charcoal filter; HS, heating system; RP, parasitoid release point; FT, high-frequency fluorescent tubes; B, bulbs; X, position of the odour source; HM, hygrometer; G, gate. Drawing by Piet Kostense.

The light source used was constructed of one half of a concave metal cylinder, hanging over the flight-compartment. For light dispersion, the inner side was covered with designed aluminium plates ('stucco'design, van Houten b.v., Ridderkerk). Inside the frame 8 fluorescent high-frequency tubes (Philips TLD 32W/84HF) and 4 bulbs (Philips Softtone 200W) were mounted. Light intensity in the flight compartment during testing was 1700 lux (2.5 $W.m^{-2}$) on the bottom, increasing to 2000 lux (2.9 $W.m^{-2}$) at the ceiling. Inside the wind tunnel, temperature varied between 21-23°C, windspeed was held constant at 20 cm/s and the relative humidity fluctuated between 40% and 60%.

The parasitoid's release site consisted of a horizontal glass cylinder (30 cm long, 15 cm diameter) with both ends open, placed on a socket of 10 cm height at a distance of 1 m from the odour sources. This cylinder was used to prevent wasps from immediately flying towards the ceiling, and keep them in the air-flow. The opened vial with an individual female was placed on the bottom of the release site and females readily walked out of the vial, initiated flight and could choose between the two odour sources at the upwind end of the wind tunnel. In all treatments the visual component of the damaged leaves (i.e. small holes) was equal, except for the undamaged cabbage leaves. Preliminary wind tunnel studies with plants in closed containers, however, showed that *C. rubecula* responds to chemical stimuli without visual cues being present. Furthermore, visual orientation in *C. rubecula* is overruled by volatiles from herbivore damaged plants (Wäckers, 1994). Different treatments were alternated after every two tested wasps. Care was taken to offer all types of odour sources of each series in a random order, and to switch the position of the odour sources per combination left-to-right or the reverse. Complete oriented flights that ended in landings on one of the two odour sources were recorded as 'choice'. Landings elsewhere in the set-up or wasps that did not take-off were recorded under 'no response'. Tests lasted until a parasitoid landed, with a maximum of five minutes after flight initiation. Each parasitoid was offered only one choice situation and within a test given one flight opportunity only.

Experimental design - Behaviour of parasitoids was examined in the following combinations. Artificially damaged leaves (AD) were tested against two alternative choices: undamaged cabbage leaves (CC) and leaves from the plant host complex (PHC). Herbivore-damaged leaves (HD) were offered together with undamaged cabbage leaves, artificially damaged leaves or leaves from the plant host complex. The faeces odour source (F) and larval odour source were offered together with uninfested cabbage leaves or a plant-host-complex. As an additional test, leaves from the PHC were offered against CC leaves. Treatments were performed over several consecutive days, 6-8 wasps a day per treatment.

Statistical analysis - Choices for odour sources were analysed using binomial probability functions. The results indicated the attractiveness of the odour sources within each choice test. Responsiveness among the choice tests was evaluated by a test for independence with Yates correction (Sokal & Rohlf, 1981).

RESULTS

Plant-host-complex - The complete PHC vs CC test yielded a response of 61.4%. Of the 44 parasitoids tested, 27 landed on the PHC leaves, whereas no landings occurred on the uninfested leaves (Fig. 2a).

Artificially damaged plants - Figure 2b shows that almost all responding parasitoids preferred the AD leaves over the CC leaves (P=0.000); only one landed on the uninfested leaves. Females of *C. rubecula* discriminated between the PHC leaves and the AD leaves, and showed a significant preference for the PHC leaves (P=0.033). The response levels of the parasitoids were equally high in both tests: 63.6% and 61.4% of the females made a choice in the AD vs. CC test and the AD vs. PHC test respectively. The presence of the plant host complex (PHC) in the latter choice situation did not lead to an increase of the responsiveness of the parasitoids compared to the choice situation without host-infested leaves.

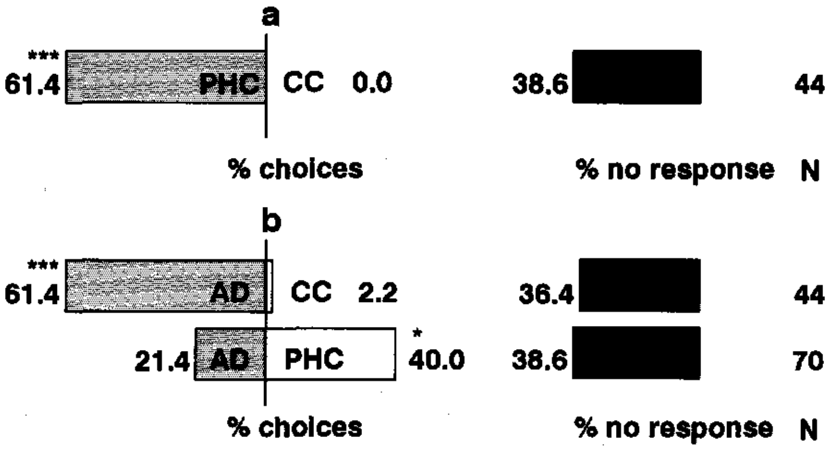

Fig. 2. Responses of *Cotesia rubecula* females to leaves from the plant-host-complex cabbage-*P. rapae* tested against undamaged leaves (a) and to artificially damaged leaves (AD) tested against two control odour sources: uninfested cabbage (CC) and a plant-host-complex (PHC) respectively (b). Asterisks indicate a significant difference within a choice test: *** P<0.0005; * P<0.05. Numbers next to bars correspond to percentage of parasitoids. Numbers at right-hand side of figure (N) indicate number of females tested.

Herbivore-damaged plants - A herbivore-damaged odour source attracted a significantly higher number of parasitoids than did the CC odour source (P=0.000)(Fig.3). When HD was tested against AD, *C. rubecula* did not show a significant preference for either one of the two odour sources offered (P=0.056). The parasitoids did not discriminate between the HD leaves and the PHC leaves (P=0.276). Analysis of responsiveness revealed no differences among tests. Parasitoids responded equally well in all three choice situations: 54.5%, 68.6% and 64.3% of the females respectively made a choice. The presence of AD or PHC next to HD leaves did not affect the responsiveness of the parasitoids in comparison to the HD vs. CC test.

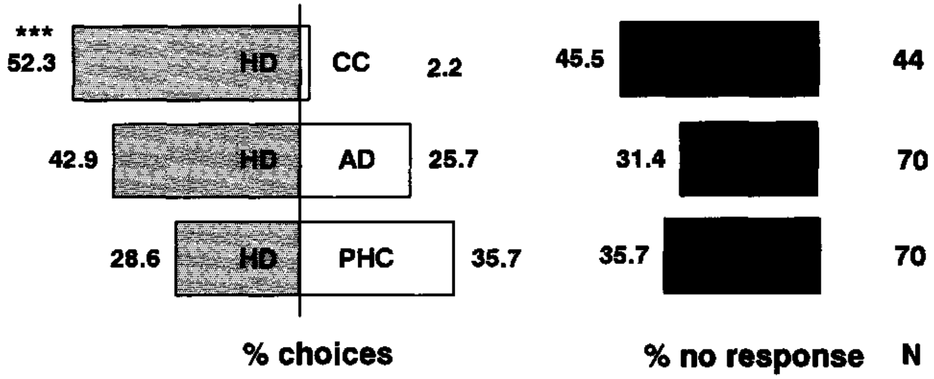

Fig. 3. Responses of *Cotesia rubecula* females to leaves from herbivore-infested plants (HD) tested against three controls: uninfested cabbage (CC), artificially damaged plants (AD) and a plant-host-complex (PHC) respectively. *** indicates a significant difference within a choice test P<0.0005. Numbers next to bars correspond to percentage of parasitoids. Numbers at right-hand side of figure (N) indicate number of females tested.

Faeces and host larvae - More *C. rubecula* females landed on leaves with faeces (F) than on uninfested leaves (CC) (P=0.029)(Fig. 4). Parasitoids did discriminate between the F odour source and the PHC leaves and showed a clear preference for the latter (P=0.000). The larval odour source (L) was more attractive to the parasitoids than uninfested leaves (P=0.033), while in the L vs. PHC test the PHC odour source attracted significantly more parasitoids than the leaves with non-feeding larvae (P=0.026). In the tests where faeces or larvae were offered against uninfested leaves, the response levels of the parasitoids were significantly lower (36.8% and 28.9%) than in the tests where PHC was present. The F vs. PHC test yielded 71.8% responding females, and in the L vs. PHC test 57.9% of the parasitoids responded.

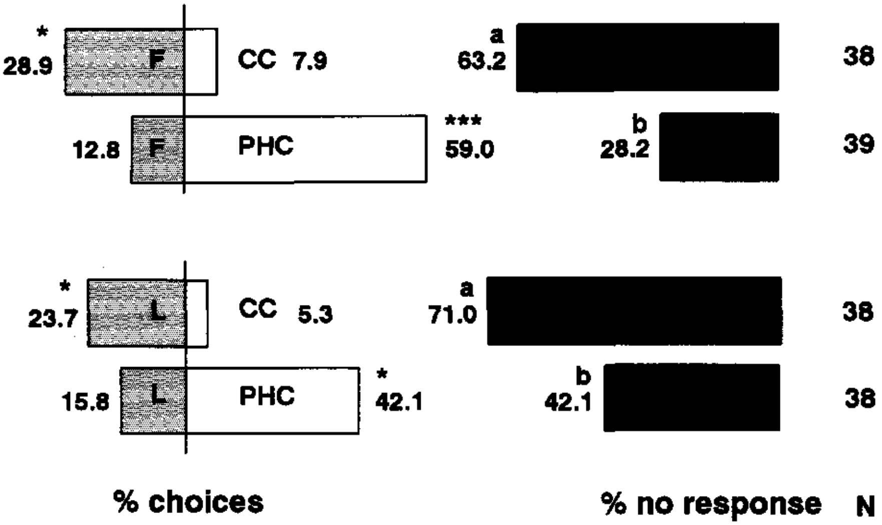

Fig. 4. Responses of *Cotesia rubecula* females to larval faeces (F) and non-feeding larvae (L) tested against two controls: uninfested cabbage (CC) and a plant-host-complex (PHC) respectively. Astersks indicate a significant difference within a choice test: *** $P<0.0005$; * $P<0.05$. Numbers next to bars indicate percentage of parasitoids. Lowercase characters left of no-response bars indicat significant difference within a pair; test for independence, $P<0.05$. Numbers at right-hand side of figure (N) indicate number of females tested.

DISCUSSION

Of all six odour sources tested, the uninfested cabbage leaves (CC) are the least attractive to *C. rubecula* females. Parasitoids did not distinguish between *P. rapae* infested leaves with feeding larvae (PHC) or leaves from which the larvae and larval by-products had been removed (HD). The PHC was more attractive to the parasitoids than the AD leaves, but just no significant preference was found for HD leaves over AD leaves ($P=0.056$). In our study leaves were artificially damaged at regular intervals of 15 min, to ascertain a release of volatiles that was continuously increased relative to the other leaves tested. Larger amounts of damaged plant tissue in this treatment may have caused quantitative differences in the release of volatiles during testing and thus an overestimation of the effect of AD. In all cases, the parasitoids preferred leaves with any damage over uninfested cabbage leaves (CC), with a comparable degree of responsiveness in the tests. Leaves with larval faeces (F) or non-feeding larvae (L) were more attractive to the parasitoids than the uninfested cabbage leaves, but they were far less attractive than the plant-host complex. Moreover, the percentage response was significantly lower when F or L were offered versus CC than when offered versus PHC. The different response levels in the respective choice tests suggest that F and L odour sources are less attractive than AD leaves. Whether F and L are equally attractive remains to be tested. Considering the results of our study, the following rank order in attractivity of the infochemical sources to *C. rubecula* was created: PHC = HD = AD > F,L > CC.

Like in our study, females of the related generalist species *C. glomerata* significantly preferred damaged plants (AD, HD and PHC) over clean cabbage plants and did not distinguish between HD and AD (Steinberg et al., 1993). The only difference between the generalist and the specialist species is, that the generalist showed a preference for the PHC plant over the herbivore damaged plant (HD), which was not found for *C. rubecula*. For *C. glomerata* a slightly different rank in preference was found to be PHC > HD = AD > F = L > CC (Steinberg et al. 1993). The herbivore used in their study was *Pieris brassicae*, the preferred host of *C. glomerata*.

This is the third study on a *Cotesia* species that shows this hierarchy in the role of volatile stimuli for the long-range host-searching behaviour. For the species *C. glomerata* (Steinberg et al., 1993) and *C. marginiventris* (Turlings et al., 1990), both being generalists on the first and the second trophic level, host-damaged plants play the most important role, followed by mechanically damaged plants, and host-derived stimuli such as faeces and larvae are less important. The specialist parasitoid *Microplitis croceipes* also preferred PHC odours over faecal odours in single- and dual choice situations in a wind tunnel (McCall et al., 1993), although for this species also long-range attraction to faeces of it's host *Heliothis zea* has been reported (Eller et al., 1988).

Considering the hypothesis, that the parasitoid's rate of specialization determines the relative role of stimuli from the first and second trophic level, in the long-range host-searching process females of the specialist *C. rubecula* appear to respond mainly to plant-derived stimuli, instead of using specific host-derived stimuli from faeces or larvae. Furthermore, only minor differences in the relative importance of stimuli from different trophic levels were found between the specialist *C. rubecula* and the generalist *C. glomerata*. This resemblance in the use of stimuli from the first and second trophic level by the two related species can be explained by a lack of specificity in the emission of volatiles by damaged cabbage plants. Comparisons of the composition of volatile blends of different plant-herbivore complexes show that the specific terpenes, the most often reported herbivore-induced attractants (for review see Dicke, 1994), were not recorded in the volatile blends of mechanically damaged cabbage plants or cabbage plants infested by either of the herbivore species, *P. brassicae* or *P. rapae* (Blaakmeer et al., 1994; Mattiacci et al., 1994). Discrimination between differently damaged cabbage plants through olfaction can be impossible, due to too subtle qualitative and quantitative differences in volatile compositions. Differences between blends emitted by mechanically damaged and *Pieris* infested cabbage plants were mainly of quantitative nature. These quantitative differences may have caused the preference of the parasitoids for PHC leaves over AD leaves, that was found for *C. rubecula* (our study) and *C. glomerata* (Steinberg et al. 1993). The cabbage plants respond to damage with an increased release of volatiles (Mattiacci et al., 1994). It is not to be expected that all plant species have the same degree of specificity in their response to herbivore attack. Some plants respond in a different way to different herbivore species or types of damage, while other plants, like cabbage, exhibit a more general response, dependent on the relative investment in defense responses (Dicke, 1994). Cabbage plants may invest more in direct defense, such as the production of glucosinolates, and less in indirect defense, e.g. the production of carnivore attractants. Specific responses to every type of damage may be constrained by cost-benefit trade-offs. If parasitoids would manage to forage efficiently for hosts by responding to any type of damaged leaf tissue, plants still would benefit, without investing in emitting specific chemical cues.

Although host-specific stimuli from larvae or their by-products, may be reliable indicators of host presence, they seem to attract parasitoids to a limited extent and thus play only a minor role in long-range host searching. These stimuli appear to be used during a later phase in the host-location process, i.e. after landing on a damaged plant, where they have an arrestant effect (Sato, 1979; Vinson, 1985; Nealis, 1986; Takabayashi & Takahashi, 1989; Turlings et al., 1991a; McCall et al., 1993; Mattiacci & Dicke, 1994a;1994b). PHC leaves were not preferred over HD leaves by the specialist parasitoid *C. rubecula*. Apparently, the two types of damage do not differ. The presence of feeding larvae and faeces on the PHC leaves does not influence the behaviour of these parasitoids. This agrees with the low responses to the F and L odour sources during long-range searching by this species. Use of stimuli from faeces in the long-range host-searching behaviour may depend on the amount produced, which is determined by the larval instar under parasitoid attack and the distribution pattern of the larvae. Chemical studies show that hardly any volatiles are detected from *Spodoptera exigua* larvae or their faeces (Turlings et al., 1991b), which is expected when selection for inconspicuousness in herbivores is severe (Vet et al., 1991). The volatiles that are released are of plant-origin (Lewis & Tumlinson, 1988; Auger et al., 1989). The low availability of specific stimuli does not allow for an effective use of this odour source during long-range host-searching.

An ability of parasitoids to discriminate between different types of damage inflicted on the same plant species may be attributed to quantitative differences in volatile production. Different concentrations of the same odour compounds may elicit different behaviours in the same organism. Also the variability in the ratio of compounds that comprise the odour blend may cause variability in a particular behaviour (Elkinton & Cardé, 1984). The composition of volatile blends emitted by plants is mostly plant-determined, and may depend on the species or the variety, and the quality, which is determined by the age and the growing conditions (Takabayashi et al., 1991a, 1994b). The probability of upwind flights by *Cotesia rubecula* to host-infested and uninfested leaves is influenced by different levels of odour concentrations (Kaiser & Cardé, 1992). Responses of *M. croceipes* parasitoids to green leaf volatiles vary with dose (Whitman & Eller, 1992), and in *Macrocentrus grandii*, a larval parasitoid of the European Corn Borer, strength of responses increases with an increase of the quantity of damaged leaf material (Ding et al., 1989a). For the cabbage - *Pieris* - *Cotesia* system studies are being carried out on the dose effects of plant infochemicals on the discriminative abilities of the parasitoids.

In addition to olfactory orientation, parasitoids may use visual cues, such as the damage pattern of the leaves, in the long-range host-searching. The role of the visual component of leaf damage was studied for *C. rubecula* (Wäckers, 1994). Innately, the parasitoids used visual cues of the damage in the absence of odours, but the visual orientation was overruled when the olfactory component of the damage was emitted. However, the parasitoids were able to learn the damage pattern of their preferred host, *P. rapae*. This indicates that the role of visual stimuli in host-searching should not be underestimated.

In conclusion, the specialist *C. rubecula* is mainly guided into the microhabitat of the hosts by infochemicals emitted by plants, the first trophic level. Host-derived chemical cues seem to play only a minor role in the long-range host-searching behaviour of this parasitoid species.

3

INNATE RESPONSES OF THE PARASITOIDS *Cotesia glomerata* AND *C. rubecula* TO VOLATILES FROM DIFFERENT PLANT-HERBIVORE COMPLEXES.

ABSTRACT

To determine and compare innate preferences of the parasitoid species *Cotesia glomerata* and *C. rubecula* for different plant-herbivore complexes, long-range (1m) foraging behaviour was studied in dual-choice experiments in a wind-tunnel. In this study we tested the hypothesis that naive females of the specialist *C. rubecula* should show more pronounced preferences for different plant-herbivore complexes than females of the generalist *C. glomerata*. The herbivore species used were the pierids *Pieris brassicae, P. rapae, P. napi* and *Aporia crataegi*, and the non-hosts *Plutella xylostella* and *Mamestra brassicae*. All herbivore species feed mainly on cabbage and wild crucifers, except *Aporia crataegi*, which feeds on species of Rosaceae.

Both parasitoid species preferred herbivore-damaged plants over non-damaged plants. Neither *C. rubecula* nor *C. glomerata* discriminated between plants infested by different caterpillar species, not even between plants infested by host- and non-host species. Both parasitoid species showed preferences for certain cabbage cultivars and plant species.

No differences were found in innate host-searching behaviour between *C. glomerata* and *C. rubecula*. The tritrophic system cabbage - caterpillars - *Cotesia* sp. seems to lack specificity on the herbivore level, whereas on the plant level differences in attractiveness to parasitoids were found.

INTRODUCTION

Herbivore-infested plants are important sources of volatile cues to insect parasitoids in their search for hosts (Vinson, 1981; Turlings et al., 1991a; Vet & Dicke, 1992; Steinberg et al., 1993; Dicke, 1994; chapter 2). These herbivory-related volatiles may facilitate host-location (Vet & Dicke, 1992). Some plants show a specific reaction to different herbivore species causing damage (Dicke et al., 1990b; Takabayashi et al., 1991a). Parasitoids should be able to discriminate between different plant-herbivore complexes, if these emit different volatile cues. The question is, to what extent the host-searching behaviour of parasitoids depends on the specificity of the signal from the plant, the ability of the wasp to distinguish different specific signals and/or the genetic, physiological and environmental influences that condition the parasitoid's response to volatiles.

To test theories on parasitoid host-searching behaviour and their interactions with plants and herbivores comparative behavioural studies on leaves put in water. Odour sources were arranged in two-choice situations. Care was taken that all odour sources of the same experimental series were offered on the same day, and that females of both parasitoid species were tested during this test period. All combinations of odour sources were offered randomly. The position of the odour sources was switched after testing one female of each parasitoid species. Tests lasted for a maximum of five minutes for each female. In the greenhouse a screen was placed in front of the plants, to minimize differences in visual stimuli between infested plants. In the wind tunnel this effort was not made, since visual stimuli were found to be overruled by volatiles from herbivore-infested plants for *C. rubecula* (Wäckers, 1994). A positive response was recorded as a plant on which a female landed, or in front of which a female landed in the greenhouse experiments. All other landings were recorded as 'no response'. Each female wasp had only one flight opportunity and was offered one choice situation only.

Experimental design. *Greenhouse experiments*. In the first experimental series, plants infested by pierids were tested against uninfested plants. In all treatments but one, the herbivores were feeding on Brussels sprouts. The exception was *Aporia crataegi* feeding on hawthorn leaves. The second series consisted of dual choice situations with different *Pieris* species infesting Brussels sprouts plants.

Wind tunnel experiments. In the third series, non-host caterpillar species infesting Brussels sprouts leaves were offered against uninfested Brussels sprouts leaves. Furthermore, a choice was given between *Pieris* sp. and the non-host species *Mamestra brassicae* feeding on Brussels sprouts leaves. In the fourth series, parasitoids were offered *P. brassicae* versus *P. rapae*, but now infesting white cabbage, red cabbage or nasturtium leaves. The fifth experimental series consisted of paired choice tests with different food plants infested by *P. brassicae* for *C. glomerata* and by *P. rapae* for *C. rubecula*.

Statistical analysis. Choices between odour sources within a test were analysed using binomial probability functions. Significance indicates preference for one of the two odour sources tested. Responsiveness among the choice tests within each series was analysed using tests for independence (Siegel, 1988).

RESULTS

Greenhouse experiments. Both *C. glomerata* and *C. rubecula* have a clear preference for *Pieris* sp.-infested Brussels sprouts plants over uninfested Brussels sprouts plants (Fig 1). *Cotesia glomerata* also showed a preference for *Aporia crataegi* infested hawthorn leaves over uninfested hawthorn. Responsiveness of *C. glomerata* ranged from 70% to 86%, and of *C. rubecula* from 46% to 56%. No significant differences in responsiveness between the different treatments were observed in both parasitoid species ($chi^2=4.19$; df=3; P=0.242 for *C. glomerata*, $chi^2=1.53$; df=2; P=0.46 for *C. rubecula*).

The choice distributions in figure 2 show that neither of the two parasitoid species responded more readily to one of the two *Pieris* species infesting Brussels sprouts plants. In *C. glomerata* 86% to 92% of the females made a choice, while in *C. rubecula* 50% to 56% of the parasitoids made a choice. Differences in responsiveness among tests were not significant ($chi^2=1.33$; df=2; P=0.51 for *C. glomerata* and $chi^2=0.49$; df=2; P=0.78 for *C. rubecula*).

Wind tunnel experiments. In figure 3a we see a clear preference of both parasitoid species for herbivore-infested leaves over uninfested leaves, even though the actual herbivores, *Plutella xylostella* and *Mamestra brassicae*, are not potential host species. Of the *C. glomerata* females tested 48% made a choice in the test with *Plutella xylostella* infested versus undamaged leaves. A significantly higher percentage (77%) made a choice in the test with *Mamestra brassicae* infested leaves versus uninfested leaves ($chi^2=5.08$; P=0.024). In the case of *C. rubecula* 50% and 57% of the females made a choice. Response percentages did not differ significantly ($chi^2=0.27$; P=0.605). The choice situations in which *P. brassicae* or *P. rapae* were offered against *Mamestra brassicae* revealed no preference for one of the two herbivore species (figure 3b). None of the two parasitoid species discriminated between a host and a non-host herbivore species feeding on Brussels sprouts leaves. Ninety one percent of *C. glomerata* and 68% of *C. rubecula* females responded.

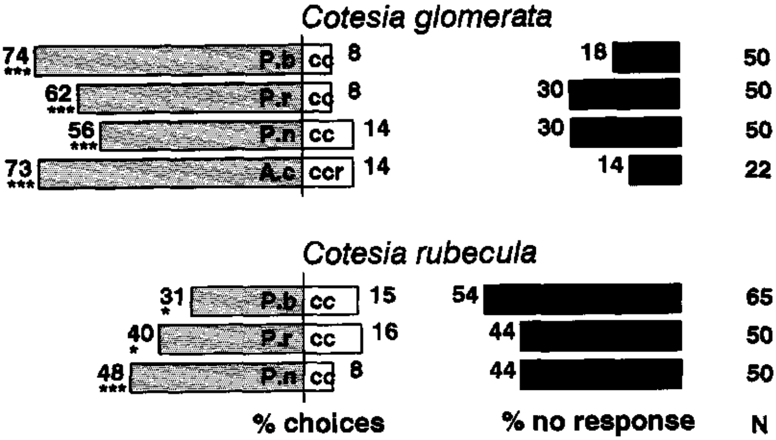

Fig. 1. Responses of *Cotesia glomerata* and *C. rubecula* females to herbivore-infested plants tested against uninfested plants. P.b, P.r, P.n and A.c refer to *Pieris brassicae, P. rapae, P. napi* and *Aporia crataegi*; cc is clean (uninfested) cabbage and ccr is clean (uninfested) *Crataegus*. Asterisks indicate a significant difference within a choice test: *** P<0.0005; * P<0.05. Numbers next to bars correspond to percentage responding and non-responding parasitoids. Numbers at the right-hand side of the figure (N) indicate number of females tested. To obtain equal amounts of damaged leaf tissue, approximately 60 first instar larvae (L1) of *P. brassicae* were used, 30 L1 of *P. rapae* and 20 L1 of *P. napi*.

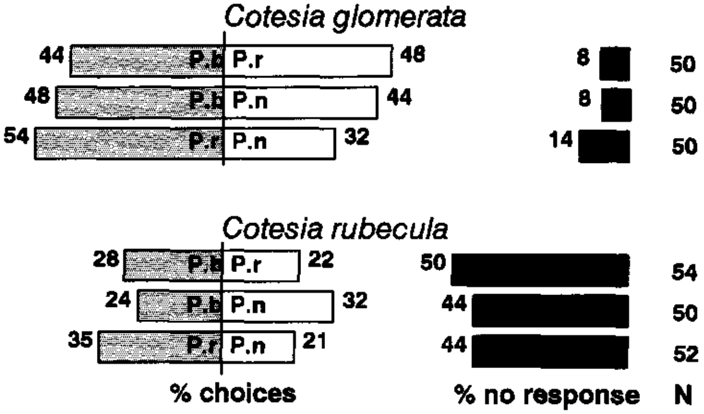

Fig. 2. Choice distribution of *Cotesia glomerata* and *C. rubecula* females over different herbivore-infested plants. P.b, P.r, and P.n refer to *Pieris brassicae, P. rapae* and *P. napi*. Numbers next to bars correspond to percentage responding and non-responding parasitoids. Numbers at the right-hand side of the figure (N) indicate number of females tested. To obtain equal amounts of damaged leaf tissue, approximately 60 first instar larvae (L1) of *P. brassicae* were used, 30 L1 of *P. rapae* and 20 L1 of *P. napi*.

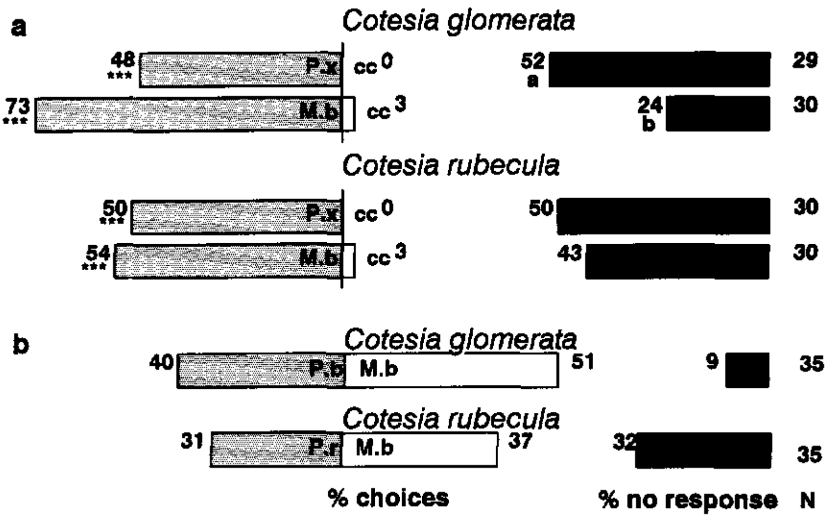

Fig. 3. Responses of *Cotesia glomerata* and *C. rubecula* females to Brussels sprouts leaves infested by non-host species (P.x, *Plutella xylostella* and M.b., *Mamestra brassicae*) tested against (a) uninfested plants (cc) and (b) *Pieris brassicae* (P.b) or *P. rapae* (P.r) infested plants. Asterisks indicate a significant difference within a choice test: *** $P<0.0005$. Numbers next to bars correspond to percentage responding and non-responding parasitoids. Lowercase characters left of no-response bars indicate significant difference within a pair. Numbers at the right-hand side of the figure (N) indicate number of females tested. To obtain equal amounts of damaged leaf tissue 20 L1 of *Plutella xylostella* and 30 L1 of *Mamestra brassicae* were used along with 30 L1 of *P. brassicae* and 15 L1 of *P. rapae* on Brussels sprouts.

Figure 4 gives the choice distribution over *P. brassicae* and *P. rapae* feeding on different cabbage cultivars or on nasturtium. *C. glomerata* discriminates between *P. brassicae* and *P. rapae* infesting white cabbage leaves, with a preference for *P. brassicae*, but no preferences were found for either of the two host species on red cabbage or on nasturtium. *C. rubecula* did not show preferences for *P. brassicae* or *P. rapae* in any of the cases. In *C. glomerata* 87% of the females made a choice in the case of white cabbage, 57% when red cabbage was offered and only 16% in the test with nasturtium. Differences in responsiveness among tests were significantly different ($chi^2=44.84$; $df=2$; $P<0.001$). In *C. rubecula* 60% and 58% of the females made a choice for white and red cabbage respectively; nasturtium evoked a significantly lower response (18%) ($chi^2=21.21$; $df=2$; $P<0.001$).

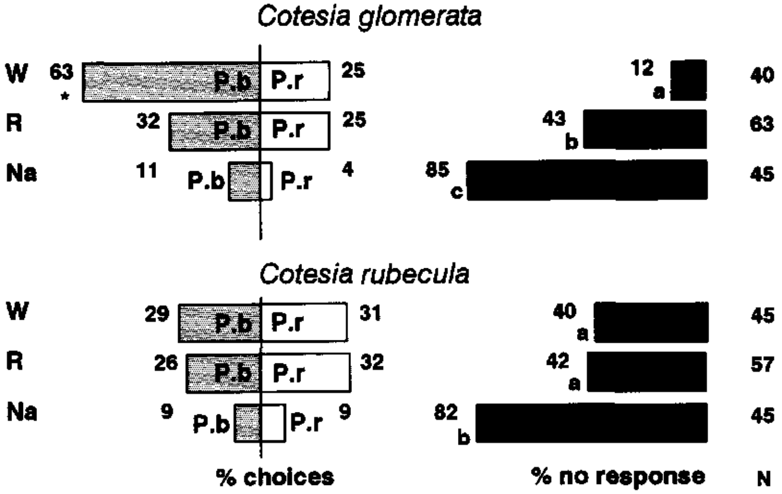

Fig. 4. Responses of *Cotesia glomerata* and *C. rubecula* females to different cabbage cultivars (W, white cabbage; R, red cabbage) and nasturtium (Na) infested by *Pieris brassicae* (P.b) or *P. rapae* (P.r). Asterisks indicate a significant difference within a choice test: * $P<0.05$. Numbers next to bars correspond to percentage responding and non-responding parasitoids. Lowercase characters left of no-response bars indicate significant difference within a pair. Numbers at the right-hand side of the figure (N) indicate number of females tested. To obtain equal amounts of damaged leaf tissue 25-30 L1 of *P. brassicae* and 15-20 L1 of *P. rapae* on nasturtium were used, along with 25 L1 of *P. brassicae* and 15 L1 of *P. rapae* on red and white cabbage.

Figure 5 shows that both parasitoid species had a preference for one of the two infested plants offered. For *C. rubecula* this preference was not significant, probably because the number of wasps that were tested was low. Responses of *C. glomerata* females were 29% to the red cabbage-nasturtium combination, 47% to the Brussels sprouts-nasturtium choice, 81% to the Brussels sprouts-red cabbage combination, and 85% to the white cabbage-nasturtium combination. Differences in responsiveness between treatments were significant ($chi^2=38.91$; $df=3$; $P<0.001$). Of the *C. rubecula* females 38% responded to the red cabbage-nasturtium combination, 57% responded to the Brussels sprouts-red cabbage combination and 67% responded to the white cabbage-nasturtium combination. Responses to different plant-combinations differed significantly ($chi^2=7.75$; $df=2$; $P=0.021$).

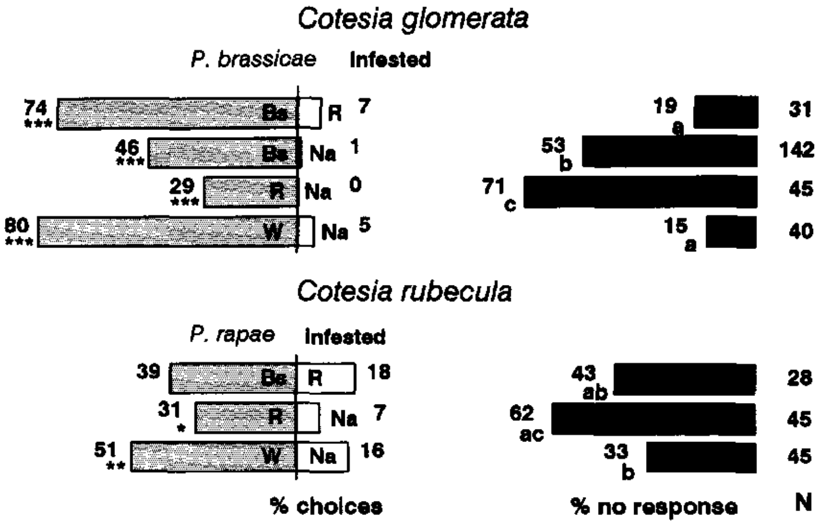

Fig. 5. Responses of *Cotesia glomerata* and *C. rubecula* females to different cabbage cultivars (Bs, Brussels sprouts; W, white cabbage; R, red cabbage) and nasturtium (Na) infested by *Pieris brassicae* (P.b) in the case of *C. glomerata* or *P. rapae* (P.r) in the case of *C. rubecula*. Asterisks indicate a significant difference within a choice test: *** $P<0.0005$; ** $P<0.005$; * $P<0.05$. Numbers next to bars correspond to percentage responding and non-responding parasitoids. Lowercase characters left of no-response bars indicate significant difference within a pair. Numbers at the right-hand side of the figure (N) indicate number of females tested. To obtain equal amounts of damaged leaf tissue 25-30 L1 of *P. brassicae* and 15-20 L1 of *P. rapae* on Brussels sprouts and nasturtium were used, along with 25 L1 of *P. brassicae* and 15 L1 of *P. rapae* on red and white cabbage.

DISCUSSION

The results show that the plants used in this study emit chemicals that are used by the parasitoids to locate the micro-habitat of the host. Herbivore-damaged plants are clearly more attractive to the parasitoids *Cotesia glomerata* and *C. rubecula* than undamaged plants. The species of herbivore does not seem particularly important, and may include both hosts and non-hosts such as *Plutella xylostella* and *Mamestra brassicae*. In earlier studies on this system (Steinberg et al., 1993; Mattiacci et al., 1994; Agelopoulos & Keller, 1994a; chapter 2), artificial damage inflicted to cabbage plants also caused the attraction of parasitoid females to these plants. This may explain why *C. glomerata* as well as *C. rubecula* show strong responses to cabbage plants infested by non-host species in choice situations with undamaged plants.

Contrary to our expectations based on host-range, neither naive *C. glomerata* nor naive *C. rubecula* females distinguished between plants infested with host or non-host species, with one exception. The only case where wasps distinguished was with *C. glomerata* which showed a preference for *P. brassicae* over *P. rapae* on white cabbage. In *C. glomerata* this almost complete absence of preferences for any of the *Pieris* species may, to some extent, reflect the generalist lifestyle of the parasitoid species. *Cotesia glomerata* can develop well in *P. brassicae*, *P. rapae* and *P. napi* (Chapter 9). In contrast, host-suitability data show that *P. rapae* is clearly the most suitable host for *C. rubecula* (Chapter 9). Based on the specialist nature of this parasitoid species a more discriminative host-searching behaviour was expected, with a preference for the cabbage - *P. rapae* complex. Instead, our study showed no evidence for fixed, innate responses to *P. rapae*-induced synomones in this specialist species.

The signals emitted by the plants apparently do not provide the wasps with specific information on which herbivore species is feeding on the plants. Volatile profiles of Brussels sprouts plants showed no qualitative differences in chemicals between *P. brassicae* and *P. rapae* infested plants. The differences are mainly of a quantitative nature (Blaakmeer et al., 1994). Even between herbivore-damaged and mechanically damaged plants no qualitative differences in volatile profiles were obtained (Mattiacci et al., 1994). Similarly, studies on a comparable system of cabbage (*Brassica oleracea capitata* cv. Green Coronet) - *P. rapae* - *C. rubecula* showed no qualitative differences in the induced volatile blends caused by mechanical damage or damage by lepidopterans (Agelopoulos & Keller, 1994b). Apparently, cabbage responds in a general way to damage, leaving naive parasitoids no opportunity to discriminate.

The lack of preferences in the behaviour of free-flying females of the two *Cotesia* species can be attributed to the fact that the female wasps used in our study had no previous experience with hosts and/or plant odours. Learning can play an important role in discrimination by parasitoids between host species (McCall et al., 1993; Turlings et al., 1993). Associative learning of host-finding cues can enhance responses to odours from the habitat of hosts and result in preferences for these odours (Vet et al., 1995). The ability to learn the odours that are associated with hosts may help the parasitoids to detect subtle differences between these cues and other nonprofitable cues (Turlings et al., 1995). Although no quantitative or qualitative differences were found in the volatiles emitted by corn infested by different herbivore species, the parasitoid *Cotesia marginiventris* was able to learn to distinguish between the cues from plants infested by larvae of the two closely related noctuids *Spodoptera exigua* and *Spodoptera frugiperda* (Turlings et al., 1995). In a previous comparative study on the cabbage - *Pieris* - *Cotesia* tritrophic system, Wiskerke & Vet (1994) showed that *C.*

rubecula does discriminate between plants infested by *P. brassicae* and plants infested by *P. rapae*, landing more often on the latter. The females used in their study had contacted a leaf previously damaged by larvae of both host species. At present research on preference learning in the two *Cotesia* species is being carried out.

Our results showed that naive females of both *Cotesia* species were clearly able to distinguish between different herbivore-infested plant species and cultivars. Based on these results a rank order in attractiveness of infested plants can be determined, which is the same for the two parasitoid species: Brussels sprouts > white cabbage, red cabbage > > > nasturtium. This agrees with the differences in responsiveness shown in figure 4. Responses to nasturtium were relatively low. Since plant-odours appear to be important to these foraging parasitoids, chemical differences between cabbage and nasturtium can be expected to cause differences in responsiveness of parasitoids to these plant species. Although nasturtium contains glucosinolates, which may have led to the addition of this plant species to the host range of *Pieris* species, it is chemically distinct form the cabbage plants. Considering the low responses to nasturtium, *Pieris* caterpillars may escape from parasitoid attack under field circumstances by exploiting nasturtium. In fact, no parasitization occurred on host-infested nasturtium plants set out in the field, by either *C. glomerata* or *C. rubecula* (chapter 10). Sato & Ohsaki (1987) and Ohsaki & Sato (1994) showed for the *Pieris - C. glomerata* system in Japan that hosts can escape from *C. glomerata* by specializing on food plant species that are not located by the parasitoids. Chemical analyses of volatiles emitted by white cabbage, red cabbage and nasturtium are needed to understand the differences between plant cultivars and plant species in the plant's responses to herbivore damage.

The fact that Brussels sprouts, the plant used in cultures of the parasitoids, elicit the strongest responses in both parasitoid species may be attributed to preadult learning or responses shaped by selection. Host diet may influence the long-range foraging behaviour of parasitoids (Hérard et al., 1988; Vet & Groenewold, 1990; Petitt et al., 1992; Kester & Barbosa, 1994). Selection may have shaped responses to this cabbage cultivar by rearing experimental wasps on Brussels sprouts plants for about 20 generations. However, recent studies on the effect of host's food plant on the host-searching foraging behaviour of *C. glomerata* showed that preferences of parasitoids did not depend of the food plant on which the host had been feeding (chapter 6).

The absence of preferences of naive *Cotesia* females on the herbivore level and their preferences for certain plant varieties correspond to results of studies on other tritrophic level interactions. Turlings et al. (1995) state that specificity of volatile induction by herbivores seems to be very limited (Takabayashi et al., 1991a), whereas distinct differences occur between odours from diffent plant species or varieties (Takabayashi et al., 1991a;

Turlings et al., 1993). On the behavioural level, similar patterns are found; parasitoids can distinguish between odours from different plants but do not always distinguish between different herbivores on the same plant (see Turlings et al., 1995).

Although expected, naive females of the two *Cotesia* species do not differ in their host micro-habitat location behaviour. It is not clear whether this is due to a constraint of the plant's general response to damage or the result of the host-searching strategies of the parasitoid species. It is possible that these parasitoids are more discriminative at lower levels in the host-searching process, and show e.g. differential response to frass or leaf damage after landing on a leaf, or differential host acceptance. Mattiacci & Dicke (1995a,b) showed that free-flying *C. glomerata* show no specific responses with respect to larval instars, whereas the wasps show a specific preference for first instar larvae over fifth instar larvae in the search for hosts on the leaf. Differences between species may become clear by comparing host-location behaviour after landing.

In conclusion, this study emphasized the importance of the plant in the host microhabitat location of some parasitoid species. Free-flying naive females of the braconid parasitoids *C. glomerata* and *C. rubecula* did not differ in their responses to plants infested by different herbivore species. They did not distinguish between different herbivore species feeding on the same type of host plant, but showed preferences for odours of specific plant species.

4

COMPARATIVE ANALYSIS OF HEADSPACE FROM DIFFERENT CATERPILLAR-INFESTED OR UNINFESTED FOOD PLANTS OF *Pieris* SPECIES.

ABSTRACT

Plants that are infested by herbivores emit volatile cues that can be used by the natural enemies of the herbivores in their search for hosts. Based on results from behavioural studies we investigated to what extent intact and herbivore-infested plant species and varieties from the food plant range of *Pieris* herbivore species differ in the composition of the volatile blends. Parasitoids of *Pieris* species, *Cotesia glomerata* and *C. rubecula*, show differential responses towards various herbivore-infested food plants, whereas differences in responses to plants infested by different herbivore species were less clear. Chemical analysis of the headspace samples of red cabbage, white cabbage and nasturtium plants, that were infested by *P. brassicae* or *P. rapae* larvae or that were intact, yielded 88 compounds belonging to the alcohols, ketones, aldehydes, esters, nitriles, terpenoids, sulfides, (iso)thiocyanates, carboxylic acids and others. The analysis revealed that herbivore-infested plants emit the largest number of compounds in the highest amounts. The plant species affected the volatile blend more than did the herbivore species, and between plant varieties differences were less pronounced than between different plant species. Differences in headspace composition between plants infested by *P. brassicae* or *P. rapae* were mainly of quantitative nature. Herbivore-infested nasturtium differed considerably from the cabbage varieties in a qualitative way. Headspace composition of red and white cabbage varieties were comparable to that of the food plant Brussels sprouts (*Brassica oleracea gemmifera* cv. Titurel) as determined in earlier studies in our laboratory. With respect to plant response to herbivory, nasturtium differed considerably from the cabbage varieties analysed so far, and shows resemblance with Lima bean, cucumber and corn, since these plant species produce more and other volatiles under herbivore attack than intact plants.
The results of this study are discussed in relation to behavioural observations on *C. glomerata* and *C. rubecula*.

INTRODUCTION.

Herbivory leads to an increased emission of volatiles by the damaged plants. These chemicals can be general plant compounds, toxins or repellents against herbivores or antibiotics against pathogens, or they can serve in the plant's indirect defense, when they are used by natural enemies during host-finding (Price, 1981; Vinson, 1981; Dicke & Sabelis, 1988; Dicke et al., 1990a; Dicke, 1994; Turlings et al., 1990; 1995; Takabayashi & Dicke, 1996).

The headspace composition of herbivore-infested plants can vary substantially (see Takabayashi & Dicke (1996) for review). Relevant factors are plant species, plant cultivar, plant parts under attack, leaf age, growing conditions of plant, time of the day, herbivore species and herbivore instar.

Volatiles from herbivore-damaged plants are an important source of information for the herbivore's natural enemies that search for their hosts (Vet & Dicke, 1992). Variation in headspace composition that is affected by herbivore species or herbivore instar may be relevant to the natural enemy, since it provides information on the herbivore identity and thus its suitability to serve as a prey or host (Vet & Dicke, 1992). This may also be true for variation due to plant species or cultivar because the plant may affect the herbivore's suitability for the natural enemy (de Moraes & McMurtry, 1987). However, the large degree of variation in information from herbivore-infested plants can easily devaluate the role of these infochemicals as the information becomes less reliable. Natural enemies are expected to have developed strategies to utilize those stimuli that most likely lead to host encounter. Apart from innate responses to infochemicals, learning plays an important role. During learning the natural enemy can associate plant volatiles with the presence of hosts (Vet & Dicke, 1992; Turlings et al., 1993; Vet et al., 1995). As a result, associative learning can lead to a temporary specialisation and variation in infochemicals is essential for this. Furthermore, learning to respond to cues from one plant-host complex can facilitate host-finding when other host-infested plants emit similar compounds or volatile blends. By generalization of learned information animals can effectively expand their foraging arena (Smith, 1993).

To assess the type and degree of variation that natural enemies face, it is important to study the variation in headspace composition among plant species and plant cultivars infested by the same herbivore species, as well as among plants of one cultivar that are infested by different herbivore species. Studies by Dicke et al. (1990b), Turlings et al. (1993), Takabayashi et al. (1991b, 1994b) and Takabayashi & Dicke (1996) show that variation in headspace composition is largest between different plant species infested by the same herbivore, and smallest between plants of one cultivar that are being infested by different herbivore species. In other words: the plant is the most important in determining the headspace composition. We have extensively studied the host-finding behaviour of two larval parasitoids of the pierids *Pieris brassicae* and *P. rapae* (Lepidoptera: Pieridae). The food plants used in these studies are the cabbage varieties Brussels sprouts (*Brassica oleracea* L. var *gemmifera* cv. Titurel), white cabbage (*Brassica oleracea capitata* L. var. *alba* cv. Langedijker de Waar) and red cabbage (*Brassica oleracea capitata* L. var. *rubra* (DC.) cv. Langedijker vroege rode) and the non cruciferous nasturtium (*Tropaeolum majus*) (*Tropeaolaceae*). The parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) clearly discriminated between different plant species or cultivars infested by *Pieris* caterpillars (Chapter 3). However, discrimination between plants from one cultivar that were infested by caterpillars of either of the two *Pieris* species was not recorded for *C. rubecula* and only occurred in *C. glomerata* after learning (Chapter 3 and 6). In addition to these laboratory studies, field studies also revealed that parasitisation of *Pieris* hosts varied with plant species: caterpillars on cabbage varieties suffered from high levels of parasitization, whereas caterpillars on nasturtium plants escaped parasitism by both parasitoid species (Chapter 10).

To understand the mechanism underlying these results we compared the headspace profiles of three food plants, i.e white cabbage, red cabbage and nasturtium, either when undamaged, or infested by first instar larvae of *P. brassicae* or *P. rapae*. Blaakmeer et al. (1994) and Mattiacci et al., (1994) already performed comparable headspace analyses of undamaged, artificially damaged, caterpillar regurgitant-treated and *P. brassicae* or *P. rapae*-infested Brussels sprouts plants (*Brassica oleracea gemmifera* cv. Titurel), the food plant on which pierid hosts are reared in our laboratory. Their studies revealed that the plants respond to damage with an increased release of green-leaf volatiles and terpenoids, and that upon caterpillar damage or application of regurgitant the emission of 'green-leaf volatiles' is enhanced (Mattiacci et al., 1994). Between headspace compositions of Brussels sprouts plants infested by either *P. brassicae* or *P. rapae* no significant differences were found (Blaakmeer et al., 1994). Our results are compared with these findings.

MATERIALS AND METHODS.

Plants. White cabbage (*Brassica oleracea capitata* L. var. *alba* cv. Langedijker de Waar), red cabbage (*Brassica oleracea capitata* L. var. *rubra* (DC.) cv. Langedijker vroege rode) and nasturtium (*Tropaeolum majus* cv. Mahogeny) (Tropaeolaceae) were reared in a greenhouse compartment at 20-25°C, 50-70% RH and a 16L:8D photoperiod. When necessary, plants were treated against aphids with DDVP (2,2 dichlorovinyl phosphate), but not within a period of four days before using them for experiments.

Insects. Stock colonies of *Pieris brassicae* and *P. rapae* were maintained on Brussels sprouts (*Brassica oleracea gemmifera* cv. Titurel) in a climatic room at 20-22°C, 50-70% RH and a 16L:8D photoperiod.

Plant treatments. Of each type of food plant, three treatments were used for volatile collection: plants were undamaged (UND), or infested by first instar larvae of either *P. brassicae* or *P. rapae*. To obtain the herbivore-infested plants, female butterflies of either host species were allowed to oviposit for 4-6 hours on plants of the desired type. Plants with eggs were kept in the culturing room, until hatching of the eggs. Early first instar larvae were gently transferred from the plant to a 4-6 weeks old plant of the same type one day before headspace-sampling. This was done to standardize feeding damage and to use fresh, small food plants that fitted in the headspace-collection flask (see below). Per plant 100 *P. brassicae* or 40 *P. rapae* were used, so that the amount of leaf damaged inflicted was approximately the same for either plant-host-complex.

Collection of headspace volatiles. Headspace samples were collected in the period of May-November 1995. Odour sources were contained in a 5-liter glass flask with a 10-cm-ID opening, that was to be closed by a glass lid with an air-inlet and an air outlet (Mattiacci et al., 1994). Plants were carefully removed from the pot soil, avoiding damaging the roots and the leaves. The root-system was gently rinsed with tap-water. The plant was carefully transferred to an erlenmeyer flask with tap-water, that was placed inside the 5-liter collection flask.

Pressurized air was filtered over silica-gel (anhydrous potassium hydroxide), molecular sieves 4A and 13X (Linde) and activated charcoal before entering the flask (Dicke et al., 1990a; Takabayashi et al., 1991; Mattiacci et al., 1994). The air-inlet, air-outlet, filters and sampling-jar were connected with 0.8 cm-diam Teflon tubing. For 3 hours the system was purged at an airflow rate of 500 ml/min to remove volatile contaminants, after which the flow was stopped for 1 hour to allow accumulation of volatiles in the flask (Mattiacci et al., 1994). The volatiles were subsequently collected on 90 mg Tenax-TA in a pyrex glass tube (160x60 mm OD), that was connected to the outlet of the flask through a Teflon-coated plastic fitting. The airflow was reset at 500 ml/min for the duration of 1 hour. After this period the Tenax tube was disconnected and closed with Teflon-lined brass caps.

Analysis of the collected headspace volatiles. The collected volatiles were released from the Tenax by heating the trap in a Thermodesorption Cold Trap Unit (Chrompack, Middelburg, The Netherlands) at 250°C for 10 min and flushing with a *He*-flow of 10 ml/min. The released compounds were cryofocused in a coldtrap (0.52 mm i.d. deactivated fused silica) at an approximate temperature of -85°C (Dicke et al., 1990a). By ballistic heating of the cold trap to 220°C the volatiles were transferred to the analytical column (Supelcowax 10, 60 m x 0.25 mm di.d., 0.25 μm film thickness), which was connected to a Finnigan MAT 95 mass spectrometer. The column was programmed from 40°C (3 min hold) to 270°C (4 min hold) at 4°C/min and the initial Helium velocity was 30 cm/sec. The mass spectrometer was operated in the 70 eV EI ionization mode and scanning was done from mass

24 to 400 at 0.7 sec/dec. Identification of the compounds was done by comparison of the mass spectra with those in the Wiley library and in the Wageningen Mass Spectral Database of Natural Products and by checking the retention index.
For each compound in a sample its contribution to the headspace was calculated as a percentage by dividing the peak area of that compound by the total peak area in that sample. For each compound the percentages determined in the replicates of a treatment were averaged.

RESULTS

Only compounds that were detected in more than 1 sample per plant species were included in the analysis (Table 1). Among treatments (23 samples) 88 compounds were detected and identified as alcohols, ketones, aldehydes, esters, nitriles, terpenoids, sulfides, (iso)thiocyanates, carboxylic acids and others.

Herbivore-infested plants versus intact plants. Herbivore-infested plants produced the largest number of compounds, although qualitative differences between infested and intact white or red cabbage plants were less pronounced than between infested and intact nasturtium plants (Table 1). Furthermore, the amounts of volatiles released from herbivore-infested plants were higher than from uninfested plants. In the cabbage varieties the average total peak area was about twice as high in infested plants compared to intact plants, while in nasturtium average total peak areas of infested plants were 10 to 17 times higher than those of intact plants.

Cabbage varieties versus nasturtium. Comparison of the headspace profiles of the two cabbage varieties white cabbage and red cabbage qualitatively shows that they resemble each other more than each of the cabbage varieties resembles nasturtium. Several striking differences in the composition of the volatile blends were found between the two cabbage varieties and nasturtium (compound numbers refer to Table 1 and Figures 1 and 2). 1) In five out of five herbivore-infested nasturtium samples two esters (methyl-2-methylpropanoate (nr. 34) and methyl-2-methylbutanoate (nr. 35) were detected, which were absent in intact nasturtium and in both cabbage varieties (intact and herbivore-infested). 2) A large number of terpenoids α-cubenene (nr. 59), ß-cubenene (nr. 60), ß-elemene (nr. 63), ß-caryophyllene (nr. 64), α-humulene (nr. 65), γ-muurolene (nr. 66), germacrene-D (nr. 67), ß-selinene (nr. 68), α-muurolene (nr. 69), α-selinene (nr. 70), γ-cadinene (nr. 71), δ-cadinene (nr. 72), calamenene (nr. 74), α-calacorene (nr. 75) and ß-calacorene (nr. 76) were detected consistently in headspace samples of *P. brassicae*-infested and *P. rapae*-infested nasturtium plants only (see also fig. 1). 3) On the other hand, the terpenoids 1,8-cineole (nr. 53) and trans-sabinene hydrate (nr. 58) were present in almost all samples of red and white cabbage, in infested and intact plants, but not at all in nasturtium samples. 4) Nasturtium plants infested by *P. brassicae* or *P. rapae* released benzyl isothiocyanate (nr. 80) that was absent in the two cabbage varieties and in uninfested nasturtium. 5) In general, fewer ketones were detected in nasturtium plants, both infested and intact, than in infested and intact red and white cabbage plants. The contribution of terpenoids to the total headspace composition of *P. rapae* and *P. brassicae*-infested nasturtium plant (40% of the total number of compounds) was higher than that of herbivore-infested red cabbage (20%) and herbivore-infested white cabbage (25%). Furthermore, (Z)-3-hexenol (nr. 9) was detected in few (2 out of 7) samples of nasturtium and (Z)-3-hexenyl-acetate (nr. 38) was detected in red and white cabbage only and in none of the nasturtium samples. It should be noted that the low number (8) of compounds detected in intact nasturtium plants accounts for the high percentages for each compound in this treatment.

White cabbage versus red cabbage. Differences in composition of volatile blends between red and white cabbage, either herbivore-infested or intact, were restricted to a few alcohols present in samples of red cabbage only (nr. 1 and nr. 2), while (3E)-4,8-dimethyl-1,3,7-nonatriene (nr. 56) was more pronounced in white cabbage samples (Table 1). Differences were mainly of quantitative nature (Table 1, fig. 1). In *P. rapae*-infested red cabbage plants (Z)-3-hexen-1-ol (nr.9) and (Z)-3-hexenyl acetate (nr. 38) were detected in higher amounts than in *P. rapae*-infested white cabbage plants (fig. 1). In contrast, decanal (nr. 30), benzonitrile (nr. 43), sabinene (nr. 48), (3E)-4,8-dimethyl-1,3,7-nonatriene (nr. 56) and hexanoic acid (nr. 85) were detected in higher amounts from *P. rapae*-infested white cabbage plants compared to red cabbage samples (fig. 1).

Pieris brassicae infested plants versus P. rapae infested plants. Differences in composition of volatile blends between headspace samples from plants infested by the two herbivore species *P. brassicae* and *P. rapae* and the intact plants were minimal (Table 1, fig. 2). Red cabbage plants infested by *P. rapae* larvae released higher amounts of (Z)-3-hexen-1-ol (nr. 9) and decanal (nr. 30) (although it was found in one sample only in both cases) than *P. brassicae*-infested plants, whereas *P. brassicae*-infested red cabbage plants produced relatively higher amounts of the terpenoids α-pinene (nr. 45), α-thujene (nr. 46), sabinene (nr. 48), limonene (nr. 51) and 1,8-cineole (nr. 53) (fig. 1b). These terpenoid compounds were also detected in relatively high amounts in samples from intact red cabbage plants, as were decanal (nr. 30) and hexanoic acid (nr. 85) (fig. 2).

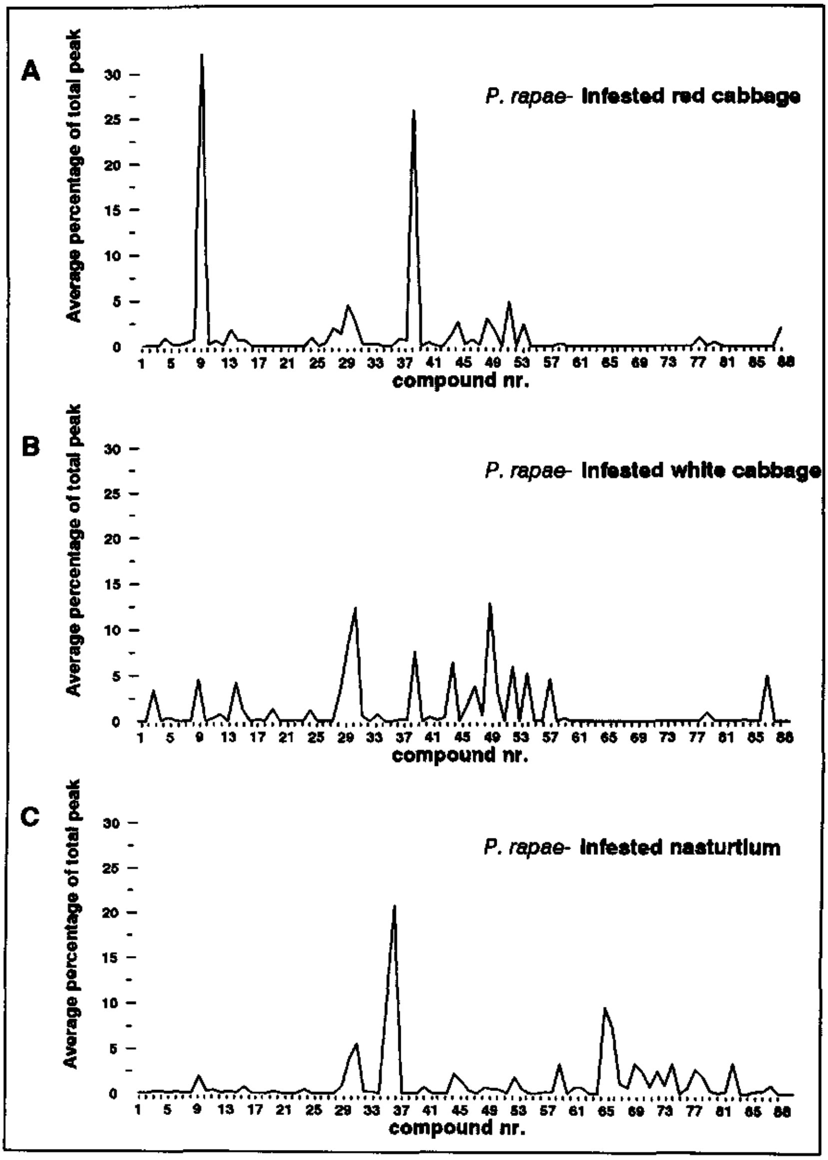

Fig. 1. Average percentages of total area of GC peaks for compounds detected in headspace samples of (A) red cabbage, (B) white cabbage and (C) nasturtium plants infested by *P. rapae* larvae (40L1). Compound numbers refer to the numbers as presented in table 1.

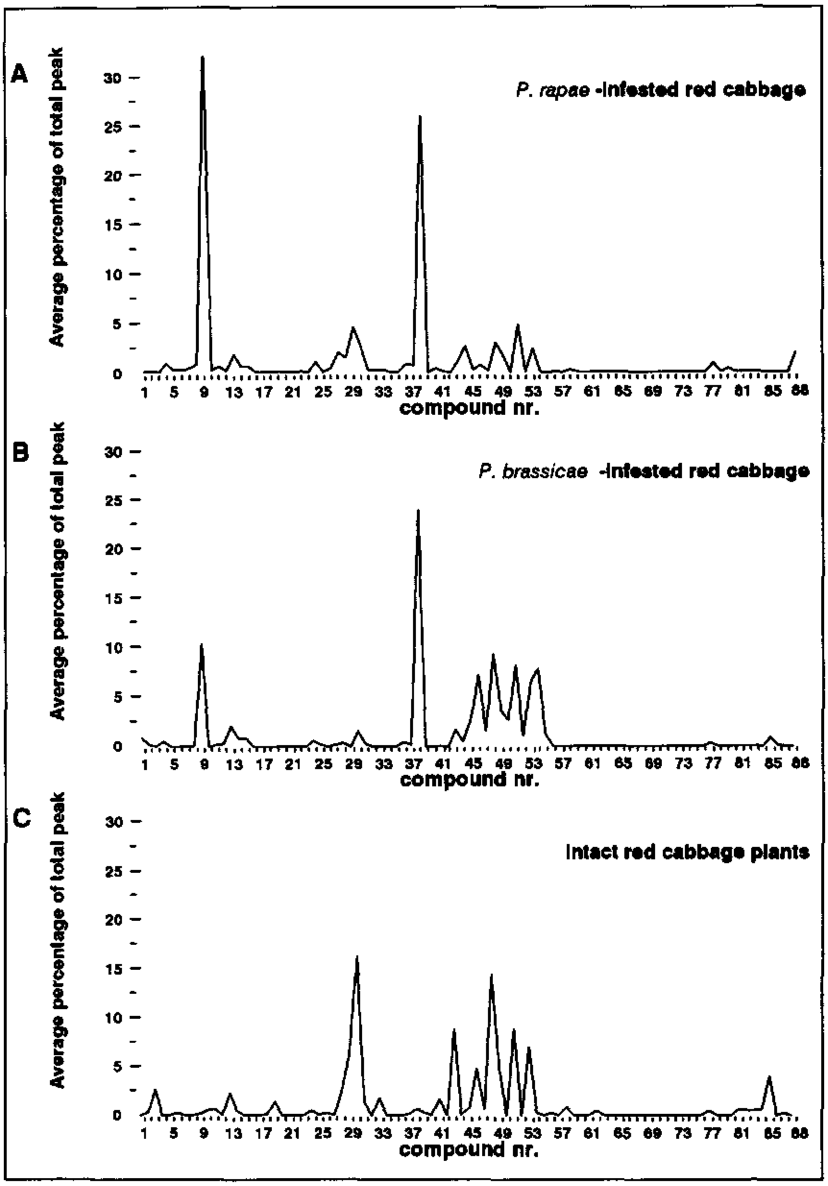

Fig. 2. Average percentages of total area of GC peaks for compounds detected in headspace samples of red cabbage plants infested (A) *P. rapae* larvae (40L1), (B) *P. brassicae* larvae (100L1) and (C) intact red cabbage plants. Compound numbers refer to the numbers as presented in table 1.

Table 1. Percentages[a] (±SE) of Total Area of GC Peaks for Compounds Detected in Headspace of Red Cabbage, White Cabbage and Nasturtium Leaves infested by *P. brassicae* larvae, *P. rapae* larvae or uninfested leaves.

	treatments[c]								
	red cabbage			white cabbage			nasturtium		
compounds[b]	*P. rapae* (N=3)	*P. brassicae* (N=2)	undamaged (N=2)	*P. rapae* (N=3)	*P. brassicae* (N=3)	undamaged (N=3)	*P. rapae* (N=3)	*P. brassicae* (N=2)	undamaged (N=2)
Alcohols									
1 3-Methyl-2-butanol	0.04±0.04'	0.9±0.9'							
2 3-Pentanol$^{*\Delta}$	0.09±0.09'	0.2±0.2'	0.3±0.3'						
3 1-Butanol	0.03±0.03'	0.07±0.07'	2.7±2.7'	3.3±3.3'	0.06±0.06'	0.6±0.5''	0.1±0.1'	0.7±0.4''	
4 1-Penten-3-ol$^{\circ*\Delta}$	0.9±0.4'''	0.6±0.6'		0.03±0.03'	0.07±0.07'		0.1±0.1'	0.3±0.3'	
5 3-Methyl-2-pentanol$^{\Delta}$	0.2±0.2'		-'	0.4±0.4'					
6 1-Pentanol$^{*\Delta}$	0.2±0.2''		0.2±0.2'	0.1±0.1'			0.2±0.2'		
7 2-Penten-1-ol$^{\Delta}$	0.4±0.4'	0.1±0.1'			0.03±0.03'				
8 1-Hexanol$^{*\Delta}$	0.8±0.3'''	0.1±0.1'		0.08±0.08'	0.08±0.08'			0.1±0.1'	
9 (Z)-3-Hexen-1-ol$^{\circ*\Delta}$	32.7±14.8'''	10.4±9.5''	0.1±0.1'	4.4±1.8'''	8.7±0.7''	0.5±0.5'	1.8±1.8'	1.0±1.0'	
10 1-Heptanol$^{\Delta}$	0.2±0.2'		0.5±0.5'		0.3±0.3'	0.3±0.3'	0 2±0.2'	0.1±0.1'	2.6±2.6'
11 1-Octanol$^{\Delta}$	0.6±0.6''	0.2±0.2'	0.7±0.7'	0.3±0.3'	0.3±0.2''	0.2±0 2'	0.3±0.2''	0.3±0.3'	
Ketones									
12 2-Pentanone$^{\Delta}$	0.1±0.1'	0.3±0.3'		0.7±0.7'	-'''	-'			
13 3-Pentanone$^{\circ*}$	1.8±0.7'''	2.1±0.06''	2.3±0.2''		-'''	0.3±0.3'	0.2±0.2'		
14 3-Methyl-2-pentanone$^{*\Delta}$	0.6±0.4''	0.9±0.9'	0.5±0.5'	4.1±3.6''	2.3±0.9'''	1.8±1.2'''			
15 1-Penten-3-one$^{*\Delta}$	0.6±0.4'''	0.9±0.9'		1.2±1.6''	1.0±0.1'''		0.7±0.5''	0.5±0.5'	
16 2-Heptanone$^{\Delta}$					0.2±0.2''	0.1±0.1'		-'	
17 3-Octanone				0.2±0.2'	0.1±0.1'				
18 1-Octen-3-one			0.1±0.1'	0.06±0.06'					
19 6-Methyl-5-hepten-2-one$^{\Delta}$	0.09±0.09'		1.4±0.3''	1.3±0.9''	0.7±0.7'	0.2±0.2'	0.2±0.2'	0.4±0.4'	
20 2-Methyl-2-cyclopenten-1-one		0.08±0.08'			0.2±0.1''				
21 2-Nonanone		0.03±0.03'			0.05±0.05'				
22 3,5,5-Trimethyl-3-cyclohexen-1-one	0.07±0.07'			0.1±0.1'	0.3±0.2''				
23 6,10-Dimethyl-5,9-undecadiene-2-one							0.4±0.4'	0.5±0.5'	
Aldehydes									
24 Hexanal$^{\circ*}$	1.0±0.6'''	0.6±0.6'	0.5±0.5'	1.2±1.1''	0.3±0.1'''	0.1±0.1'		0.3±0.2''	
25 2-Pentenal*	0.02±0.02'	0.2±0.2'			0.06±0.06'				
26 1-Heptanal$^{\Delta}$	0.4±0.4''		0.2±0.2'		0.2±0.2''			-'	
27 (E)-2-Hexenal$^{*\Delta}$	2.0±1.9''	0.2±0.2'						0.3±0.3'	
28 Octanal$^{\circ*\Delta}$	1.4±0.9'''	0.4±0.4'	2.7±1.1''	3.3±3.1''	0.6±0.5''	1.0±1.0''	0.8±0.2'''	0.7±0.4''	3.7±3.7'
29 Nonanal*	4.5±3.0'''		6.3±6.3'	8.0±6.1''	3.6±4.3''	3.9±4.3''	3.8±1.5'''	-''	
30 Decanal*	2.6±3.7'	1.6±1.6'	16.3±10.4''	12.1±9.6''	4.0±2.4'''	0.9±0.9''	5.4±2.4'''	4.6±4.2''	23.3±16.6''
31 Undecanal	0.3±0.4'	0.3±0.3'	1.4±1.4'	0.6±0.6'	0.2±0.2'		0.2±0.2'	0.4±0.4'	
32 Phenylacetealdehyde$^{\Delta}$	0.2±0.2'						0.2±0.2'		1.3±1.3'
33 Dodecanal	0.3±0.3'		1.8±1.8'	0.7±0.7'	0.5±0.5'				

Table 1. *(continued)*

compounds	treatments								
	red cabbage			white cabbage			nasturtium		
	P. rapae (N=3)	*P. brassicae* (N=2)	undamaged (N=2)	*P. rapae* (N=3)	*P. brassicae* (N=3)	undamaged (N=3)	*P. rapae* (N=3)	*P. brassicae* (N=2)	undamaged (N=2)
Esters									
34 Methyl-2-methyl-propanoate							10.1±2.2***	9.9±3.0**	
35 Methyl-2-methyl-butanoate							20.1±1.8***	8.5±1.2**	
36 3-Methyl-3-buten-1-yl-acetate^{a}	0.8±0.7**	0.5±0.5*		0.2±0.2*	0.5±0.3***				
37 Hexyl-acetate^{ab}	0.7±0.8**	0.2±0.2*	0.1±0.1*	0.2±0.2*	0.3±0.2**				
38 (Z)-3-Hexen-1-yl-acetate^{ab}	25.9±9.6***	24.0±24.0*	0.7±0.2**	7.4±7.4*	30.7±5.1*	0.5±0.5*			
39 Methylbenzoate			0.3±0.3*			0.1±0.1*	0.7±0.5***	0.9±0.5**	
40 Methylsalicylate	0.4±0.5*			0.4±0.4*	0.2±0.2**	4.4±0.2**		0.4±0.4*	
Nitriles									
41 3-Methylbutanenitrile	0.04±0.05***	0.06±0.06*	1.7±1.7*	0.1±0.1*	0.3±0.3*				
42 2-Methylbutanenitrile				0.4±0.4*	0.05±0.05*				
43 Benzonitrile	1.1±0.8***	1.7±1.0**	8.9±7.8**	6.2±5.6***	1.3±0.8***	3.6±3.3***	2.1±0.9***	3.0±0.6**	53.9±33.7**
44 Benzylcyanide	2.6±2.0**	0.5±0.5*			0.1±0.1*		1.3±0.3***	15.8±10.8**	
Terpenoids									
45 α-Pinene^{a}	0.2±0.2**	2.8±2.7**	0.8±0.3**	1.7±2.4**	1.0±1.0***	2.1±1.3***	0.3±0.3**	0.2±0.2*	
46 α-Thujene^{ab}	0.7±0.3***	7.3±6.3**	4.8±1.9**	3.7±4.3**	5.0±3.3***	6.7±1.6***			
47 β-Pinene^{ab}	0.1±0.1**	1.6±1.4**	0.7±0.2**	0.6±0.7**	0.6±0.3***	1.0±0.4***	0.6±0.6***	0.3±0.3**	
48 Sabinene^{ab}	3.0±0.8***	9.4±6.3**	14.5±6.3**	12.5±13.6***	11.6±5.8***	22.5±11.1***	0.5±0.5***	0.2±0.2*	
49 *Myrcene*^{ab}	1.7±0.8***	3.7±2.7**	4.8±2.2**	3.0±2.5**	4.7±1.8***	7.5±3.5***	0.4±0.4**	0.1±0.1*	
50 α-Terpinene		2.8±2.8*				0.05±0.05*			
51 Limonene^{ab}	4.8±3.1***	8.2±5.5**	8.9±3.2**	5.8±4.4**	9.3±1.9***	16.0±3.8***	1.8±1.6***	4.1±3.9**	0.3±0.3*
52 β-Phellandrene^{a}	0.05±0.04**	1.1±1.1*	-*	0.02±0.02*	0.2±0.1***	0.2±0.2**	0.5±0.7**	0.1±0.1*	
53 1,8-Cineole^{ab}	2.4±1.3***	6.6±3.4**	7.1±0.8**	5.1±5.3**	5.5±1.7***	10.9±1.7***			
54 'gamma'-Terpinene		7.9±7.9*	0.3±0.3*		0.03±0.03*	0.1±0.1*			
55 p-Cymene		1.3±1.3*			0.6±0.3***		0.2±0.2*		
56 (3E)-4,8-dimethyl-1,3,7-nonatriene	0.06±0.06*		0.2±0.01**	4.5±5.9**	2.2±1.4***	0.6±0.5**	0.2±0.2**		
57 α-Copaene							3.2±1.0***	0.7±0.07**	
58 Trans-sabinene-hydrate^{a}	0.3±0.2**		0.9±0.1**	0.3±0.3*	0.7±0.3***	1.3±0.7***			
59 α-Cubenene							0.7±0.6**		
60 β-Cubenene							0.7±0.2***	0.2±0.2*	
61 Linalool^{cd}					0.3±0.2**				
62 Longifolene			0.5±0.5*			0.2±0.2*			
63 β-Elemene^{a}							9.3±1.8***	4.4±3.4**	
64 β-Caryophyllene							7.1±1.2***	2.0±1.3*	
65 α-Humulene							1.1±0.2***	0.3±0.3*	
66 'gamma'-Muurolene							0.7±0.6**	0.1±0.1*	
67 Germacrene-D							3.3±2.5**	2.8±2.8*	

Table 1. (continued)

	treatments								
	red cabbage			white cabbage			nasturtium		
compounds	*P. rapae* (N=3)	*P. brassicae* (N=2)	undamaged (N=2)	*P. rapae* (N=3)	*P. brassicae* (N=3)	undamaged (N=3)	*P. rapae* (N=3)	*P. brassicae* (N=2)	undamaged (N=2)
Terpenoids (continued)									
68 β-Selinene							2.4±1.4***	0.5±0.5*	
69 α-Muurolene							0.8±0.2***	0.2±0.1**	
70 α-Selinene							2.5±2.1***	0.6±0.6*	
71 'gamma'-Cadinene							0.9±0.3***	0.1±0.1*	
72 δ-Cadinene							3.3±1.7***	1.4±0.4**	
73 (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene					0.04±0.04*	0.4±0.4*			
74 Calamenene							0.6±0.4**		
75 α-Calacorene							2.6±1.3***	0.2±0.2*	
76 β-Calacorene							1.9±0.2***	-*	
Sulfides									
77 Dimethyldisulfide°*Δ	0.9±0.9***	0.3±0.02**	0.5±0.2**	0.9±0.9**	0.5±0.3***	3.3±2.2***	0.3±0.2**	-**	
78 Dimethyltrisulfide°*Δ		0.02±0.02*		0.05±0.05*	0.2±0.2**	0.5±0.3***	0.06±0.06*	0.06±0.06*	
(Iso)thiocyanates									
79 Methyl(iso)thiocyanate*Δ	0.4±0.3***	-*			0.4±0.3**		0.2±0.2*	0.2±0.2*	
80 Benzylisothiocyanate							3.3±3.2**	26.6±26.6*	
Carboxylic acids C-Z									
81 Propanoic acid			0.7±0.7*			0.3±0.3*		0.2±0.2*	
82 Butanoic acid			0.5±0.5*	0.2±0.2*		0.3±0.3*			
83 3-Methyl butanoic acid			0.5±0.5*				0.3±0.3*		
84 Pentanoic acid			0.6±0.6*			1.1±1.1*	0.3±0.3*	0.6±0.6*	
85 Hexanoic acid		0.9±0.9*	4.1±4.1*	4.8±5.3**		6.9±6.9*	0.9±1.0**	2.9±2.9*	7.0±7.0*
others									
86 2-Pentylfuran		0.07±0.07*						0.1±0.1*	
87 Furfural			0.2±0.2*						7.9±2.3**
88 Indole	2.0±2.0**				0.03±0.03*				
Number of compounds	47	42	41	41	51	37	50	49	8
Average total peak area	5,783	4,707	2,995	3,090	4,323	2,530	2,526	4,037	235
(SE)	(2,534)	(3,099)	(568)	(2,633)	(1,085)	(740)	(102)	(1,420)	(151)

[a] Average peak areas are calculated by dividing the peak area of a compound in every sample by the total peak area of that sample. Percentages are averages of the samples in a treatment.

[b] ° compound detected by Blaakmeer et al., 1994; * compound detected by Mattiacci et al., 1994; Δ compounds detected by Agelopoulos et al., 1995.

[c] each * indicates the presence of a compound in one sample; - indicates no peak areas determined

DISCUSSION

Herbivore-infested plants versus intact plants. Plant-herbivore complexes are known to elicit strong behavioural responses in natural enemies of the herbivores (Dicke et al., 1990a,b; Turlings et al., 1990; Steinberg et al, 1993; Chapter 2). Comparisons of chemical blends emitted by herbivore-infested plants and intact plants demonstrated qualitative and/or quantitative changes in the emission of infochemicals by the plant-herbivore complexes (Dicke et al., 1990a,b; Dicke, 1994; Turlings et al., 1990, 1993; Blaakmeer et al., 1994; Mattiacci et al., 1994; Takabayashi et al, 1994; Takabayashi et al, 1995; Takabayashi & Dicke, 1996). Our data show as well that herbivore-infested plants produced the highest number of compounds, although qualitative differences between infested and intact cabbage plants are less pronounced compared to the differences between infested and intact nasturtium plants (Table 1). Differences between intact and infested cabbage plants are mainly of quantitative nature, since infested plants produce similar compounds but in larger amounts than intact plants. In herbivore-plant interactions two defense options can be recognized (Price et al., 1980; Dicke & Sabelis, 1988; Dicke, 1994; 1996). Plants can have traits by which they directly prevent herbivores to attack or develop successfully, or they may have intrinsic defensive traits by which they promote effectiveness of natural enemies of the herbivores. Cabbage varieties belong to the group with a more direct defense strategy, by producing glucosinolates that deter many herbivorous species (Chew, 1988). Considering the differences in volatiles between intact and herbivore-infested nasturtium plants, this plant species seems to employ the intrinsic defense strategy, like plant species such as Lima bean, cucumber and corn that produce compounds upon herbivory, that are not demonstrated in volatile profiles from intact plants (Dicke et al., 1990b; Turlings et al., 1990, 1993; Dicke, 1994; 1996).

Role of plant and herbivore in determining headspace composition. Laboratory and field studies on the parasitoid species *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) demonstrated that females of both species show differential responses to food plants infested by pierid hosts. Differences in responsiveness were most clearly shown between different plant species or cultivars infested by the same host-species; discrimination between different herbivore species feeding on the same plant cultivar was less clear (Chapter 3) and had to be learned (Chapter 6). Herbivore-infested nasturtium elicited low responses in naive *Cotesia* parasitoids that search for hosts compared to the various herbivore-infested cabbage varieties (Chapter 3).

For *Brassicaceae*, Tollsten & Bergström (1988) studied headspace volatiles from intact and mechanically damaged crucifer species and found qualitative and quantitative differences between the various *Brassica* species. The comparative study on Brussels sprouts plants infested by different *Pieris* species by Blaakmeer et al. (1994) revealed minimal differences in volatile profiles, that were mainly of quantitative nature. Takabayashi et al. (1991) and Turlings et al. (1995) stated that the plant is more important in affecting the composition of the volatile blend than the herbivore. Our comparative study on the volatile profiles from the cabbage varieties red and white cabbage and the non-cruciferous food plant nasturtium supports this. Samples of plants (cabbage varieties and nasturtium) infested by different herbivore species (*P. brassicae* or *P. rapae*) showed less variability in composition of volatile blends, and differences were mainly of quantitative nature. On the other hand, herbivore-specific responses have been reported for the corn-*Pseudoletia separata* system (Takabayashi et al., 1995), where large differences in volatile compositions were found between plants infested by different instars of the same herbivore species. This difference in herbivore-specific responses between plant species may indicate differences in the defensive strategies against herbivory between plant species (Dicke, 1994; 1996).

Role of plant species vs plant cultivar in determining headspace composition. As expected (Takabayashi et al., 1991), our data support the suggestion, that differences between the plant species are more pronounced than between plant varieties. Composition of volatile blends from herbivore-infested plants or intact plants clearly differed between the two cabbage varieties and nasturtium, both qualitatively and quantitatively, but not so much between the two cabbage varieties, either infested or intact. Samples of plant varieties infested by different herbivore species showed less variability in composition of volatile blends, and variation was mainly of quantitative nature. Furthermore, comparison of the volatile profiles from Brussels sprout plants (Blaakmeer et al., 1994; Mattiacci et al., 1994) with those from red and white cabbage reveals that (Z)-3-hexen-1-ol (nr. 9) and (Z)-3-hexenyl acetate (nr. 38) were also found in high amounts in headspace samples of *P. brassicae*-infested Brussels sprouts leaves (Mattiacci et al., 1994). The alcohol (nr. 9) comprised 31% of the total in the headspace of the Plant-Host-Complex, and only 4-9% in that of undamaged, artificially damaged or caterpillar-regurgitate-treated leaves (Mattiacci et al., 1994). In our analysis, herbivore-infested red and white cabbage released considerably higher percentages of this compound than intact plants. In our study 'green leaf volatiles' such as (Z)-3-hexenyl isovalerate, (Z)-3-hexenyl butyrate, (Z)-3-hexenyl isobutyrate that were detected in herbivore-infested Brussels sprouts (Blaakmeer et al., 1994; Mattiacci et al., 1994) and that are reported to be involved in the attraction of natural enemies, were not detected. This may account for the reported preference for herbivore-infested Brussels sprouts over infested red cabbage in naive *C. glomerata* and *C. rubecula* (Chapter 3).

Nasturtium versus cabbage varieties. In nasturtium samples (Z)-3-hexen-1-ol was detected in considerably lower amounts than in red and white cabbage and (Z)-3-hexenyl acetate was absent. The latter 'green-leaf volatile' may be important for host-searching by natural enemies (Whitman & Eller, 1990). It was found in high amounts in *P. brassicae*-infested Brussels sprouts plants (Mattiacci et al., 1994), that are highly attractive to *C. glomerata* females. Terpenoids are a major class among the herbivore-induced synomones, that play a role in host-searching behaviour by natural enemies (Dicke et al., 1990a,b; Turlings et al., 1993; Takabayashi et al., 1991). In the emission of this class of compounds, nasturtium showed distinct responses, such as the lack of 1,8-cineole and trans-sabinene hydrate, two compounds that were found consistently in samples of herbivore-infested and uninfested leaves of Brussels sprouts (Blaakmeer et al., 1994; Mattiacci et al., 1994), red cabbage and white cabbage (this study). On the other hand, in samples of herbivore-infested nasturtium a high number of terpenoids was detected that were not found in infested red cabbage or white cabbage (this study), Brussels sprouts infested by *P. brassicae* or *P. rapae* (Blaakmeer et al., 1994; Mattiacci et al., 1994) or in cabbage (*Brassica oleracea capitata* cv. Green Coronet) infested by *P. rapae* or *Plutella xylostella* (Agelopoulos & Keller, 1994c). The two esters methyl-2-methyl propanoate and methyl-2-methyl butanoate (nr. 34 and 35) detected in nasturtium were not detected in red or white cabbage, neither were they reported from cabbage in other studies (Blaakmeer et al., 1994; Mattiacci et al, 1994; Agelopoulos & Keller, 1994c), nor from other plant-herbivore complexes. They are natural products that are reported from analyses of nutritional chemicals (M.A. Posthumus, pers. comm.).

Pieris species feed on plants that contain glucosinolates, which are found in cruciferous plants and in nasturtium (Tropaeolaceae). These secondary plant compounds are the precursor of the volatile isothiocyanates. Besides methyl (iso)thiocyanate also benzyl isothiocyanate was detected in samples of herbivore-infested nasturtium leaves, which was absent in the samples of the cabbage varieties, accounting for another qualitative difference between nasturtium and cabbage.

The qualitatively and quantitatively clearly distinct volatile profiles released by nasturtium in comparison to those known from the cabbage varieties studied so far, are likely to account for the different behaviour of the parasitoids towards this plant species. The lack or the presence of compounds can make the plant 'unrecognizable' for naive parasitoids, and learning is needed to get increased responses and motivated search by the female parasitoids. In field situations, parasitization of host larvae feeding on nasturtium plants placed amongst herbivore-infested cabbage varieties hardly occurs, whereas parasitization levels on Brussels sprouts and red cabbage were high (Chapter 10). On the other hand, learning can change the preference of females for certain plant-host complexes, the effect being stronger with initially less attractive plants such as nasturtium. In parasitoids that had their first experience with infested nasturtium, learned responses to nasturtium are not easily overruled by experience with cabbage (Chapter 8). It remains to be tested whether field release of nasturtium-experienced parasitoids would lead to parasitization of *Pieris* hosts on nasturtium plants.

Epilogue. Our chemical data support the findings in behavioural studies in the system cabbage - *Pieris* - *Cotesia*. Parasitoids that search for hosts have to deal with variability in the availability of chemical cues emitted by the food plants of their hosts. It was demonstrated that volatile profiles of plants infested by different (related) herbivore species show few differences, mainly of quantitative nature. It is unknown whether the sensory sensitivity of parasitoids is increased with experience, i.e. whether naive parasitoids are less able to detect these differences compared to experienced females. On the other hand, parasitoids may actively choose not to discriminate between such subtle differences, until they become more alert to these differences through experience and discriminate (Vet et al., 1997). Based on results found for *Leptopilina heterotoma*, a parasitoid of *Drosophila* flies, a recent hypothesis (Vet et al., 1997) states that qualitative differences in (chemical) information between different substrates are more easily being learned by parasitoids than quantitative differences. Wasps may perceive qualitative differences as relevant information, and use this information to discriminate between different substrates, whereas quantitative differences may be regarded as irrelevant 'noise'. Parasitoids may generalize similarities between herbivore-infested plant varieties to effectively expand their host-foraging arena. Discrimination between substrates with quantitative differences may occur, when these differences inform parasitoids about e.g. high or low host densities (Chapter 8) or host presence or absence (Papaj et al., 1994). In our system, naive *Cotesia* females were able to discriminate between infested Brussels sprouts and infested red cabbage or infested nasturtium (Chapter 3), that showed qualitatively different volatile profiles (this study). *Cotesia glomerata* females did not discriminate between infested Brussels sprouts and infested white cabbage (Vet & van Aaken, unpubl. results), although qualitative differences between volatile profiles of Brussels sprouts and white cabbage are found in this study. Although different with respect to volatile profiles, Brussels sprouts and white cabbage are very similar with respect to plant colour and shape, which may cause female parasitoids to actively decide not to discriminate between these plants during their search for hosts.

It is known that parasitoids use visual cues such as colour and shape while searching for hosts (Wäckers & Lewis, 1993) and *C. glomerata* females were able to learn to discriminate between herbivore-infested Brussels sprouts and herbivore-infested red cabbage differ in colour (Chapter 6). Additional behavioural experiments with plant-herbivore complexes, that only differ quantitatively and/or qualitatively in emitting information are needed to test the hypothesis given by Vet et al. (1997), that parasitoids more easily learn to pay attention to qualitative differences than to quantitative differences between odour bouquets and that parasitoids initially decide to neglect quantitative differences between substrates, until the parasitoid through experience learns that such differences are reliably associated with essential variation in host presence.

5

THE ROLE OF HOST SPECIES, AGE AND DEFENSIVE BEHAVIOUR ON OVIPOSITIONAL DECISIONS IN A SOLITARY SPECIALIST AND A GREGARIOUS GENERALIST PARASITOID (*Cotesia* SPECIES).

ABSTRACT

The main objective of this study was to determine the extent to which host acceptance behaviour as related to host species, age, and defensive behaviour might explain the differences in host use that exist between two congeneric and sympatric species of parasitic wasps. *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) is gregarious and generalist on several species of Pieridae, whereas *C. rubecula* (Marshall) is solitary and specific to *Pieris rapae* (L.). *Cotesia* species differed in their responses to host species (*P. brassicae* (L.), *P. napi* (L.) and *P. rapae*) and developmental stage (early and late 1st, 2nd and 3rd instars). In no-choice tests, host acceptance by *C. rubecula* was higher for *P. rapae* and females did not distinguish among the 6 host ages. In contrast, when foraging for *P. brassicae* and *P. napi*, *C. rubecula* females more readily attacked early first instar. *Cotesia glomerata* showed a higher degree of behavioural plasticity towards acceptance of *Pieris* species and host age than did *C. rubecula*. *Cotesia glomerata* females parasitized the three *Pieris* species and showed higher acceptance of first and second instars over third instar. Oviposition success was also influenced by host defensive behaviour. The frequency and the effectiveness of defensive behaviour rose with increasing age of the host, *P. brassicae* being the most aggressive *Pieris* species. Furthermore, the mean duration of *C. glomerata* oviposition was significantly reduced by the defesive reactions of *P. brassicae*, which would likely affect parasitoid fitness as oviposition time is positively correlated to clutch size in *C. glomerata*. Acceptance frequencies corresponded well to field reports of *Pieris-Cotesia* associations and to patterns of parasitoid larval performance, suggesting that the acceptance phase might be used as a reliable indicator of *Cotesia* host-specificity.

INTRODUCTION

Whereas feeding niche is one of the most frequently studied aspects of insect herbivore ecology, relatively little is known on host use by parasitoids. Scarcity of extensive field records of host-parasitoid present and past associations (Askew & Shaw, 1986; Shaw, 1994), unreliability of host-parasitoid lists in the literature (Askew & Shaw, 1986; Noyes, 1994), and lack of quantitative comparative studies have prevented the development of a general model of host range in parasitoids.

The host specificity of parasitoids is generally thought to reflect phylogenetic differences and to be determined by the physiological capacity of immatures to exploit the host and the behavioural ability of adults to find the resource. Typically, parsitoids are strongly constrained by the physiological suitability and nutritional value of the host, as reflected by immature survival and overall adult fitness components (Slansky, 1986). Hosts also differ in spatial and temporal availability as well as in detectability for foraging parasitoids (Vet & Dicke, 1992). Furthermore, a variety of other determinants such as the effectiveness of behavioural defenses of the host and the presence of natural enemies, as suggested for insect herbivores (Gilbert & Singer, 1975), could also affect the expression of host use by parasitoids.

To better understand parasitoid host specificity it is of interest to estimate the extent to which each component of the above determinants are involved. The purpose of the present study was to examine the role of host acceptance behaviour. Even though foraging parasitoid females respond to stimuli closely associated with potential hosts (Lewis et al., 1990; Vet & Dicke, 1992), they are likely to encounter individuals that differ in their behavioural and immune defenses and in their nutritional value. As the acceptance phase represents the ultimate step of a hierarchy of types of behaviour in a parasitoid's search for hosts, it might be used as a reliable indicator of parasitoid host-specificity.

To date, most studies have focused on single host-parasitoid associations or on single parasitoid-multiple host associations. A more powerful procedure is to investigate multiple host-parasitoid associations (van Alphen & Vet, 1986). Specifically, such an approach has recently proved to be profitable in characterizing the biological attributes of generalist and specialist parasitoids (Poolman Simons et al., 1992; Wiskerke & Vet, 1994; Brodeur & Vet, 1995; chapter 3).

The system we have been using, *Cotesia glomerata* (L.) and *C. rubecula* (Marshall) (Hymenoptera: Braconidae), which potentially parasitize larvae of three pieris butterflies, *Pieris brassicae* (L.), *P. rapae* (L.) and *P. napi* (L.) (Lepidoptera: Pieridae), has favourable attributes for the study of parasitoid host-specificity from a comparative perspective. The gregarious *C. glomerata* is a generalist larval endoparasitoid that attacks and develops successfully in several hosts belonging to a few genera of the Pieridae (Laing & Levin, 1982). In contrast, the solitary *C. rubecula* is known primarily as a specialist on *P. rapae*, but has been sporadically recovered from *P. brassicae* (Richards, 1940).

The main objective was to determine the extent to which host acceptance behaviour, as related to host species, age, and host defensive behaviour, might explain the differences in host use that exist between *C. glomerata* and *C. rubecula*. An *a priori* hypothesis would be that *C. rubecula* is more absolute than *C. glomerata* in its oviposition decision. Acceptance should therefore be higher in *C. rubecula* parasitizing *P. rapae* than in any other host-parasitoid combination. Given that younger larvae are the most profitable stages for the development of both *Cotesia* species (Nealis et al., 1984; Sato, 1980), we also predicted that younger larvae should be the most readily accepted hosts in all host-parasitoid combinations. Furthermore, mobile hosts often defend themselves against attacks by parasitoids. A general pattern is that the effectiveness of the behavioural defenses of the host increases with age (Gross, 1993). We predicted that each of the *Pieris* species we tested follow this pattern and that the consequences in terms of host acceptance are the same for both the specialist *C. rubecula* and the generalist *C. glomerata*. Part of this study also examined the relationship between host defensive behaviour and oviposition time for the gregarious *C. glomerata*, and considered how this interaction may influence the fitness of the parasitoid.

We tested these hypotheses by measuring host acceptability, oviposition time, and susceptibility of ovipositing females to host defensive behaviour. Because we were promarily interested in acceptance behaviour (rather than in host preference), we used non-choice tests for ovipositional responses. We also chose to use experienced *Cotesia* females as it is well documented that parasitoid foraging behaviour and motivation to oviposit often depend on genetically fixed and learned components (Mangel, 1989; Vet & Groenewold, 1990). Of special significance, oviposition experience with an actual host has been shown to affect the subsequent response (acceptance and oviposition duration) of parasitoid females (Ikawa & Suzuki, 1982; Drost & Cardé, 1990).

MATERIALS & METHODS

Insects. Parasitoid and butterfly cultures were established from individuals collected near Wageningen, The Netherlands. Caterpillars were maintained on Brussels sprouts plants (*Brassica oleracea*) at 25°C, 50-70% r.h. under a 18:D6 photoregime. *Cotesia glomerata* and *C. rubecula* were reared on *P. brassicae* and *P. rapae*, respectively. Parasitoid cocoons were held in large Petri dishes under the same temperature and humidity conditions, but under a L16:D8 photoregime.

Following eclosion, males and females were caged together to allow mating. They had access to honey, but not to hosts. All females used for experimentation were selected at random; they were 3-5 days old, an optimal age for maximum searching behaviour in response of *C. glomerata* (Steinberg et al., 1992). Females had had a foraging experience 6 h prior to the test. In this instance, females were introduced for 1 h in a large cage with Brussels sprouts plants infested with hosts (at a parasitoid:host ratio of 1:5) from the same species and from the same age class as the ones to be tested. Between the experience treatment and the test, females were kept individually in a petri dish of 5 cm in diameter and 1.5 cm in depth, and provided with honey. All females were only trained and tested once.

Treatment. For each of the six host-parasitoid associations: *C. glomerata* and *C. rubecula* on *P. brassicae*, *P. rapae* and *P. napi*, we tested the parasitoid response to six different classes of caterpillar: early and late first, early and late second and early and late third instar of *Pieris* hosts. Hosts of a specific age class were obtained by transferring neonate larvae to fresh Brussels sprouts plants and rearing them as a synchronous cohort until they reache the desired stage. Early instars were newly moulted caterpillars (< 12 h) with individuals having a head wider than the width of the body; late instarts were characterized by fully grown caterpillars having an asymmetric swollen abdomen compared with the head capsule. Table 1 gives the range of fresh weight of a sample (n = 20 per type) of the *Pieris* larvae used in this study.

Table 1. Range of fresh weights (mg) of *Pieris brassicae*, *P. rapae* and *P. napi* larvae used in this study (N = 20).

Instar	*Pieris* host species		
	P. brassicae	*P. rapae*	*P. napi*
Early I	0.15-0.20	0.14-0.16	0.14-0.18
Late I	1.0-1.2	0.5-0.6	0.6-0.9
Early II	2.0-3.0	0.6-1.0	0.7-1.0
Late II	6.0-7.0	2.2-2.5	2.6-3.0
Early III	10.0-12.0	2.6-3.4	2.8-3.8
Late III	26.0-30.0	10.0-12.0	10.0-15.0

Bioassay. Host acceptance and the outcome of the *Cotesia* - *Pieris* interaction were examined using no-choice tests in the laboratory. Tests started by introducing a parasitoid female onto a cabbage leaf disk (2.5 cm in diameter) along with a feeding host in the center of the petri dish. In order to increase female motivation to search for the host, leaf disks were bearing host-derived products (e.g., frass, silk) as well as leaf damage areas to provide females with the corresponding stimuli from a particular host type. We assessed acceptability of caterpillarsto *Cotesia* by observing female wasps under a stereomicroscope (20-40X). The following types of behaviour were noted:

Antennal encounter: touching the host with the tips of the antennae.

Ovipositional attempt: the wasp stands still beside the host, raises her antennae and wings, and bends her abdomen into position to oviposit.

Oviposition: insertion of the ovipositor into the host.

Host defense: head and thorax thrashing, biting, regurgitation of fluid droplets or crawling away.

The duration of oviposition was measured with a stopwatch following every ovipositional attempt by *C. glomerata*. The outcome (successful or not) of the host's defensive behaviour was also noted. For *C. glomerata* we made a distinction between complete parasitoid failure to oviposit (ovipositor insertion is prevented) and partial parasitoid failure to oviposit (oviposition is interrupted) as a result of the host's defensive behaviours. Each test lasted 5 min, or ended when 3 antennal contacts or an oviposition had been recorded. Thirty females were tested for each combination of larval stages and host species.

Acceptance of a host is here defined as an attempt by the parasitoid to oviposit into the host. This definition takes into account oviposition failure due to the behavioural defenses of the host and is therefore broader than the one of Arthur (1981) who stated that host acceptance concurs with oviposition. furthermore, ovipositor insertion was assumed to be a reliable indicator of host acceptance as more than 99% and 89% of the oviposition insertions by *C. glomerata* (N = 140 observations) and *C. rubecula* (N = 288 observations), respectively, resulted in egg laying (data from Brodeur & Vet, 1995). Acceptability was calculated by dividing the number of oviposition attempts by the number of antennal contacts per female and converted to percentages by multiplying per 100. Probing behaviour by *C. glomerata* (ovipositor insertion of less than 2 s; see le Masurier, 1990) was rare and was not considered in the calculation of the acceptability.

Statistical analysis. The effects of parasitoid species, host species and host developmental stage on the frequency of host acceptance were examined using a logistic regression analysis (Aitkin et al., 1989; GLIM procedure). Percentage data were transformed by logit transformation prior to analysis. Significant main effects and interactions were analysed by G-tests for goodness-of-fit to test for differences between species and host-age within and among the factors (Sokal & Rohlf, 1981). For the effect of host defensive reactions on oviposition time by *C. glomerata*, treatment means were compared using Student's t-test. Significance levels were $P = 0.05$ for all tests.

RESULTS

The pattern of host acceptance of *Pieris* hosts by *C. glomerata* varied considerably from that of *C. rubecula*. The significant Parasitoid X Host interaction (Table 2) indicates that parasitoids differ in their relative responses to the three hosts. *Cotesia glomerata* accepted and oviposited in all three host species (Figure 1). It showed a higher acceptance of *P. brassicae* than of *P. rapae*, while *P. napi* was the least accepted species. By contrast, *C. rubecula* was more specialized in its choice of hosts for oviposition (Figure 2). the frequency of acceptance toward *P. rapae* was striking; more than 95% of the caterpillars were accepted following an antennal contact.

Both *Cotesia* species showed similar patterns of host-stage acceptance (non-significant Parasitoid X Instar interaction, Table 2). However, a significant interaction was found between the host and their stage, young larvae being more frequently accepted by wasps than older larvae (Figures 1 and 2), except for *C. rubecula* parasitizing *P. rapae* where all six host developmental stages were readily accepted (Figure 2).

Table 2. Logistic regression analysis of the significance of parasitoid species, host species and host stage, together with the resultant interactions, in host acceptance of *Cotesia glomerata* amd *C. rubecula*.

Source	Deviance[1]	Df	Chi^2 Prob.
Parasitoid	15.4	1	<0.001
Host	290.7	2	<0.0001
Instar	154.4	5	<0.0001
Parasitoid X Host	147.4	2	<0.0001
Parasitoid X Instar	9.2	5	0.10
Host X Instar	37.4	10	<0.001
Parasitoid X Host X Instar	27.3	10	0.002

Pieris caterpillars responded to *Cotesia* attacks with defensive behaviour. Due to the small number of ovipositions obtained from large caterpillars, data from early and late third instar were pooled. For each host-parasitoid association, the frequency of defensive behaviour rose with increasing age

4). The first instar of *P. brassicae* showed a stronger reaction than those of *P. rapae* and *P. napi*. The efficiency of aggressive behaviour followed similar patterns. Large caterpillars were more effective in physically repelling attacks by *Cotesia* wasps than younger ones (Figures 3 and 4), and the percentage of *P. brassicae* that successfully overcame parasitoid attacks was higher than the two other *Pieris* species. Remarkably, most of the *P. rapae* and *P. napi* caterpilars which displayed defensive behaviour were not capable of interrupting oviposition of either *Cotesia* species.

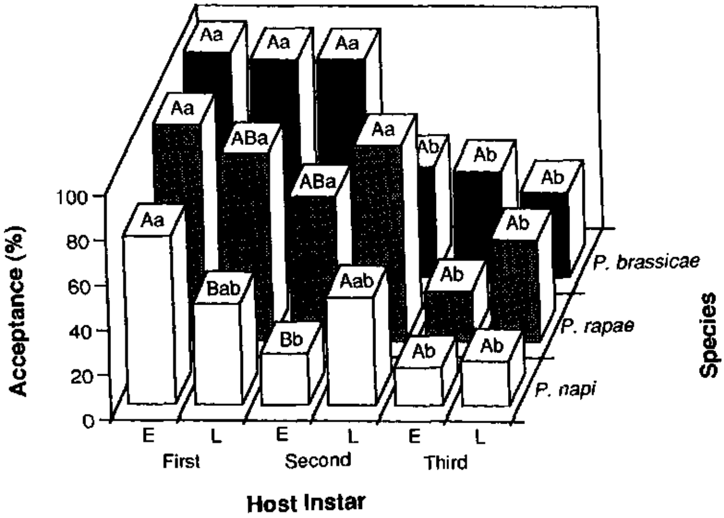

Fig. 1. Distribution of the percentage of acceptance by *Cotesia glomerata* of three different *Pieris* host species at six different host stages. Lower case and capital letters above bars indicate differences within rows and columns, respectively. Means followed by different letters are significantly different (G-test for goodness of fit, $P<0.05$). Thirty females were tested for each treatment. E and L correspond to early and late instar, respectively.

The *C. glomerata* oviposition sequence may be interrupted by host defensive reactions. Figure 3a showed that a significant proportion of *P. brassicae* aggressive behaviour resulted in partial oviposition failure. As a result, the mean duration of oviposition recorded for each host developmental stage tested was significantly reduced, except for early first instar (Figure 5). Too few successful host defensive reactions by *P. rapae* and *P. napi* were recorded to warrant a similar analysis.

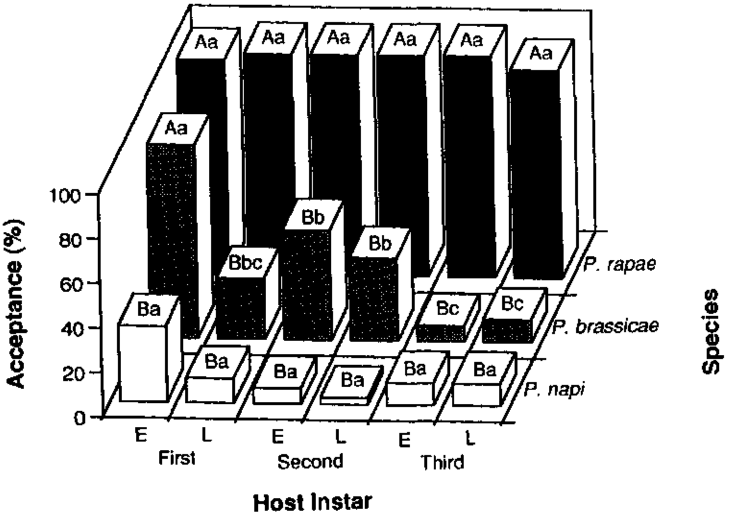

Fig. 2. Distribution of the percentage of acceptance by *Cotesia rubecula* of three different *Pieris* host species at six different host stages. Lower case and capital letters above bars indicate differences within rows and columns, respectively. Means followed by different letters are significantly different (G-test for goodness of fit, $P < 0.05$). Thirty females were tested for each treatment. E and L correspond to early and late instar, respectively.

DISCUSSION

The results support the hypothesis we initially proposed on the expected concordance between *Cotesia* host range and host acceptance. Acceptance is higher in the specialist *C. rubecula* parasitizing *P. rapae* than in any of the other *Cotesia* - *Pieris* associations, except for *C. glomerata* attacking early first instar of *P. rapae* and *P. brassicae*. On encountering *P. rapae*, all *C. rubecula* females directly showed oviposition behaviour, whereas only a small proportion of interactions with *P. brassicae* and *P. napi* led to oviposition. On the other hand, *C. glomerata* showed a higher degree of

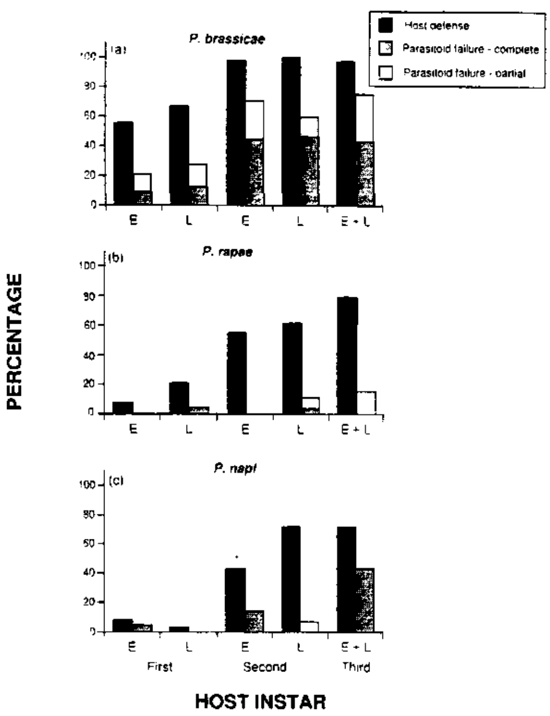

Fig. 3. Defensive reactions (%) shown by different host stages of *Pieris brassicae* (a), *P. rapae* (b) and *P. napi* (c) in response to an attack by *Cotesia glomerata*, together with the proportions of interaction that result in parsitoid complete or partial failure to oviposit. E and L correspond to early and late instar, respectively. * N < 5.

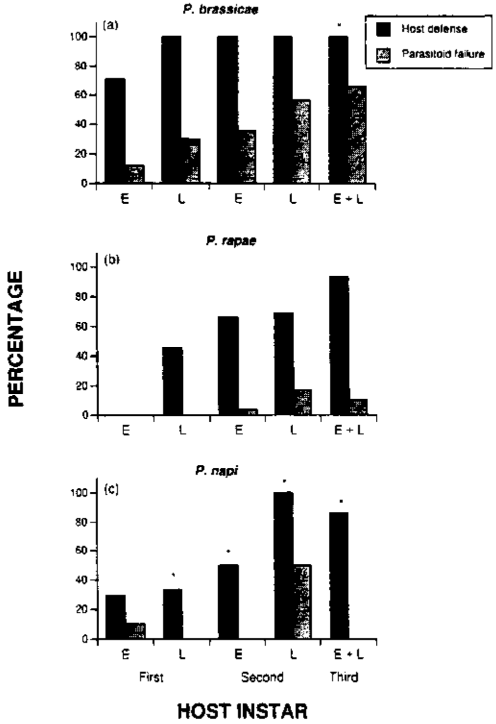

Fig. 4. Defensive reactions (%) shown by five different host stages of *Pieris brassicae* (a), *P. rapae* (b) and *P. napi* (c) in response to an attack by *Cotesia rubecula*, together with the proportions of interaction that result in parasitoid failure to oviposit. E and L correspond to early and late instar, respectively. * N < 5.

behavioural plasticity towards acceptance of *Pieris* host sepcies than did *C. rubecula*. Consistent with reliable field reports about *C. glomerata* specificity (see Laing & Levin, 1982), *C. glomerata* females were motivated to parasitize all three *Pieris* species tested.

Our prediction that younger and smaller caterpillars would be the most readily accepted hosts in all *Pieris* - *Cotesia* associations is partially supported. Overall, *C. glomerata* discriminate between hosts of different ages, showing a higher oviposition acceptability of first and second instars than of third instar. This pattern corresponds well with the attack rate of *C. glomerata* in the field, as 13 times as many ovipositions occurred on first instar as on either second or third instar *P. rapae* introduced in cabbage plots (Van Driessche, 1988). In contrast, no consistent patterns are observed between host age and acceptability in *C. rubecula*. Noticeabley, *C. rubecula*

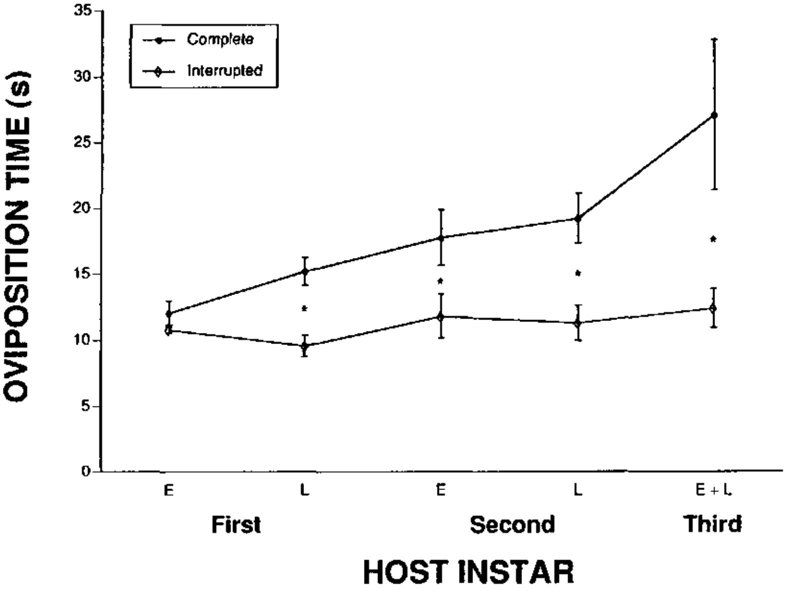

Fig. 5. Changes in mean duration of *Cotesia glomerata* oviposition interrupted by defensive reactions of five different host stages of *Pieris brassicae*. * indicate significant differences between means (t-tests, $P<0.05$). Error bars represent SE, E and L correspond to early and late instar, respectively.

accepted and oviposited in early and later 1st, 2nd and 3rd instars of *P. rapae* . These results are in agreement with those from other studies where *C. rubecula* was found to oviposit in all larval instars of *P. rapae* (Parker & Pinnell, 1973; Nealis et al., 1984). At this point, we might hypothesize that the flexibility in the use of hosts of different ages by *C. rubecula* may be an attribute of specialist larval parasitoids, in which the number of host species included in the diet is limited, but the range of host age accepted for oviposition is extended.

Within the *Cotesia - Pieris* system that we have in culture in Wageningen, the observed frequencies of host acceptance of both *C. glomerata* and *C. rubecula* appear to be adaptive, as females more readily accept host species that maximize survival of the immature and weight of the offspring (Chapter 9).

During the host acceptance phase, and the egg laying phase, parasitoid females are exposed to physical host defenses. Our results show that *Pieris* species differ in their vulnerability to both *Cotesia* species. *Pieris brassicae* possesses effective behavioural defenses against *Cotesia*, whereas *P. rapae* and *P. napi* display relatively weak and inefficient defensive responses. Such a difference could even be greater for gregarious *P. brassicae*, as caterpillars may also benefit from communal defense (Stamp, 1982). Secondly, in accordance with the general pattern observed in most arthropods, the effectiveness of *P. rapae* behavioural defeses increase with age. However, even though the *P. rapae* and *P. napi* weight distribution tested in this study varies over the range of 0.14 to 15.0 mg, there is no clear indication that *Cotesia* are less successful in attacking larger caterpillars of these two species. Third, the frequency of *Pieris* defensive reactions increased with the host age (size), the increase being more pronounced for *P. rapae* and *P. napi* than for *P. brassicae*. These variations in the nature and effectiveness of *Pieris* sp. aggressive behaviour might be related to their overall defensive strategy towards predators and parasitoids. *Pieris rapae* and *P. napi* caterpillars are cryptic, solitary feeders and appear to rely mostly on avoidance to reduce mortality from natural enemies. In contrast, *P. brassicae* caterpillars are aposematic, they feed gregariously during the first three larval instars and display aggressive behaviour towards natural enemies.

Behavioural interactions between host and ovipositing parasitoid would not only determine the outcome of the interaction, but may also contribute to shape host species and host age preference by parasitoids (Taylor, 1988; Allen, 1990). All other aspects of host availability and suitability for development being equal, if *Cotesia* females experience different levels of risk when attacking hosts of different species and developmental stage, they shoud evolve a preference for the less menacing hosts. Hence, the effect of *P. rapae* and *P. napi* weak defensive behaviour seems negligible in determining host utilisation by *Cotesia*. Head-thrashing, biting and regurgitation by *P. brassicae* caterpillars occasionally injured parasitoids and may have influenced patterns of host use by *Cotesia*. Furthermore, we showed that the mean duration of oviposition is significantly reduced by the defensive reactions of *P. brassicae*. This has obvious implications for parasitoid fitness, as there is a positive correlation between oviposition time in non-parasitized hosts and the size of the clutch in *C. glomerata* (Ikawa & Suzuki, 1982).

Determining parasitoid host specificity is complex and involves various levels of investigation. Parasitoid-host associations reflect differences in phylogeny or in selective pressure and may result from interactions at ecological, behavioural and physiological levels. So far, in our quest for significant determinants of *Cotesia*-host associations, we showed that some factors, both physiological and behavioural, do not provide conclusive explanations for *C. glomerata* and *C. rubecula* host specificity. First, experimental evidence indicates that the specialist *C. rubecula* and the generalist *C. glomerata* do not differ significantly in the long-range host-searching strategy with regards to infochemicals (Steinberg et al., 1992; Agelopoulos & Keller, 1994a; Chapter 2 and 3). For both species, plant-derived information was more attractive than stimuli associated with the host larvae, the latter playing a major rolein the short-range host location phase (Sato, 1979; Nealis, 1986; Mattiacci & Dicke, 1995; Agelopoulos et al., 1995). Thus, processes involved in habitat selection do not appear to predominate in determining the specificity of *Cotesia* toward *Pieris* hosts. Second, the differences in the observed *Cotesia* host ranges are not primarily based on immunological compatibility of the *Pieris* hosts (Brodeur & Vet, 1995); levels of encapsulation of *C. rubecula* eggs are not consistent with host preference. Third, an imperfect concordance exists between *Cotesia* host use and overall host suitability. The specialist *C. rubecula* is physiologically able to develop in host species (*P. brassicae*) that are rarely attacked under field conditions (Chapter 9 and 10).

The present study indicates that acceptance frequencies are reliable indicators of parasitoid specificity, suggesting that interspecific differences in the degree of specialization in *Cotesia* could arise from differences occurring at the acceptance phase. Acceptance, as an 'active' behavioural selection of host types by parasitoid females, involves the detection of physical and/or chemical stimuli produced by the host (Arthur, 1981; Vinson, 1985). The type of behavioural response, more or less innate, of *Cotesia* females towards these stimuli may therefore contribute substantially to the probability of oviposition, given an encounter with a *Pieris* host. Further work is required on the mechanisms and heritability of host acceptance behaviour by *C. glomerata* and *C. rubecula* to explain the observed relation with host range.

II. LEARNING AND HOST-SEARCHING BEHAVIOUR

immature or emerging insect may learn cues resulting in preferences for these cues in the adult (Hopkins, 1917; Hérard et al., 1988; Kester & Barbosa, 1992; Cortesero & Monge, 1994). During the adult stage, parasitoids may gain experience by associating certain stimuli with the presence of hosts, and use these stimuli in subsequent host-finding activities (Lewis & Tumlinson, 1988; Vet & Groenewold, 1990; Vet et al., 1990b; de Jong & Kaiser, 1991; Turlings et al., 1993). This experience can induce behavioural changes, such as increased or new responses towards certain stimuli. The mechanisms involved in these changes are priming and preference learning respectively. A temporary enhancement of general responsiveness to host-finding cues present can lead to an increase in host encounter rate (Papaj & Vet, 1990; Martin & Lewis, unpublished study). Preference learning can be expected when an individual parasitoid has to make a large number of foraging decisions during its lifetime, when variation of the environment is predictable and when the species is not specialized on one host species or host microhabitat (Vet et al., 1995; Potting et al., 1996). The relation between the degree of specialization of parasitoids and their learning abilities was the subject of the present study.

The adaptive value of possible interspecific differences in learning can be elucidated through a comparative approach (Vet & Dicke, 1992; Poolman Simons et al., 1992). In this paper the role of experience in parasitoid host-seeking behaviour will be discussed for two closely related parasitoid species of *Pieris* caterpillars (Lepidoptera: Pieridae), that differ in their diet breadth. *Cotesia glomerata* is considered to be a generalist on both the host level and the food plant level (Laing & Levin, 1982), and *C. rubecula* is more specialized at the host level and the plant level (Shenefelt, 1972). Both parasitoid species have overlapping niches. The pierid hosts of *C. glomerata* feed on cruciferous food plants, but food plants have also been reported from the families Tropaeolaceae, Rosaceae and Berberidacae. Food plants of the preferred host of *C. rubecula* is more restricted to plant species that contain glucosinolates (Terofal, 1965; Feltwell, 1982; Tax, 1989).

The hypothesis we tested for this system is whether the effect of experience differs for species that differ in the degree of specialization. The value of behavioural modification through learning is expected to be higher for the generalist *C. glomerata* than for the specialist *C. rubecula*, as *C. glomerata* can potentially exploit more host species with a broader food plant range.

The first question we want to answer is to what extent the preferences of naive *C. glomerata* for plant-herbivore complexes are shaped by the food plant of the host in which the parasitoid developed. Early adult learning may affect the host-finding behaviour of naive parasitoids (Hopkins, 1917; Hérard et al., 1988; Cortesero & Monge, 1994), or responses can be shaped by selection. In our laboratory, experimental wasps have been reared for a maximum of 20 generations on host larvae that feed on Brussels sprouts plants. Naive females showed a preference for this cabbage cultivar in wind tunnel experiments (Chapter 3). In a selection experiment we studied the host-finding behaviour of *C. glomerata* females that emerged from *P. brassicae* larvae feeding on a non-cruciferous plant.

The second question is, to what extent preference learning by the host-seeking adult of both parasitoid species plays a role in the expression of preferences for host and plant species.

MATERIALS & METHODS

Insects. Parasitoid and herbivore species originated from individuals collected in Brussels sprouts fields near Wageningen, The Netherlands. *Pieris brassicae*, *P. rapae* and *Mamestra brassicae* larvae were obtained from colonies maintained on Brussels sprouts plants in a climatic room at 20-22°C, 50-70% RH and a L16:D8 photoregime. Caterpillars used for the experiments were always late first instar larvae. *Cotesia glomerata* and *C. rubecula* colonies had been maintained on *Pieris brassicae* and *P. rapae* larvae respectively, feeding on Brussels sprouts plants (*Brassica oleracea* gemmifera cv. Titurel) under the same conditions. Caged parasitoid females were offered Brussels sprouts leaves with first instar host larvae for parasitization, after which the leaves were transferred to cages with Brussels sprouts plants. Cocoons were kept in petri dishes (i.d. 9 cm) at 20-22°C, 50-70% RH and a 16L:8D photoperiod. Upon emergence males and females were caged together to allow mating. Cages (40*30*30 cm) were supplied with wet cotton wool and honey.

For the adult learning experiments 3-5 days old females were collected from these cages. The day prior to testing the females were given an experience (see *Experimental design*), after which individual females were kept overnight at 15°C in cotton wool-stopped glass vials with water and honey until they were tested.

For the early adult learning experiment *C. glomerata* were reared for several generations on *P. brassicae* in a similar way as described above, but instead of Brussels sprouts, nasturtium (*Tropaeolum majus* L. cv. Emperor of India) (Tropaeolaceae) was used as food plant for the parasitized host larvae. Cocoons were placed in a cage until emergence of the adult parasitoids. For wind tunnel tests, 3-5 day old, mated females were collected individually in glass vials, just prior to testing. For the experiments naive wasps were used, i.e. they had not contacted host or plant material. Two generations of wasps were tested. Females that chose the *P. brassicae*-infested nasturtium in these tests were allowed to parasitize *P. brassicae* larvae feeding on nasturtium leaves, to create an additional colony on nasturtium. Subsequently 3-5 days old, naive daughters of this F1 generation were used for wind tunnel experiments, and those females that preferred infested nasturtium over infested Brussels sprouts were used for parasitization, to create an F2 generation.

Odour sources. Food plants used in this study were the cabbage varieties Brussels sprouts (*Brassica oleracea* gemmifera cv. Titurel), white cabbage (*Brassica oleracea capitata* L. var. alba cv. Olympiade, cv. Carlton and cv. Bartelo) (see results), cauliflower (*Brassica oleracea* L. var botrytis L. cv. Alpha of Record), red cabbage (*Brassica oleracea capitata* L. var. rubra (DC.) cv. Langedijker vroege rode) and nasturtium (*Tropaeolum majus* L. cv. Emperor of India) (Tropaeolaceae). Food plants were reared in a greenhouse, at 20-25°C., 50-70% RH and a L16:D8 photoperiod. When necessary, plants were treated against aphids with dichlorophosphate (DDVP), but not within a period of four days before the experiments. To obtain the odour sources for the greenhouse experiments (see below), plants were infested by transferring eggs of the desired herbivore species from Brussels sprouts plants to the test plants. For the wind tunnel experiments (see below) odour sources were obtained by transferring early first instar larvae of the desired species from the plant on which they hatched to the same kind of test-plant. Larvae were allowed to feed for 24 hours. To avoid quantitative differences in volatile emission, the number of larvae of each species was chosen in such a way that the amount of damage inflicted - measured as the periphery of the damaged spot times leaf thickness - was approximately the same for each plant-herbivore complex.

Bioassay. The wind tunnel as described in Chapter 2 was used for all experiments except one. The effect of experience on the food plant preference was tested in the greenhouse flight set-up as described by Steinberg et al. (1992), but without the screen between parasitoid release point and odour sources. For the *wind tunnel* experiments, odour sources consisted of herbivore-damaged leaves and were offered in two-choice situations between two plant-host complexes that had been used for the experience. Care was taken that all odour sources of the same experimental group were offered on the same day. The position of the odour sources was switched after testing four females (two of each parasitoid species). Tests lasted for a maximum of five minutes for each female. A 'choice' was recorded as the leaves on which a female landed. All other landings were recorded as 'no response'. Each female wasp had only one flight opportunity within a test and was offered one test combination (treatment) only. For the experiments in the *greenhouse set-up* odour sources consisted of herbivore-infested individually potted plants. Test methods were the same as in the wind tunnel experiments, except that *C. glomerata* and *C. rubecula* were not tested in the same test period.

Experimental design.

Early adult learning was studied by offering *P. brassicae*-infested Brussels sprouts leaves or red cabbage leaves versus nasturtium leaves in a dual choice test to naive F1 and F2 *C. glomerata* females of the general rearing on nasturtium. Furthermore, naive females of the F1 and F2 generations of the additional nasturtium-reared colony (based on females that showed an innate preference for *P. brassicae*-infested nasturtium over *P. brassicae*-infested Brussels sprouts in wind tunnel tests) were tested in the nasturtium - Brussels sprouts situation as well.

Adult learning was tested by giving females experience with a particular plant-host complex (as described below). Half of the females in a group experienced one plant-host complex of the combination to be tested, the other half the other plant-host complex. Experienced wasps were transferred to glass vials and kept as described before.

Preference for host species. The effect of experience on the preference for host species infesting Brussels sprouts leaves was tested for females that were experienced in the following ways:
1) leaf-damage experience: for 15-30 s female wasps were allowed to contact an herbivore-damaged leaf from which larvae had been removed. Females of *C. glomerata* were offered leaves infested by *P. brassicae* (host) or by *M.brassicae* (non-host). *Cotesia rubecula* females were offered leaf damage by *P. rapae* (host) or by *M. brassicae* (non-host); 2) single oviposition experience: individual females were allowed to parasitize one larva of either *P. brassicae* or *P. rapae* on an herbivore-infested leaf; 3) multiple oviposition in the same host species: individual *C. glomerata* females were allowed to oviposit in two *P. brassicae* or in two *P. rapae* larvae on an herbivore-infested leaf; *C. rubecula* was offered two or three *P. rapae* larvae for oviposition on an herbivore-infested leaf. 4) leaf-damage experience on a leaf infested by both *P. brassicae* and *P. rapae*; 5) double oviposition in different host species: individual females were allowed to parasitize one larva of *P. brassicae* plus one larva of *P. rapae*, or in the reverse order on herbivore-damaged leaves.

For oviposition experience, *P. brassicae* were offered in clusters of 20-25 first instar larvae per leaf and the solitary *P. rapae* larvae had a scattered distribution over the leaf. After the desired number of larvae had been parasitized, wasps were transferred individually to glass vials.

Preference for plant cultivar or species. In all tests Brussels sprouts was one of the plant species offered, next to red cabbage, white cabbage or nasturtium. For *C. glomerata*, experience and tests were conducted on *P. brassicae*-infested leaves. The following experience groups were created:

1) leaf-damage experience on Brussels sprouts or white cabbage; 2) single oviposition on Brussels sprouts or white cabbage; 3) multiple oviposition: females were allowed 5 subsequent ovipositions on Brussels sprouts or white cabbage; 4) a combination of leaf-damage experience, a flight and five subsequent ovipositions on Brussels sprouts or white cabbage: in the greenhouse set-up individual females were allowed to contact herbivore-infested leaf material for 15-30 s, subsequently fly to a herbivore-damaged plant of the same type, on which they were allowed to oviposit in five host larvae; 5) leaf-damage experience on Brussels sprouts or cauliflower; 6) leaf-damage experience on Brussels sprouts or red cabbage; 7) leaf-damage experience on Brussels sprouts or nasturtium and
8) a combination of leaf-damage experience, a flight and five subsequent ovipositions on Brussels sprouts or nasturtium.

For *C. rubecula* all leaves were infested by *P. rapae* larvae and the experience treatments were 1) leaf-damage experience on Brussels sprouts or white cabbage; 2) a single oviposition on Brussels sprouts or white cabbage; 3) leaf-damage experience on Brussels sprouts or cauliflower; 4) leaf-damage experience on Brussels sprouts or red cabbage and 5) three ovipositions on Brussels sprouts or red cabbage.

Statistical analysis. In all experiments choices within a test were analysed using binomial probability functions. Significance indicates preference for one of the two odour sources tested. Parasitoid choices in different treatments within each group were analysed using tests for independence (Siegel, 1988).

RESULTS

Early adult learning. The majority of the females of nasturtium-reared *C. glomerata* offspring of the first and second generation chose infested Brussels sprouts leaves (Fig 1a). The same is true for the first and second generation of the females with mothers that chose nasturtium (Fig 1b). In the choice test with red cabbage vs nasturtium again most females of the two generations preferred the *P. brassicae*-infested cabbage variety over infested nasturtium (Fig 1c). Rearing the parasitoids in larvae that feed on nasturtium did not lead to a preference for this host food plant. Instead, parasitoids preferred cabbage varieties over nasturtium. Hence, we were not able to show an effect of the rearing plant on the preference of naive *C. glomerata*.

Adult learning. Preference for host species. Experience with leaf damage which was caused by only one herbivore species did not influence the choices of *C. glomerata* or *C. rubecula* for Brussels sprouts leaves infested by different herbivore species. No preference for *P. brassicae*- or the non-host species *M. brassicae*-infested leaves was found in *C. glomerata* (Fig 2a).

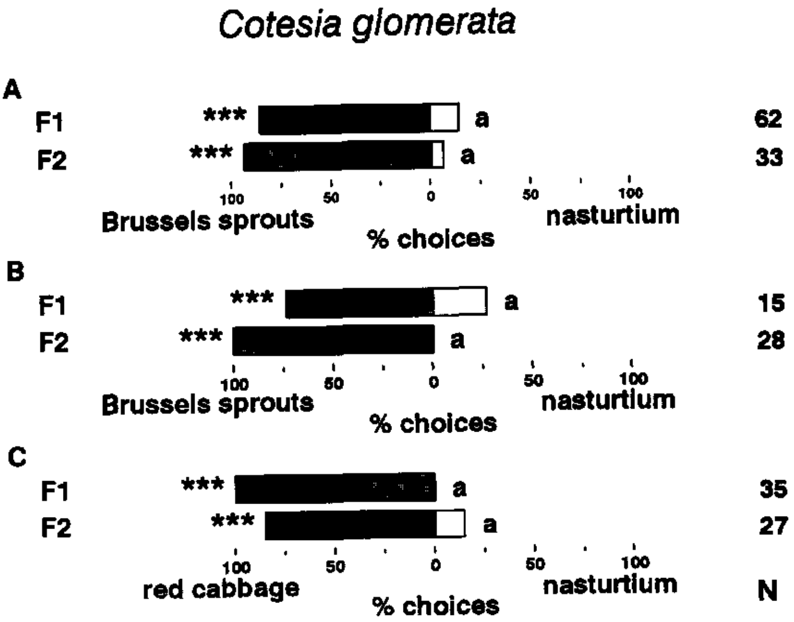

Fig. 1. Choice distribution of responding naive *C. glomerata* females over different *P. brassicae*-infested host food plants. (A) F1 and F2 generation reared in *P. brassicae* larvae feeding on nasturtium; (B) as (A), but mothers chose for nasturtium in flight tests; (C) as (A) but in tests red cabbage and nasturtium were offered as odour sources. Bars with the same characters indicate no significant differences in parasitoid choices between tests. Asterisks indicate a significant difference within a choice test: *** $P<0.0005$. Numbers of responding females are indicated by N.

After 1 oviposition on *P. brassicae* or *P. rapae* no preference for the experienced plant-host complex was found either (Fig 2b). Two ovipositions on *P. brassicae* led to preference for Brussels sprouts infested with this host species, but so did an experience of two ovipositions on *P. rapae*. The latter preference was as pronounced as that in the group that experienced *P. brassicae* (Fig 2c). The majority of the *C. glomerata* females that had contacted leaf material infested by both *P. brassicae* and *P. rapae* chose *P. brassicae*-infested leaves in their subsequent flight (Fig 2d). After multiple experience of an oviposition on *P. brassicae* and one on *P. rapae*, *C. glomerata* showed a clear preference for *P. brassicae*-infested leaves. The

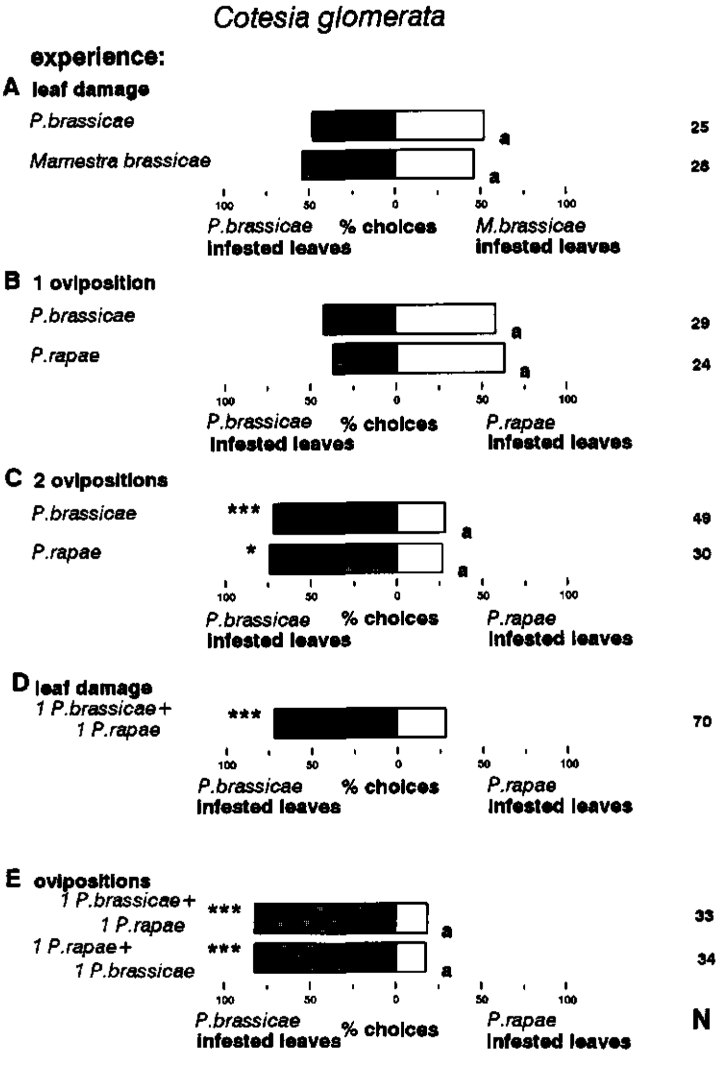

Fig. 2. Choice distributions over Brussels sprouts leaves infested by different herbivore species of responding *C. glomerata* females with different types of experience: (A) leaf-damage experience with leaves infested either by *P. brassicae* or *Mamestra brassicae*; (B) 1 oviposition in either *P. brassicae* or *P. rapae* on a herbivore damaged Brussels sprouts leaf; (C) 2 ovipositions in *P. brassicae* or *P. rapae*; (D) leaf-damage experience with a leaf infested by both *P. brassicae* and *P. rapae*; (E) 1 oviposition in *P. brassicae* followed by 1 oviposition in *P. rapae* or the reverse order. Bars with the same characters indicate no significant differences in parasitoid choices between tests. Asterisks indicate a significant difference within a choice test: *** $P<0.0005$; * $P<0.05$. Numbers of responding females are indicated by N.

order in which they had parasitized the host larvae did not affect their preference (Fig 2e). Hence, in *C. glomerata* discrimination between *P. brassicae*- and *P. rapae*-infested Brussels sprouts leaves can be induced. Experience with a single host species does not necessarily lead to preference for the experienced host.

Unlike *C. glomerata*, the specialist *C. rubecula* did not show any preference for *P. rapae*- or *P. brassicae*- damaged leaves regardless the type of experience (Fig 3). In all tests females chose as often for *P. rapae*-infested leaves as for *P. brassicae*-infested leaves.

Preference for plant-cultivars or species. Leaf-damage experience led to a preference of *C. glomerata* for Brussels sprouts when this was the experienced cabbage cultivar, but experience with white cabbage did not lead to a preference for white cabbage. The choice distribution of this last group differed significantly from the Brussels sprouts experienced wasps (Fig 4a). Perhaps at first glance surprising, one oviposition experience of *C. glomerata* on Brussels sprouts (after which the female was removed from the cluster of hosts) did not lead to a preference for infested Brussels sprouts, whereas females with only one oviposition experience on white cabbage preferred the non-experienced Brussels sprouts plant in their subsequent flight (Fig 4b). Choice distributions did not differ significantly between the two *C. glomerata* groups. Probably the removal from the cluster of hosts after one oviposition is experienced as 'negative' (see discussion). Five consecutive ovipositions (figure 4c) or an experience of leaf damage, flight and five ovipositions (figure 4d) showed the same pattern as experience with leaf damage only: 1. it led to a significant preference for Brussels sprouts in Brussels- sprouts-experienced females, 2. females with experience on white cabbage showed no preference for one of the cabbage varieties, 3. the choice distributions of the two groups within a test were significantly different.

Leaf-damage experience with cauliflower did not result in a preference of *C. glomerata* for cauliflower in the Brussels sprouts-cauliflower combination (figure 4e). However, *C. glomerata* females with leaf-damage experience on red cabbage (figure 4f) or nasturtium (figure 4g) showed significant preferences for the experienced plant cultivar or species, with significant differences between treatment groups. The same result was obtained in females with the more extensive experience of leaf-damage experience plus a flight plus five subsequent ovipositions (figure 4h).

For *C. rubecula* experience on different cabbage varieties infested by *P. rapae* larvae in no case resulted in a difference in preference between treatments (figure 5). Leaf-damage experience on white cabbage resulted in a preference for Brussels sprouts, but the choice distributions of the two treatment groups showed no significant difference (figure 5a). Females with

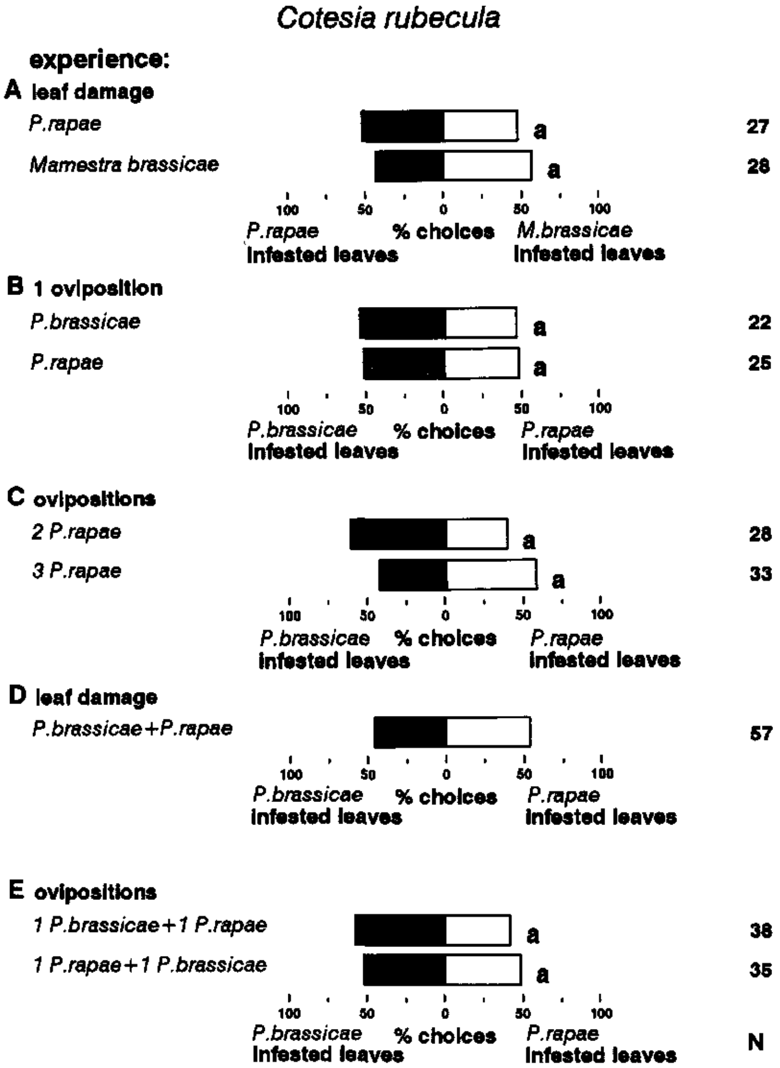

Fig. 3. Choice distributions over Brussels sprouts leaves infested by different herbivore species of responding *C. rubecula* females with different types of experience: (A) leaf-damage experience with Brussels sprouts leaves infested either by *P. rapae* or *Mamestra brassicae*; (B) 1 oviposition in either *P. brassicae* or *P. rapae* on a herbivore damaged Brussels sprouts leaf. (C) 2 ovipositions in *P. rapae* or 3 ovipositions in *P. rapae*; (D) leaf-damage experience with a leaf infested by both *P. brassicae* and *P. rapae*; (E) 1 oviposition in *P. brassicae* followed by 1 oviposition in *P. rapae* or the reverse order. Bars with the same characters indicate no significant differences in parasitoid choices between tests. Numbers of responding females are indicated by N.

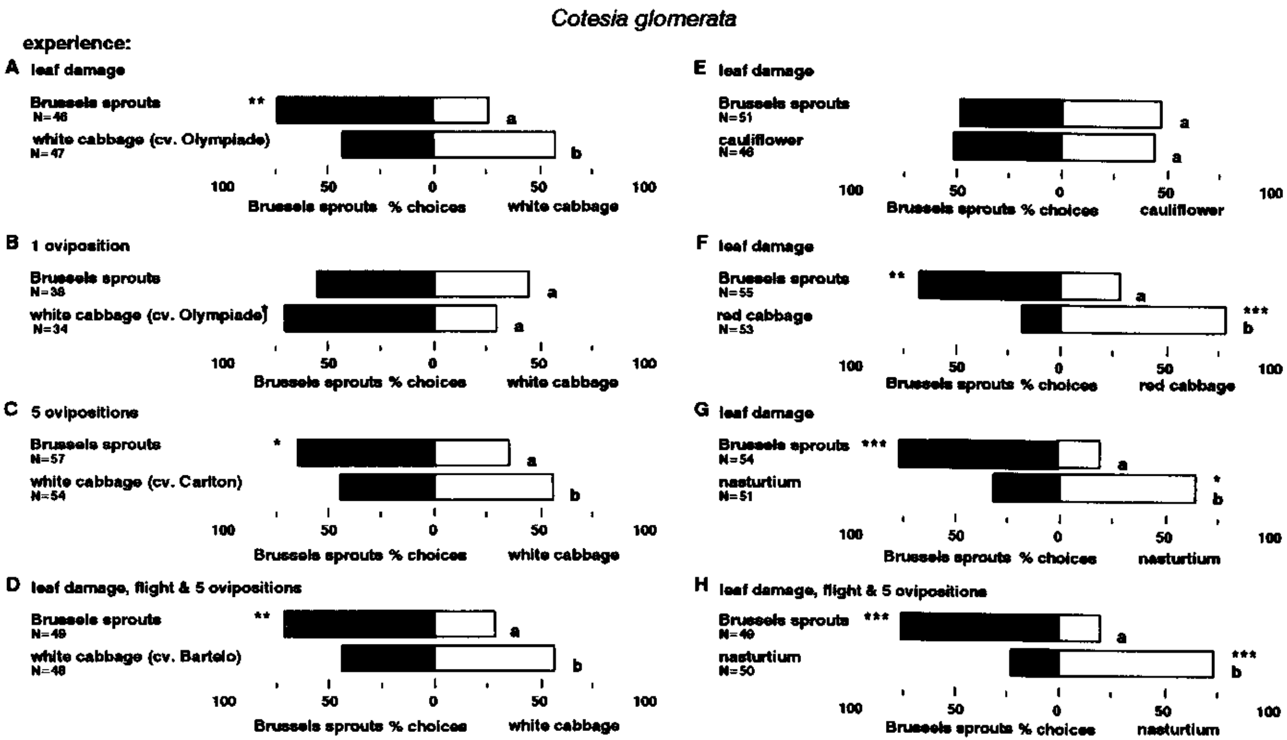

Fig. 4. Choice distributions over *P. brassicae*-infested Brussels sprouts or white cabbage of responding *C. glomerata* females with different types of experience: (A) leaf-damage experience with *P. brassicae*-infested Brussels sprouts or white cabbage; (B) 1 oviposition in *P. brassicae* on an infested Brussels sprouts leaf or an infested white cabbage leaf; (C) 5 ovipositions in *P. brassicae* on a Brussels sprouts leaf or a white cabbage leaf; (D) a combination of leaf damage, a flight and 5 ovipositions in *P. brassicae* either on Brussels sprouts or white cabbage; (E) leaf-damage experience with *P. brassicae*-infested Brussels sprouts or cauliflower; (F) leaf damage with *P. brassicae*-infested Brussels sprouts or red cabbage; (G) leaf damage with *P. brassicae*-infested Brussels sprouts or nasturtium; (H) a combination of leaf damage, a flight and 5 ovipositions in *P. brassicae* either on Brussels sprouts or nasturtium.. Bars with different characters indicate significant differences in parasitoid choices between tests. Asterisks indicate a significant difference within a choice test: *** $P<0.0005$; ** $P<0.005$; * $P<0.05$. Numbers of responding females are indicated by N.

an oviposition experience on the Brussels sprouts-*P. rapae* complex preferred this plant-host combination, but again the choice distributions of females in the two treatment groups did not differ significantly (figure 5b). Leaf-damage experience on Brussels sprouts or cauliflower had no effect on plant preference (figure 5c). Leaf damage or oviposition experience with Brussels sprouts or red cabbage both resulted in significant preferences for Brussels sprouts (figure 5d and 5e respectively).

DISCUSSION

In general, early adult- or emergence experience seems to be of less influence on adult host-searching behaviour than adult experience (Turlings et al., 1993). The same is true for *C. glomerata* for which we studied the effect of both kinds of experience.

Early adult learning.

Learning during immature parasitoid development has frequently been suggested as a mechanism for behavioural changes, although the effect may be the result of early adult learning, rather than learning by the immatures (Hérard et al., 1988; Caubet & Jaisson, 1991; Turlings et al., 1993; Cortesero & Monge, 1994). Potting et al. (1996) found no early adult learning (or any learning) in *Cotesia flavipes*, whereas for the parasitoid species *Campoletis sonorensis*, and *Microplitis croceipes* rearing environment did not seem to affect their subsequent responses, but the effect of adult learning was however easily shown (Müller, 1983; McAuslane et al., 1990). From the early adult learning experiments with *C. glomerata* in the present study it appears that the host's diet does not influence preference for plant-host odours in *C. glomerata* females. Rearing *C. glomerata* for two generations on the *P. brassicae*-nasturtium complex did not reverse the preference for *P. brassicae*-infested Brussels sprouts of naive *C. glomerata* females (Chapter 3), which might have been too few.

Adult learning.

Preference for host species. In both *Cotesia* species, leaf damage or single oviposition experience with only one host species did not change preference for a particular host. A single experience may not have been sufficient to affect the parasitoid's behaviour and so multiple experiences were given. It was clearly shown that for *C. glomerata* these multiple experiences led to a preference for *P. brassicae*. Host acceptance (Chapter 5) and host-suitability studies (Brodeur & Vet, 1995; Chapter 9) showed that *C. glomerata* females more readily accepted *P. brassicae*, a host species that maximizes survival

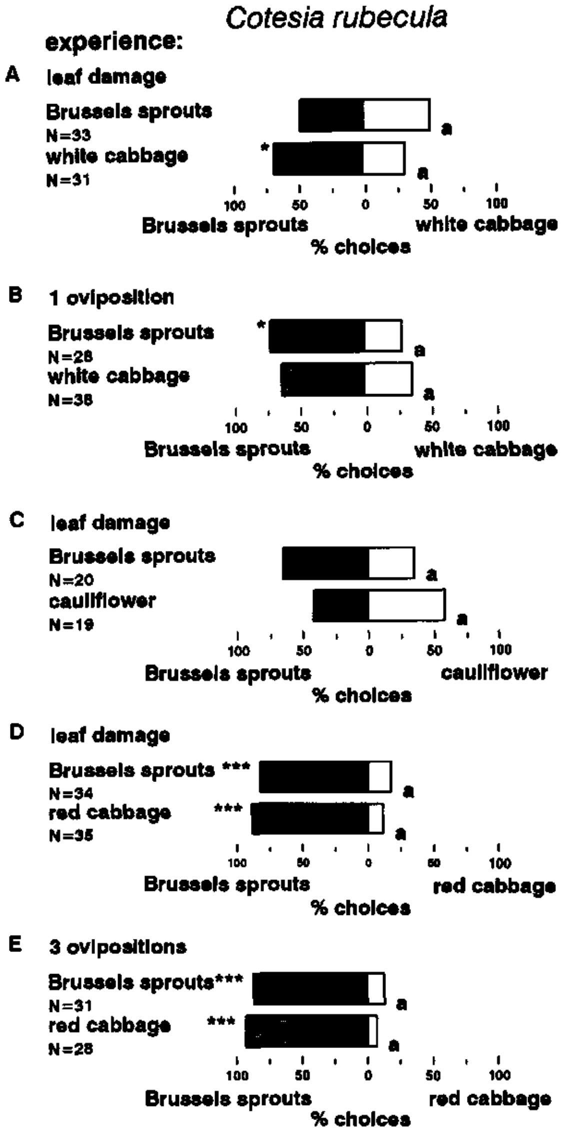

Fig. 5. Choice distributions over *P. rapae*-infested Brussels sprouts or white cabbage of responding *C. rubecula* females with different types of experience: (A) leaf-damage experience with *P. rapae*-infested Brussels sprouts or white cabbage; (B) 1 oviposition in *P. rapae* on an infested Brussels sprouts leaf or an infested white cabbage leaf; (C) leaf-damage experience with *P. rapae*-infested Brussels sprouts or cauliflower; (D) leaf damage with *P. rapae*-infested Brussels sprouts or red cabbage; (E) 3 ovipositoins in *P. rapae* on Brussels sprouts or red cabbage. Bars with the same characters indicate no significant differences in parasitoid choices between tests. Asterisks indicate a significant difference within a choice test: *** $P<0.0005$; * $P<0.05$. Numbers of responding females are indicated by N.

of the immatures and weight of the offspring, than *P. rapae* or *P. napi* larvae. Apparently, this parasitoid species prefers the damage caused by caterpillars that are more suitable as a host, but it needs to acquire information to do so. Multiple experience may have a stronger effect on the female's motivation to search and the establishment of preferences than a single experience. In *M. croceipes*, a single experience was found to be insufficient to establish a preference, whereas repeated experience did result in preferences (Eller et al., 1992). Such phenomena may be mediated by sensitivity changes in the olfactory receptors as reported for parasitoids of *Drosophila* (Vet et al., 1990a). In the case of experience with both *P. brassicae* and *P. rapae*, complete information about the host species involved was offered. In parasitoids of *Drosophila* there is a striking difference in the effect of experience with complete and incomplete information (Vet et al., 1996). The opportunity to compare the suitability of the host species present and subsequently choose for the most suitable one can help to optimize host selection in parasitoids.

In generalist parasitoids, learning is supposed to enhance flexibility of the host-seeking behaviour (Vet & Dicke, 1992; Vet et al., 1995), leading to a preference for the experienced host species. Instead, *C. glomerata* prefers *P. brassicae*-infested leaves after experience on *P. rapae*. This may have been an artefact of rearing conditions. Although the laboratory colony of *C. glomerata* was replaced with parasitoids collected in the field every season, *C. glomerata* may be well adapted to *P. brassicae*, interfering with the results in this study. On the other hand, learning to concentrate on *P. brassicae* may be functional for this parasitoid species, considering the possible competition between *C. glomerata* and *C. rubecula* larvae in *P. rapae*. Parasitization experiments showed that *C. glomerata* is the inferior competitor in the case of multiparasitism of *P. rapae* by both *C. rubecula* and *C. glomerata* (Laing & Corrigan, 1987). In The Netherlands competition is likely, since both parasitoid species and their hosts occur simultaneously in the same habitats. Due to this risk of competition, *P. rapae* will be a less suitable host for *C. glomerata*. Learning to concentrate on *P. brassicae* can result in a 'competitor-free' space for *C. glomerata* provided that *C. rubecula* does not parasitize this host under natural conditions. Since it is a specialist on *P. rapae*, it is assumed that this does not occur. Field observations confirm that *C. rubecula* was only collected from *P. rapae* larvae (Chapter 10).

For *C. rubecula*, preference learning could not be shown. None of the experience types given to the females resulted in a landing preference for any of the host species experienced. This supports the hypothesis that learning plays a less important role in this more specialized parasitoid species.

Preference for plant cultivars or species. For *C. glomerata*, clear preferences for the experienced plant-host complex were found in the Brussels sprouts-red cabbage combination and in the Brussels sprouts-nasturtium combination. Chapter 3 showed that responses of naive *C. glomerata* to red cabbage and nasturtium were weak, compared to those to Brussels sprouts and white cabbage. Furthermore, naive *C. glomerata* females showed preferences for *P. brassicae*-infested Brussels sprouts leaves over infested red cabbage leaves or over infested nasturtium leaves (Chapter 3). It has been argued that initially weak responses are the most easily reinforced through learning (Cornell & Pimentel, 1978; Vet et al., 1990b; 1995; Sheehan & Shelton, 1989). The effects of learning are largest for the least preferred microhabitats and hosts (Vet & van Opzeeland, 1984; McAuslane et al., 1991; Kaiser et al., 1989; Sheehan & Shelton, 1989). The results of the present study provide additional evidence for this phenomenon.

Experience with *P. brassicae*-infested Brussels sprouts, white cabbage or cauliflower showed less clear learning effects. Together with the fact that naive females did not distinguish between Brussels sprouts and white cabbage or Brussels sprouts and cauliflower, this may indicate that stimuli from these cabbage varieties closely resemble stimuli from infested Brussels sprouts. Learned cues may then be generalized in subsequent flights, resulting in similar responses to these plant-host complexes. To confirm this, the composition of the volatile blend from caterpillar-infested Brussels sprouts, white cabbage, red cabbage and nasturtium are currently being investigated.

A single oviposition experience in a *P. brassicae* larva on an infested leaf resulted in choices for the non-experienced plant-host complex of *C. glomerata* females. It has been suggested that the Dutch *C. glomerata* is most adapted to parasitizing in clusters of larvae (Wiskerke & Vet, 1994). The removal after one oviposition of *C. glomerata* females from the cluster of *P. brassicae* larvae in this treatment, which is a gross interruption, may have been experienced by the wasps as 'negative', leading to an induced preference for the other host plant in the tests. In those treatments with multiple oviposition, wasps were given the opportunity to stay longer on a cluster of host larvae, until the desired number of ovipositions was observed. Besides positive associative learning, negative experiences are thought to be important in shaping foraging behaviour (Mangel, 1993; Papaj et al., 1994). Natural disturbance of a parasitoid searching for hosts (e.g. by competitors or predators) could cause a negative perception of the availability of hosts. Wind tunnel studies showed that *C. glomerata* avoids *P. rapae*-infested Brussels sprouts leaves on which *C. rubecula* is parasitizing *P. rapae* larvae (see Chapter 10). Furthermore, results of experiments on unrewarding experiences in *Leptopilina heterotoma*, parasitoids of *Drosophila* larvae, suggest that unsuccessful foraging enhances responses to novel stimuli (Papaj et al., 1994).

In all tests with different cabbage varieties visual stimuli were present. To the human eye, the colour of red cabbage and nasturtium differ considerably from that of Brussels sprouts, white cabbage and cauliflower, and the habitus of nasturtium differs from that of the cabbage varieties. These visual factors may play a role in parasitoid host-finding (McAuslane et al., 1991b; Wäckers and Lewis, 1993) and may be important for *C. glomerata*. Without visual stimuli present, experienced *C. glomerata* females responded to the odour plume of the experienced plant-host complex, but did not land. In the absence of olfactory stimuli, experienced females did not respond to the experienced visual stimulus at all and showed undirected flights (Geervliet, unpublished results). However, tests with different plant odours blown over plants in a wind tunnel in the absence of visual differences showed that *C. glomerata* is able to learn odours from host-infested plants and develop a preference for the experienced odour (Zwaan, Geervliet, Vet & van Loon, unpublished results). Thus a visual stimulus is needed for landing, but is not necessary for discrimination between odours.

No preference learning at the plant level was found in *C. rubecula* females. Naive females show no preference for Brussels sprouts, white cabbage or cauliflower (data not shown). Experience with these cabbage varieties did not lead to preferences for the experienced plant-host complex. Naive females do prefer Brussels sprouts over red cabbage (Chapter 3), but this preference is not affected by experience with red cabbage. For the host-finding behaviour the differences in learning between *C. glomerata* and *C. rubecula* are in agreement with the hypothesis that in specialists the behaviour is less modified by experience than in generalists. In *C. glomerata*, preferences can be changed through experience both at the herbivore and at the plant level, and evidence is accumulating that they learn to concentrate on the most profitable host or patch, which is expected to enhance host-seeking efficiency (Chapter 8). *Cotesia rubecula* seems to show a more fixed host-finding behaviour. Both its host-searching and food-searching are governed by a set of innate preferences (Wäckers, 1994). Although the species is specialist on *P. rapae* however, females do not show innate (Chapter 3) or induced preferences (this study) for plants infested by this host species over plants infested by other herbivores. Chapter 5 showed that interspecific differences in the degree of specialization in *Cotesia* could arise from differences occurring at the acceptence phase of the host-searching behaviour. This phase seems to be crucial for *C. rubecula* to distinguish *P. brassicae* from *P. rapae*. Furthermore, recently Agelopoulos et al. (1995) detected an important role of caterpillar frass in the host-seeking behaviour of *C. rubecula*. Plant-derived information may be used as an indication of damage (Steinberg et al., 1993; Agelopoulos & Keller, 1994; Chapter 2), with a possibility to distinguish between more or less preferred food plants, while frass at shorter distances may be used as the host species distinguishing cue.

7

HOST-LOCATION BEHAVIOUR OF THE LARVAL PARASITOIDS *Cotesia glomerata* AND *C. rubecula*: THE EFFECT OF HOST AND PLANT SPECIES.

ABSTRACT

After arriving in a potential host-microhabitat, parasitoids enter the host-location phase, and by responding mainly to host-derived cues they may be able to estimate the suitability of the patch they are sampling. These cues can be recognized by the naive parasitoid or they can be learned to discriminate between different plant complexes in future searching. The aim of the present study was to investigate the host-location behaviour of two closely related parasitoid species, *Cotesia glomerata* and *C. rubecula*, larval endoparasitoids of *Pieris* species, that differ in their diet breadth.

In a free-ranging wind tunnel experiment, individual *C. glomerata* and *C. rubecula* females were given the opportunity to sample a set of Brussels sprouts plants, bearing *P. brassicae*- or *P. rapae*-infested leaves and uninfested leaves. Behaviour was recorded continuously for one hour and it was analysed for the first and the second half hour to determine whether behaviour changed with time. Number of visits and residence times were host-specific for both parasitoid species. In *C. glomerata* a higher number of visits and longer residence time on *P. brassicae*-infested leaves compared to the other leaf types, were accompanied by a higher number of ovipositions in *P. brassicae* larvae than in *P. rapae* larvae. For *C. rubecula* more visits and longer residence times were observed on *P. rapae*-infested leaves, but no ovipositional preferences were found.

Furthermore, individual naive females were offered leaves of Brussels sprouts, red cabbage or nasturtium plants with feeding damage of the hosts *P. brassicae* or *P. rapae*. Residence times on leaves of one plant species with different types of herbivore damage were compared, as well as residence times on leaves of different plants damaged by one of the two herbivore species. Search times of *C. glomerata* and *C. rubecula* did not differ on leaves infested by different herbivore species, but significantly longer search times were observed on damaged Brussels sprouts leaves than on damaged leaves of red cabbage or nasturtium.

The correlation between host-habitat location behaviour, host-location behaviour and host-suitability is discussed for *C. glomerata* and *C. rubecula*.

INTRODUCTION

Searching for hosts by insect parasitoids involves several phases (Vinson, 1984). After emergence, adult parasitoids often have to tide over a certain distance between their place of birth and the prospective place of birth of their offspring. To find suitable host microhabitats, they use stimuli from their environment, related to the host, the food of the host and other host-related organisms, and host-by products. During this phase in the host-searching process, plant-derived stimuli have been reported to be more important than host-derived stimuli (Weseloh, 1981; Vinson, 1985), because these type of stimuli are more detectable for the parasitoids (Vet & Dicke, 1992). Once a parasitoid female has located a potential host-microhabitat, her search for hosts continues by responding mainly to host-derived cues (Sato, 1979; Nordlund & Sauls, 1981; Weseloh, 1981; Vinson, 1985; Ding et al., 1989b; Wäckers, 1994; Mattiacci & Dicke, 1995a,b; Agelopoulos et al., 1995 and references therein). The presence of these cues, that ideally reveal e.g. the identity of the herbivore species present, the stage of the host present, and its density, will determine the female's preparedness to search in and exploit the patch (Waage, 1978; Strand & Vinson, 1982; Sabelis & Dicke, 1985; van Roermund et al., 1993; Driessen et al., 1995; van Steenis et al., 1996). Contact with host-derived products generally leads to behavioural changes in the searching female, such as shorter turns, oviposition attempts, moving the antennae downwards, and changes in walking speed (Nealis, 1984, 1986; Waage, 1978; Strand & Vinson, 1982; Vet & van der Hoeven, 1985; Wiskerke & Vet, 1994; Mattiacci & Dicke, 1995a,b).

By these cues parasitoids may recognize the suitability of the patch they are sampling, either innately and/or these cues may be learned to distinguish between different plant-host complexes in future searching bouts (Vet, 1983; 1990; Lewis & Tumlinson, 1988; Sheehan et al., 1993).

The present study was initiated to investigate the host-habitat location and host-location by two closely related parasitoid species, *Cotesia glomerata* and *C. rubecula*, larval endoparasitoids of *Pieris* species. These parasitoid species differ in their diet breadth: *C. glomerata* is a generalist that can attack several pierid hosts (Laing & Levin, 1982) that occur on cruciferous food plants, but also on plants from the families Tropaeolaceae, Rosaceae and Berberidaceae (Terofal, 1965; Feltwell, 1982). *Cotesia rubecula* is more specialized at the host and plant level, since its preferred host is *Pieris rapae* that feeds on a less broad food plant spectrum than the potential hosts of *C. glomerata* (Terofal, 1965). Behaviour of generalists was hypothesized to be more flexible and to be influenced by learning more than that of specialists, that are expected to show a more fixed innate behaviour (Vet & Dicke, 1992).

In earlier studies, the host-microhabitat location by both species was examined in wind tunnel experiments. Preferences for host species or food plants were determined by recording first landings of females in two-choice experiments. Naive females of neither species distinguished between food plants infested by different herbivore species, but they had preferences for certain host-infested food plants over others (Chapter 3). Preferences for plants infested with host species were only found in *C. glomerata* females

after multiple experience, that led to a preference for *P. brassicae*-infested leaves (Chapter 6). In contrast to what we expected, naive *C. rubecula* females did not show preferences for plants infested with their host, *P. rapae* in flight experiments (Chapter 3), and even multiple oviposition experience with this host on host-damaged leaf material did not result in preferences for *P. rapae*-infested plants in subsequent flights (Chapter 6). Lack of major herbivore-specific infochemicals in this system (Blaakmeer et al., 1994; Mattiacci et al, 1994; Agelopoulos & Keller, 1994c) may constrain naive parasitoids to distinguish from a distance between different herbivores feeding on a plant. However, *C. glomerata* is capable of learning subtle (quantitative) differences with experience (Chapter 6) and experienced females show a preference for *P. brassicae*-infested leaves over *P. rapae*-infested leaves. Contact with damaged leaves, feces and larval silk during the experience treatment may have caused this preference.
From semi-field studies (Wiskerke & Vet, 1994) and field studies (Chapter 10) it was concluded that *C. rubecula* searched most successfully for the solitary *P. rapae*, whereas *C. glomerata* seems to be more adapted to search for the gregarious *P. brassicae*. Furthermore, host-suitability experiments showed that clear fitness penalties exist for *C. rubecula* parasitizing *P. brassicae* or *P. napi* larvae, while *C. glomerata* developed well in the three host species *P. brassicae*, *P. rapae* and *P. napi* (Chapter 9). In these studies parasitoids could use non-volatile cues present on the leaves during host-location, which were not contacted in the in-flight wind tunnel experiments described above.

This discrepancy between wind tunnel studies and observations from the (semi)-field and rearing experiments urged us to study the host-habitat location and host-location behaviour (i.e. after landing on the leaf) of the two parasitoid species in more detail. The present study aimed to investigate the effect of habitat sampling on the discriminative abilities of the parasitoids. We tested whether initially naive females of *C. glomerata* and *C. rubecula* show differences in the number of visits to leaves of different plant-host complexes and search times on these leaves. Through foraging experience gained in the course of time, females of both species are expected to become more discriminative with respect to host species. Furthermore, we investigated to what extent plant species and herbivore species lead to differential search times of females when contacting leaf material with damage and host-derived products of different herbivore species. For *C. rubecula* we tested its reported 'specialization on' *P. rapae* (Shenefelt, 1972) and for the generalist *C. glomerata* a more flexible behaviour in searching for and accepting the different host species present is expected than for the specialist *C. rubecula*.

MATERIALS & METHODS

Rearing procedures. Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* cv. Titurel), red cabbage (*Brassica oleracea* L. var. *rubra* (DC.) cv. Langedijker vroege rode) and nasturtium (*Tropaeolum majus* L. cv. Emperor of India) (Tropaeolaceae) plants were reared in a greenhouse, at 20-25°C, 50-70% RH and a L16:D8 photoregime. When necessary, plants were treated with DDVP (2,2 dichlorovinylphosphate) against aphids, but not within a period of four days before the experiments. The pierid hosts *P. brassicae* and *P. rapae* (Lepidoptera: Pieridae) were reared on Brussels sprouts plants in a climatic room at 20-22°C, 50-70% RH and a L16:D8 photoregime. For experiments late first instar larvae were used. The parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) were maintained on *P. brassicae* and *P. rapae* respectively. Wasps were offered Brussels sprouts leaves with host larvae for parasitization. Parasitized larvae were reared on Brussels sprouts plants in cages in greenhouse compartments at 20-25°C, 50-70% RH and a L16:D8 photoregime. Parasitoid cocoons were collected in petridishes and transferred to emergence cages (40*30*30 cm) that were supplied with water and honey. Male and female wasps were kept together to allow mating. Cocoons were daily transferred to empty cages to obtain groups of wasps of known age. Wasps were kept in the absence of hosts and food plants. For experiments 3-5 day old naive females were used that were individually collected from the cages in a vial (5 cm, 1.5 cm i.d.) with water and honey prior to testing.

Free-ranging experiment. This experiment was carried out in the wind tunnel described in chapter 2. Wind speed was 25-30 cm/s, light intensity 1700-2000 lux (bottom-top) and 40-60% RH.

<u>Herbivore damaged Brussels sprouts plants</u> were obtained by transferring *P. brassicae* or *P. rapae* larvae to leaves of intact Brussels sprouts plants, per leaf one cluster of 10 *P. brassicae* larvae or 10 solitary *P. rapae* larvae. Per plant two opposite leaves in the third leaf-layer from the top were infested with host larvae, the rest of the leaves (6-8) was left uninfested. Four infested plants were placed in the windtunnel in a square, the *P. brassicae*-infested plants (and so the *P. rapae*-infested plants) diagonally opposite to each other.

<u>Observations</u>. An opened vial with an individual naive *C. glomerata* or *C. rubecula* female was introduced in the wind tunnel, downwind of the plants. Behavioural recordings were started after flight initiation, and were recorded using 'The Observer' (Noldus, 1991). Females were allowed to search for hosts freely in the wind tunnel for one hour. This observation time was analysed for the first and the second half hour separately, to test the effect of experience. For each period the following parameters were quantified

(Sato, 1979; Nealis, 1986; Wiskerke & Vet, 1994; Mattiacci & Dicke, 1995):

1. Behaviour of the parasitoid

fly
walk: the wasp moves along the leaf surfaces with her antennae extended in front of the head, alternately moving up and down.
search: the wasp moves the antennae up and down, sometimes bending them or sweeping them along the leaf surface. The abdomen is regularly bent towards the leaf surface as if the wasp is trying to contact larvae.
oviposit: the wasp draws her abdomen between her legs and raises her antennae and wings. She inserts her ovipositor into a host larva.
groom: the wasp grooms the antennae, wings and abdoment with her legs.
stand: the wasp stands motionless on the leaf, keeping her antennae in an upright position.
total time: the total time the wasp spent on the leaf before flying away.

2. patch type:
a wasp was on a *P. brassicae*-infested leaf of plant 1 or plant 2, a *P. rapae*-infested leaf of plant 1 or plant 2, or on an undamaged leaf, either of a *P. brassicae*-infested plant (1 or 2) or a *P. rapae*-infested plant (1 or 2). All other locations in the wind tunnel were recorded as 'elsewhere'.
For *C. glomerata* a parasitization was considered to have occurred when the characteristic oviposition behaviour lasted for 7 s or longer. Ovipositor insertions lasting less than 7 seconds were not treated as ovipositions in the analysis (le Masurier, 1987 check). For *C. rubecula* ovipositions were recognized by the characteristic short jab at the host with its ovipositor, with the wings in an upright position (Nealis, 1986).

Contact experiment. Herbivore damaged leaves were prepared by placing one plant of Brussels sprouts, red cabbage and nasturtium in the oviposition cage of *P. brassicae*, *P. rapae* or *P. napi* butterflies one week before testing, except the Brussels sprouts - *P. napi* combination, so that in total eight plant-host combinations were included in the experiment. The number of eggs per leaf were reduced to one cluster of 10-15 eggs of *P. brassicae* and five to eight solitary eggs of *P. rapae* and *P. napi*. Plants with host eggs were kept in a climatic room at 20-22°C, 50-70% RH and a L16:D8 photoregime. After hatching, larvae were allowed to feed for 24-36 hours. On the test days, leaves were cut from the stem with a surgical knife and placed with their petiole in an erlenmeyer flask (100 ml) filled with water and the opening sealed with parafilm. Larvae were gently removed from the leaves, without disturbing the host by-products (silk and frass), just prior to the bioassay. Continuous observations were made of female *C. glomerata* or *C. rubecula* on a herbivore-damaged leaf of one of the eight plant-herbivore complexes as described above. Observations were made under natural light conditions (ca 750-1750 lux), 20±2°C and 45-50% RH and were started by allowing a naive female of either *C. glomerata* or *C. rubecula* to walk out of the vial and step onto the herbivore-damaged leaf. Behaviour was recorded on a portable computer using the event recording program 'The Observer' (Noldus, 1991). Observations ended either when a wasp flew away or when it had been standing motionless on the leaf for 2 minutes. Preliminary studies revealed that *Cotesia* seldomly resumed searching after 2 minutes of standing (Mattiacci & Dicke, 1995ab). These 2 minutes were left out of the analysis. For this experiment the following parameters were quantified (Sato, 1979; Nealis, 1986; Wiskerke & Vet, 1994; Mattiacci & Dicke, 1995ab):

search: the time spent walking and walking while antennating over the leaf surface.
groom: the wasp grooms the antennae, wings and abdoment with her legs.
stand: the wasp stands motionless on the leaf, keeping her antennae in an upright position.
total time: the total time the wasp spent on the leaf before flying away or before becoming inactive for 2 minutes.

Females of both parasitoid species were tested on the same day. It was not possible to do one replicate of all 8 treatments on the same day. Therefore, the host species were offered on the same day, whereas plant species were offered in 'blocks'.

Statistics. Durations of behaviours in the free ranging experiment were analyzed using the Mann-Whitney U test and numbers of visits and ovipositions were analyzed using the Wilcoxon matched-pairs signed-rank test. To analyze overall differences in time allocations and durations of behaviours between treatments in the 'contact experiment' the Kruskall-Wallis one-way ANOVA was used. The Mann-Whitney U-test with Bonferroni inequality was used for pairwise comparisons.

RESULTS

Free-ranging experiment.

Visits. For each parasitoid species the number of visits paid to the various leaf types (undamaged or damaged by either *P. brassicae* or *P. rapae* larvae) was measured for the two time periods (fig. 1). Both *C. glomerata* and *C. rubecula* pay significantly more visits to herbivore damaged leaves than to undamaged leaves throughout the experiment. In both observational periods *C. glomerata* pays more visits to *P. brassicae*-infested leaves than to the other leaf types (fig 1a), whereas *C. rubecula* females pay significantly more visits to *P. rapae*-infested leaves than to any other leaf type (fig 1b).

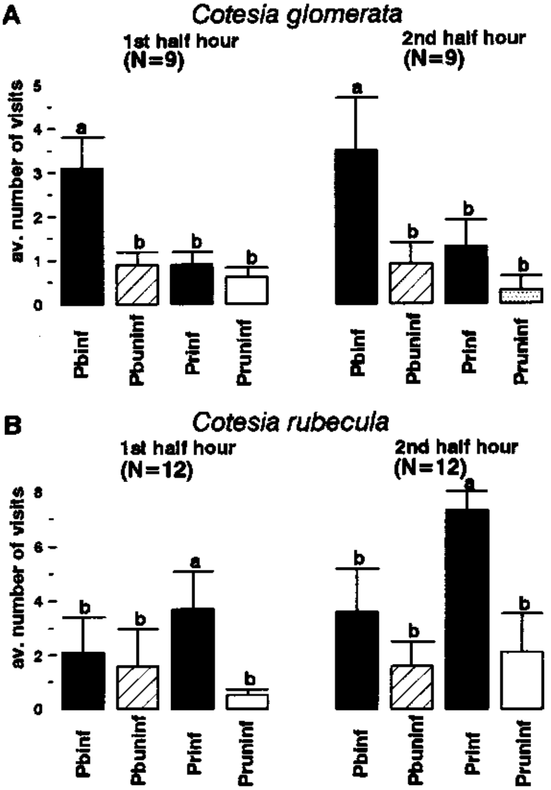

Fig. 1. Average number of visits ± SE per period per leaf type for (A) *C. glomerata* and (B) *C. rubecula*. Bars within a period with different letters are significantly different (Wilcoxon matched pairs signed rank test, $P<0.05$). Abbreviations: Pbinf = *P. brassicae*-infested Brussels sprouts leaves, Pbuninf = uninfested leaves of a *P. brassicae*-infested Brussels sprouts plant; Prinf = *P. rapae*-infested Brussels sprouts leaves; Pruninf = uninfested leaves of an *P. rapae*-infested Brussels sprouts plant. N indicates numbers of females tested per period.

Time allocation. Time allocation on the various leaf types and duration of behaviours was measured during the two foraging periods (Table 1). For both parasitoid species absolute durations of behaviour did not change with time, except for the time spent 'elsewhere' by *C. rubecula*, which was significantly shorter in the second than in the first period.

For *C. glomerata* searching, grooming and total time on *P. brassicae*-infested leaves were significantly higher than on any of the uninfested leaf types both periods (Table 1). Average search times were higher on *P. brassicae*-infested leaves than on *P. rapae*-infested leaves in the second period only (Table 1, Fig. 2a). For *C. rubecula* searching, grooming and total times were significantly higher on *P. rapae*-infested leaves than on any of the undamaged leaf types and *P. brassicae*-infested leaves in the two periods (Table 1, Fig. 2b).

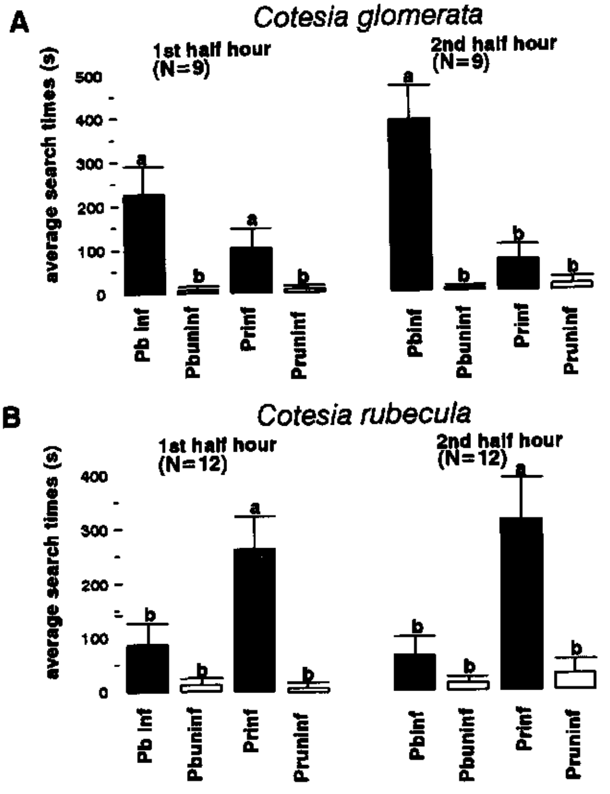

Fig. 2. Average search times ± SE per period per leaf type for (A) *C. glomerata* and (B) *C. rubecula*. Bars within a period with different letters are significantly different (Mann-Whitney U test $P<0.05$). Abbreviations: Pbinf = *P. brassicae*-infested Brussels sprouts leaves, Pbuninf = uninfested leaves of a *P. brassicae*-infested Brussels sprouts plant; Prinf = *P. rapae*-infested Brussels sprouts leaves; Pruninf = uninfested leaves of an *P. rapae*-infested Brussels sprouts plant. N indicates number of females tested per period.

Table 1. Average durations (s) (±SE) of behaviours of *Cotesia glomerata* and *C. rubecula* on different leaf types

parasitoid species	behaviour / parameter (s)[a]			behaviour / parameter (s)[a]	Leaf type							
					P. brassicae-infested		*P. brassicae* undamaged		*P. rapae*-infested		*P. rapae* undamaged	
C. glomerata		period 1 (N=12)	period 2 (N=9)		period 1 (N=12)	period 2 (N=9)	period 1 (N=12)	period 2 (N=9)	period 1 (N=12)	period 2 (N=9)	period 1 (N=12)	period 2 (N=9)
	fly	109.3±23.5	104.0±40.4	search	227.3±61.8	389.8±79.2	7.1±4.4	4.7±3.8	103.1±41.8	71.6±33.3	7.2±4.7	14.2±14.2
	elsewhere	473.8±164.1	171.7±147.9	walk	25.1±16.2	20.6±10.6	4.8±4.8	0.6±0.6	12.0±6.4	0.7±0.7	1.7±1.7	0.0±0.0
				groom	316.9±86.8	490.6±91.4	5.9±2.3	41.3±29.3	195.8±85.6	65.4±27.9	11.2±7.1	2.1±2.1
				stand	143.0±76.7	127.6±95.1	1.1±1.4	19.1±16.2	43.7±21.8	24.5±15.9	3.5±2.4	3.4±3.4
				oviposit	81.1±26.1	154.7±43.1	-	-	33.5±15.9	7.1±3.6	-	-
				total	793.4±185.4	1183.0±149.5	19.1±8.0	65.6±43.0	380.3±145.3	169.3±77.9	23.6±12.9	19.7±19.7
C. rubecula		period 1 (N=12)	period 2 (N=12)		period 1 (N=12)	period 2 (N=12)	period 1 (N=12)	period 2 (N=12)	period 1 (N=12)	period 2 (N=12)	period 1 (N=12)	period 2 (N=12)
	fly	101.3±24.4	192.7±25.8	search	87.0±40.7	64.2±32.8	12.6±12.4	13.8±9.6	261.7±60.7	314.1±74.4	7.7±5.5	29.4±25.2
	elsewhere[b]	667.9±150.8a	71.7±57.5b	walk	3.6±3.6	1.3±0.9	44.7±41.8	0.3±0.3	3.1±1.2	6.6±5.6	6.1±5.5	0.0±0.0
				groom	138.7±68.7	114.0±47.0	17.3±12.7	31.4±20.1	206.9±59.1	404.4±75.8	10.2±6.9	28.1±27.1
				stand	32.6±22.5	95.8±40.6	17.1±12.5	50.0±34.1	141.5±55.6	279.8±88.8	11.4±10.6	86.7±82.3
				oviposit	6.6±3.4	4.5±2.8	-	-	9.7±3.0	11.3±6.3	-	-
				total	268.5±120.1	279.7±118.6	91.6±62.9	95.5±55.3	626.7±136.8	1016.0±172.6	35.4±18.7	144.2±134.3

[a] Mean ± SE

[b] Times within a row followed by a different letter are significantly different (Mann-Whitney U; $P<0.05$).

Ovipositions were counted per host species for each period of 30 min and per period the average number of ovipositions per female was calculated (Fig 3). In the course of time *C. glomerata* females get an ovipositional preference for *P. brassicae* larvae (Fig. 3a). Although *C. rubecula* females oviposit more in *P. rapae* larvae, differences in ovipositions between *P. rapae* and *P. brassicae* larvae are not significant (Fig. 3b).

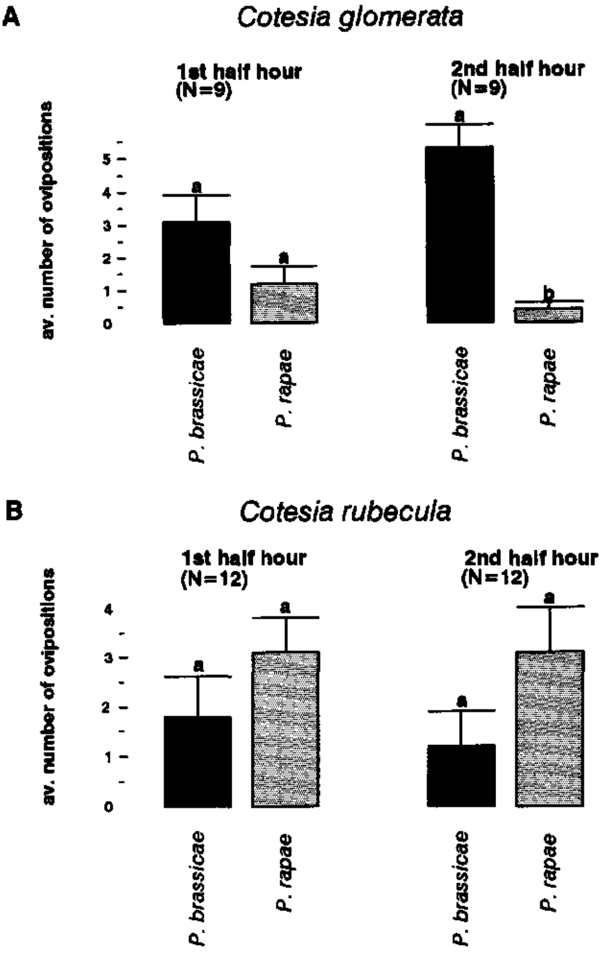

Fig. 3. Average number of ovipositions ± SE per period per leaf type for (A) *C. glomerata* and (B) *C. rubecula*. Bars within a period with different letters are significantly different (Wilcoxon matched pairs signed rank test, $P<0.05$). N indicates number of females tested per period.

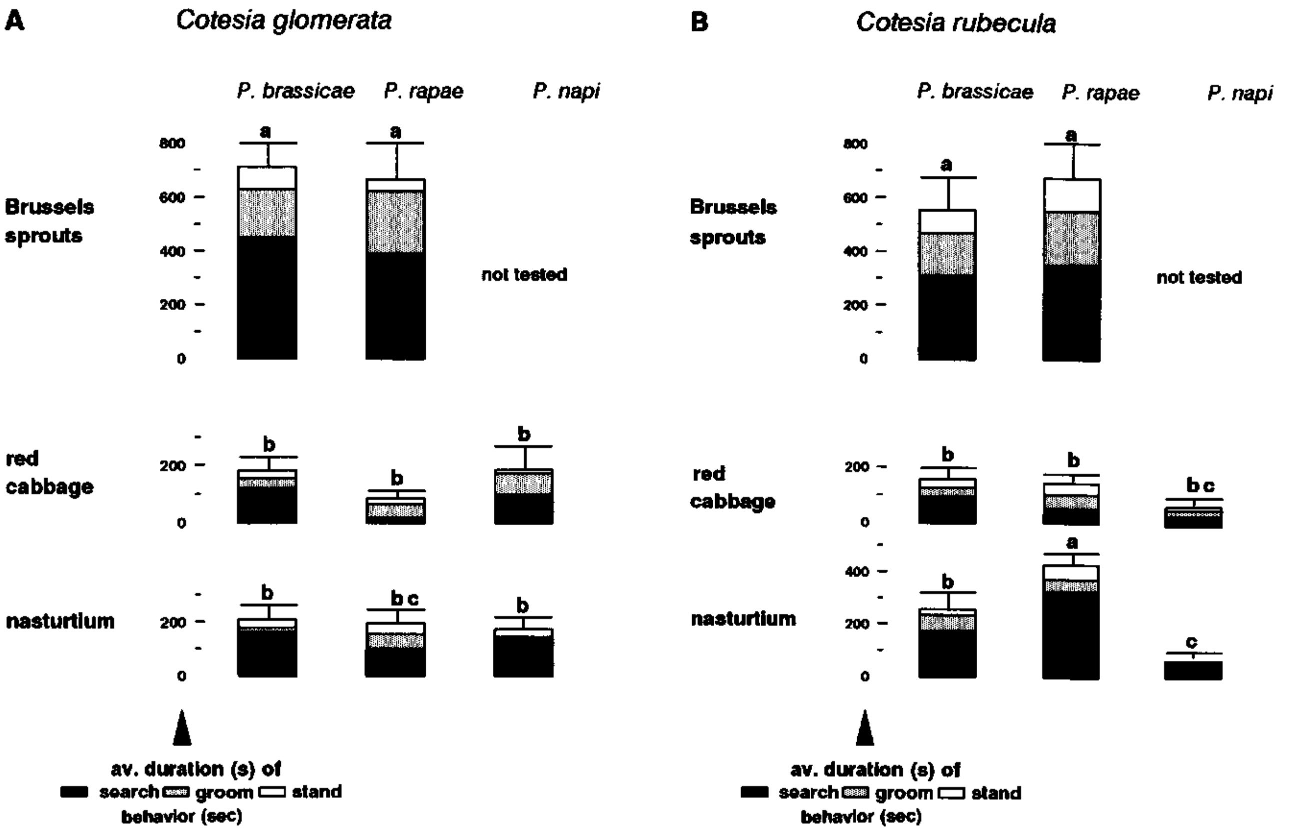

Fig. 4. Average durations (s) ± SE of behaviours by naive females of (A) *C. glomerata* and (B) *C. rubecula* on Brussels sprouts, red cabbage or nasturtium leaves damaged by *P. brassicae*, *P. rapae* or *P. napi* larvae. Bars within a row and bars within a column with different letters have significantly different search times (Kruskal-Wallis, Mann-Whitney U with Bonferroni inequality for multiple pairwise comparisons; $P<0.05$).

Contact experiment.
Continuous observation of behaviour of naive *C. glomerata* and *C. rubecula* females on herbivore-damaged Brussels sprouts, red cabbage or nasturtium leaves, revealed that females spent most of their time searching (average percentages of 50-70% for *C. glomerata* and 38-80% for *C. rubecula*) (fig 4a and 4b). Therefore, search times were used to analyze differences in females' responses for the different treatments. Naive *C. glomerata* females searched equally long on leaves damaged by *P. brassicae* or *P. rapae* on Brussels sprouts. There was no difference in search times of *C. glomerata* on leaves infested by *P. brassicae*, *P. rapae* or *P. napi* larvae on red cabbage and nasturtium (fig. 4a). Females of *C. rubecula* did not distinguish between host species in searching times on Brussels sprouts or red cabbage leaves, but on nasturtium leaves damaged by *P. rapae*, significantly more time was spent searching than on nasturtium leaves damaged by the two other host species (fig. 4b).
For both parasitoid species search times were significantly higher on herbivore-damaged Brussels sprouts leaves compared to search times on herbivore-damaged red cabbage or nasturtium leaves.

DISCUSSION

Free-ranging experiment
Leaving tendencies and other foraging decisions of parasitoids may be affected by actual host encounters rather than by host products such as leaf damage only (Waage, 1978; Strand & Vinson, 1982; Driessen et al., 1995; Vos et al., 1996), and distinguishing between host species may only occur after parasitization, through ovipositional experience. Therefore the free-ranging experiment was carried out. Summarizing the results of this experiment, each observational period shows a similar behavioural output of the wasps on the various leaf types, with a clear preference of each parasitoid species for one of the two *Pieris* species. Per period both parasitoid species paid more visits to and spent more time on infested leaves than on uninfested leaves. Females of both species showed a landing preference for leaves infested by one of the two host species present: *C. glomerata* paid more visits to *P. brassicae*-infested leaves, whereas *C. rubecula* visited *P. rapae*-infested leaves more often. Furthermore, both parasitoid species had longer search times on these host-infested leaves than on the other leaf types they visited. For *C. glomerata* the larger number of visits and the longer residence times on the *P. brassicae*-infested leaves is accompanied by more ovipositions in *P. brassicae* larvae than in *P. rapae* larvae in the second period (fig. 4a), but for *C. rubecula* the differences in oviposition percentages between *P. brassicae* and *P. rapae* were not

significant. Between periods, absolute duration of behaviours on a certain leaf type did not change. One exception was observed, but the importance of the difference in time spent 'elsewhere' by *C. rubecula* is considered to be low.

The great similarity between the two subsequent test periods in behaviour of the two parasitoid species and the clear discrimination between the host species from the first period on, indicate that the *Cotesia* females either gained their foraging experience very fast, in the first half hour of their search for hosts, or that they both showed innate responses, that were hardly changed during the following search period. The only change was observed for ovipositional decisions in *C. glomerata* (Fig 3a). Throughout the test periods, *C. glomerata* shows a preference for *P. brassicae*-infested leaves and *P. brassicae* larvae, and *C. rubecula* for *P. rapae*-infested leaves and *P. rapae* larvae.

Innate preferences of *C. glomerata* for host species could not be shown in two-choice wind tunnel experiments or in the above described contact bioassay, but fast learning capacities of *C. glomerata* were clear (Chapter 3 and 6). Contact experience with herbivore-damaged leaf material is sufficient to get a preference for that plant, whereas double oviposition experience on either *P. brassicae* or *P. rapae* leads to a preference for *P. brassicae*-infested Brussels sprouts. Furthermore, the first experience *C. glomerata* females get, was found to be important for the effect of subsequent experiences on the behaviour (Chapter 8). In the free-ranging experiment of this study, the first experience of *C. glomerata* often consisted of *P. brassicae*, because the first landing of *C. glomerata* females occurred in most cases (80%) on *P. brassicae*-infested leaves. In all cases this was followed by one or more ovipositions on this host species (data not shown). The few (20%) first landings on *P. rapae*-infested leaves by *C. glomerata* did not always lead to host encounter or ovipositions, because in approximately 75% of the cases females left the leaf soon after landing (data not shown).

In *C. rubecula* no innate preference for *P. rapae*-infested plants nor any effect of experience with this host on the subsequent choices was found so far in two-choice wind tunnel experiments (Chapter 3) or in the contact experiment of this study. In this experiment a landing preference for *P. rapae* in *C. rubecula* was demonstrated, and although ovipositional preferences were not found for the separate observational periods ($P=0.11$ and $P=0.14$ for the first and the second period respectively), pooling the number of ovipositions over the total observational period of one hour reveals a significant preference for *P. rapae* ($P=0.03$). Wiskerke and Vet (1994) showed landing and ovipositional preferences of *C. rubecula* for *P. rapae*. Their set-up was comparable to our free-ranging set-up, but wasps were released from a leaf with damage inflicted by both *P. brassicae* ánd *P. rapae*,

whereas we used naive parasitoid females, and Wiskerke & Vet used higher doses of infested leaf material than we did.
The outcome of the free-ranging experiment supports our observations in the field (Chapter 10), where *P. brassicae* was found to be parasitized mainly by *C. glomerata* and *P. rapae* by *C. rubecula*. As also concluded by Wiskerke & Vet (1994), *C. glomerata* seems to be adapted to *P. brassicae* and *C. rubecula* to *P. rapae*. Furthermore, host distributions (gregarious versus solitary) appear to play a role in foraging success of the parasitoids. Considering the low number of offspring of *C. glomerata* from *P. rapae* and of *C. rubecula* from *P. brassicae* in the field, it is of interest to further investigate the chances of host encounter for both parasitoid species, and their flexibility in patch leaving decisions and in dealing with patch perception. With this objective, proportional hazard models are currently being applied to behavioural data (Vos et al., 1997).

Contact experiment

Naive females of *C. glomerata* and *C. rubecula* show similar responses when searching on herbivore-damaged leaves. Both parasitoids spend most of the time searching on the damaged spots. For *C. glomerata* the average percentages (50-70%) of time spent searching on the different leaf types resemble those percentages found for *C. glomerata* searching on Brussels sprouts leaves damaged by different instars of *P. brassicae* larvae (Mattiacci & Dicke, 1995a). Even in the absence of hosts, females remain searching on the damaged spots, apparently because other cues (synomones, kairomones and physical cues such as silk or frass particles) are present that play a role in the parasitoid's foraging decisions.
However, females of both species did not distinguish between hosts and host by-products of *P. brassicae*, *P. rapae* or *P. napi*, except for *C. rubecula* females on nasturtium leaves, where longer search times were observed on sites damaged by *P. rapae* than on sites damaged by the other two herbivore species. For both parasitoid species comparison of search times between plant species showed longer search durations on damaged Brussels sprouts leaves than on red cabbage or nasturtium leaves damaged by the same host species, except for *P. rapae*-damaged Brussels sprouts and nasturtium leaves, where search times of *C. rubecula* were not significantly different.

In conclusion, the parasitoids *C. glomerata* and *C. rubecula* need information consisting of a combination of olfactory cues, visual cues, kairomones and physical cues (actual encounter with hosts, frass particles and silk) in their search for hosts, to sample their habitat and to base their host searching decisions on. With this set of cues both species appear to be capable of finding the most suitable host for development of their offspring. For *C. glomerata* all three host species are suitable for development (Chapter 9), but under field circumstances *P. brassicae* is the main host resource for this species (Feltwell, 1982; Peters, 1992; Chapter 10). Probably through competition with *C. rubecula* for *P. rapae* (Laing & Corrigan, 1982) this host is not available and in fact not suitable. The free-ranging experiment of the present study adds evidence for a correlation between the host-finding behaviour of *C. glomerata* and *C. rubecula* and the suitability of *Pieris* hosts for larval development.

8

LONG-DISTANCE ASSESSMENT OF PATCH PROFITABILITY THROUGH VOLATILE INFOCHEMICALS BY THE PARSITOIDS *Cotesia glomerata* AND *C. rubecula*.

ABSTRACT

Using two closely related larval parasitoids (*Cotesia* sp.) of *Pieris* caterpillars we tested the hypothesis that parasitoids are capable of assessing patch profitability from a distance by showing differential responses to odours from plants infested with different host densities. We furthermore tested whether experience improves this assessment process. The effect of host densities on the olfactory responses of naive and experienced females was studied in two-choice wind tunnel experiments. Naive females of *Cotesia glomerata* and *C. rubecula* discriminated between odours from plants with high and low densities. Responsiveness of naive females to odours from host-infested leaves increased with increasing the total number of feeding hosts. In *C. glomerata* sensitivity to low host densities increased after experience.

The effect of multiple oviposition experiences of *C. glomerata* on different plants with different host densities was measured in two-choice situations. Females indeed use experienced host density of a patch as a cue to establish a preference, but the sequence of the experienced host densities influences the behaviour to a great extent. The first experience does not entirely fix their behaviour. For *C. glomerata*, the retention of learned odours was found to be three days.

This study illustrates the importance of quantitative differences in infochemicals to host-foraging decisions from a distance. It further demonstrates how experience can modify the parasitoid's response to variation in resource availability.

INTRODUCTION

Adult female parasitoids spend a great proportion of their lifetime searching for hosts. During this process they use a variety of cues in decisions such as where to search and how long to search at a certain location (van Alphen & Vet, 1986; Godfray, 1994; Vet et al., 1995). Natural selection should lead to the evolution of host-searching strategies that ensure a high reproductive success. We therefore expect female parasitoids to search for and select those patches that are highly profitable in terms of host suitability, host encounter rate and quality of food for the host, while minimizing risk (Vinson, 1976; van Alphen & Vet, 1986; Vet & Dicke, 1992; Godfray, 1994). Most foraging models conveniently assume that parasitoids are omniscient with respect to patch profitability (Charnov, 1976). Other models more realistically assume that parasitoids need to contact (sample) patches first to estimate the presence or density of hosts through the use of contact kairomones (Waage, 1979; Driessen et al., 1995). However, when differences in patch profitability provide the parasitoid with detectable differences in volatile cues at an earlier moment in their host-foraging sequences, this would help to optimize patch selection decisions even further. Using closely related larval parasitoids of *Pieris* caterpillars (*Cotesia* sp.) we tested the hypothesis that parasitoids are capable of assessing patch profitability from a distance by showing differential responses to odours from plants infested with different host densities.

The habitat and host resources with which parasitoids are faced are variable both within and among parasitoid generations (Vet et al., 1990b, Lewis et al., 1990; Vet & Dicke, 1992; Takabayashi et al., 1994; Takabayashi & Dicke, 1996). Parasitoids that attack plant-feeding hosts have to cope with variation in major cues, and differences in volatile cues can be a) qualitative or b) quantitative, or both. Qualitative differences in cues between plant-host complexes can be the result of specific chemical cues, such as host-derived cues (e.g. faeces), or cues that are produced by the plant in response to herbivory (herbivore-induced synomones) (Vet & Dicke, 1992; Turlings et al.,1993; Dicke, 1994; Takabayashi et al., 1994). Visual cues such as damage pattern and image of specific plant structures (e.g. flowers, leaves, buds) may also yield qualitative differences in cues (Wäckers, 1994; Harris & Foster, 1995). Quantitative differences between cues can be caused by differences in host or plant characteristics (e.g. species, age (Takabayashi et al., 1991a; 1995), or densities). Higher quantities are likely to be better detectable to parasitoids (Kaiser & Cardé, 1992; Whitman & Eller, 1992).

Animals can adapt to varying circumstances through behavioural plasticity. To maximize their host-finding success, parasitoids can adjust their behaviour to changing availability and quality of hosts (Turlings et al., 1993). Our hypothesis is that parasitoids show differential responses to odours from plants with different host densities and that experience may help them to estimate the informational value of differences in volatile cues between patches that differ in profitability. The parasitoid's degree of specialization is expected to determine the adaptive value of behavioural plasticity and so the behaviour of generalists is likely to be more flexible and affected by learning than that of specialists (Vet & Dicke, 1992). We tested the diet breadth - learning hypothesis through a comparative research on two closely related larval endoparasitoids of *Pieris* sp., *C. glomerata* and *C. rubecula* (Hymenoptera; Braconidae). *Cotesia glomerata* is a generalist that can attack several pierid hosts (Laing & Levin, 1982) that occur on cruciferous food plants, but also on plants from the families Tropaeolaceae, Rosaceae and Berberidaceae (Terofal, 1965; Feltwell,1982). *Cotesia rubecula* is more specialized at the host and plant level, since it is mainly parasitizes *Pieris* *rapae* that feeds on a narrower food plant spectrum (Terofal, 1965; Shenefelt, 1972; Feltwell, 1982; Tax, 1989).

In the present study we investigate how naive and experienced parasitoids deal with volatile cues of patches that differ in profitability. We investigate: (i) the influence of infochemical quantity as represented by host density, and (ii) the influence of infochemical quality, as represented by different plant-host complexes. The generalist *C. glomerata* is supposed to deal in a flexible way with qualitative or quantitativedifferences in patch profitability, responding to the most profitable patches. Through learning this species is expected to adjust its behaviour to the most profitable sites. Because *C. rubecula* is specialized on *P. rapae*, a solitary feeding host that can occur in low host densities on plants (Root & Kareiva, 1984; Davies & Gilbert, 1985), females of this parasitoid species are hypothesized to be adapted to search for low host densities and to be better adjusted to deal with low quantity cues. Furthermore, for *C. glomerata* we investigate (iii) whether females remember odours from different host plant species and learn to concentrate on the odours from the most profitable plant and (iv) whether and how fast learning effects wane.

MATERIALS & METHODS

Insects. Parasitoid colonies were initiated with individuals from the field collected near Wageningen. *Cotesia glomerata* was maintained on *Pieris brassicae* and *C. rubecula* on *P. rapae* larvae, feeding on Brussels sprouts plants. For the stock colonies, parasitoid females were offered Brussels sprouts leaves with first instar host larvae for parasitization. Leaves with parasitized hosts were transferred to cages with Brussels sprouts plants in greenhouse compartments, at 20-22°C, 50-70% RH and a 16L:8D photoregime. After parasitoid egression, cocoons were kept in petri dishes (i.d. 9 cm) in a climate controlled room, at 20-22°C, 50-70% RH and a 16L:8D photoperiod, until emergence. Males and females were caged together to allow mating. Cocoons were transferred daily to new cages to assure parasitoid groups of uniform age. Gauze cages (40*30*30 cm) were supplied with wet cotton wool and honey. Unless stated otherwise, just prior to experience or test procedures 3-5 day old females were collected from the emergence cage and kept individually in cotton-wool stoppered glass vials, provided with water and honey. *Pieris brassicae* and *P. rapae* colonies were maintained on Brussels sprouts plants (*Brassica oleracea* gemmifera cv. Titurel) in a climatic room, at 20-22°C, 50-70% RH and a L16:D8 photoregime. Caterpillars used for the training procedures and tests were always late first instar larvae.

Odour sources. For training and choice tests, herbivore-infested Brussels sprouts (*Brassica oleracea* gemmifera cv. Titurel) or nasturtium (*Tropaeolum majus* L. cv. Emperor of India) (Tropaeolaceae) plants were used. Food plants were reared in different greenhouse compartments, at 20-25°C., 50-70% RH and a L16:D8 photoregime. When necessary, dichlorophosphate (DDVP) was used to control aphids, but not within a period of four days before experiments. Infested leaves were obtained by placing individually potted foodplants in *P. brassicae* or *P. rapae* cages and allowing female butterflies to deposit their eggs for 4-6 h. The desired number of eggs per leaf was obtained by removing the excess of eggs with a small paintbrush. After hatching, host larvae were allowed to feed for 16-24 hours. For experiment 1 (see below) the stem of the plant was cut below the top 5 or 6 leaves with a surgical knife and immediately transferred to vials filled with fresh tapwater. For experiments 2 and 3 (see below), individual leaves containing larvae were excised from the plant with a surgical knife. Stems of the cut leaves were put in a vial (1.5 cm i.d) filled with water, that was closed with parafilm. For each odour source the vial with the leaf was placed in a horizontal glass cylinder (12 cm i.d., 21 cm long) with both ends open. This cylinder was placed on a glass socket (18 cm high) at the upwind end of a wind tunnel, the opening facing the parasitoid release site, allowing air to pass through.

Bioassays. All tests were conducted in a wind tunnel as described in Chapter 2. Test-plants and leaves in glass cylinders were offered in a dual-choice situation. All tests within a group of experiments were carried out on the same day, using four to five females per test. Tests were repeated on several consecutive days, until the total number of females tested was >20. In the first experiment *C. glomerata* and *C. rubecula* females were tested on the same day and the position of the odour sources was switched after the flight of four females (two of each species). In the second and third experiment the odour sources were switched after five *C. glomerata* females. Invididual females were gently introduced into the wind tunnel by allowing them to step over from the preservation vial into a glass vial at the release site. Tests lasted for a maximum of five minutes for each female. After flight initiation, choices for one of the two odour sources were recorded. Landings elsewhere in the wind tunnel were recorded as 'no response'. Each female wasp had only one flight opportunity within a test and were tested once.

Experiment 1. Host densities. To test responses of females of both species to different host densities on Brussels sprouts, the following three groups of tests were conducted.

a. Naive parasitoids. Naive females were tested for their discriminative abilities with regard to different host-densities. Both parasitoid species were offered the following combinations of host densities: 1) one host larva versus zero larvae (uninfested leaves); 2) three larvae versus one larva; 3) 24 larvae versus six larvae; 4) 90 larvae versus 24 larvae and 5) 90 larvae versus six larvae. For this group of tests, *Pieris brassicae*-infested plants were offered to *C. glomerata* and *P. rapae*-infested plants to *C. rubecula* females. Furthermore, to test the effect of host species on the parasitoids' choices, 6) naive *C. glomerata* were offered 24 *P. rapae* larvae versus six *P. brassicae* larvae and 7) *C. rubecula* were offered 24 *P. brassicae* larvae versus six *P. rapae* larvae.

b. Experienced parasitoids. To test whether experience enhances responsiveness to low host densities, parasitoid females were allowed to oviposit in one single host larva on a herbivore-damaged Brussels sprouts leaf one day prior to testing. *Cotesia glomerata* females were offered *P. brassicae* larvae, whereas *C. rubecula* were offered *P. rapae* larvae. After oviposition, females were individually kept overnight in a cotton-wool stoppered vial provided with water and honey at 15°C. As a control, naive females were collected in a vial and kept in the same way. The next day naive and experienced females were offered a dual choice situation in the wind tunnel, consisting of Brussels sprouts leaves infested by either three host larvae or one host larva of the same species as used in the experience procedures.

Since hardly any effect of experience on the behaviour of *C. rubecula* could be found in this or earlier studies (experiment 1b above; chapter 6), the following experiments were limited to *C. glomerata*.

Experiment 2. Multiple experience with different plant species infested by different host numbers.

To test the effect of experienced patch quality on odour preference, six groups of experienced females were created. Individual 3-5 days old *C. glomerata* females were offered a *P. brassicae*-infested Brussels sprouts leaf (S), 1 hour later followed by a *P. brassicae*-infested Nasturtium leaf (N) or in the reverse order. On these leaves females encountered high (20-25 L1) or low (3 L1) host densities. The amount of herbivore-damaged leaf tissue was correlated with the number of larvae present that had been feeding for 16-20 hours. For each of the two subsequent preflight treatments females had to oviposit in three *P. brassicae* larvae. At the end of the experience procedure, wasps were transferred to glass vials as described earlier. Choice tests were conducted in two parts. The first part was carried out with females that had experienced only high host densities: 1) high density on Brussels sprouts + high density on nasturtium (highS + highN) and 2) high density on nasturtium + high density on Brussels sprouts (highN + highS). The second part was conducted with females that had experienced a high host density plus a low host density on Brussels sprouts or on nasturtium: 1) high density on Brussels sprouts + low density on nasturtium (highS+lowN), 2) high density on nasturtium + low density on Brussels sprouts (highN+lowS), 3) low density on Brussels sprouts + high density on nasturtium (lowS+highN) and 4) low on nasturtium + high on Brussels sprouts (lowN+highS). Daily, four females of each training group were tested, over several consecutive days. Choices consisted of a Brussels sprouts leaf and a Nasturtium leaf, both equally damaged by a clutch of ca 20-25 *P. brassicae* larvae. Total leaf area was 40-50 cm^2 for both types.

Experiment 3. Retention of learned odour preferences.
For the experiment on retention, 3-day old females of *C. glomerata* were trained individually in a petri dish provided with a host-infested Nasturtium leaf. Females were allowed to oviposit in three of the available *P. brassicae* larvae, after which they were kept in a nylon gauze cage provided with water and honey until testing. Choice tests were conducted with females of different groups of wasps, one, two, three, four and five days after oviposition experience and with naive wasps (3-8 days old) as control. Choice options consisted of a Brussels sprouts leaf and a Nasturtium leaf, both equally damaged by a clutch of ca 20-25 *P. brassicae* larvae. Total leaf area was 40-50 cm^2 for both types.

Statistical analysis. In all tests, preferences for odour sources were analysed using binomial tests. Overall differences in parasitoid choice distributions between groups within experiments and differences in responsiveness were analysed using chi-square tests for independent samples, where necessary followed by partitioning degrees of freedom (Siegel, 1988). Data of the second part of experiment 2 were further analysed using GLIM procedure.

RESULTS

Experiment 1. Host densities
a. Naive parasitoids. For naive females of *C. glomerata* and *C. rubecula* an overall effect of host densities on responsiveness was found (Fig 1). Percentages of non-responding females decreased with increasing total host densities (Fig 1a & 1b). Furthermore, *C. glomerata* showed significant preferences for the high host density in the two combinations with the largest difference between host numbers (Fig 1a, two lower bars), and *C. rubecula* showed preferences for the highest host density within a test in the three highest host-density combinations (Fig 1b, three lower bars). When two different host species are present in different densities, *C. glomerata*

and *C. rubecula* significantly prefer plants infested by the highest number of larvae (Fig. 1c).

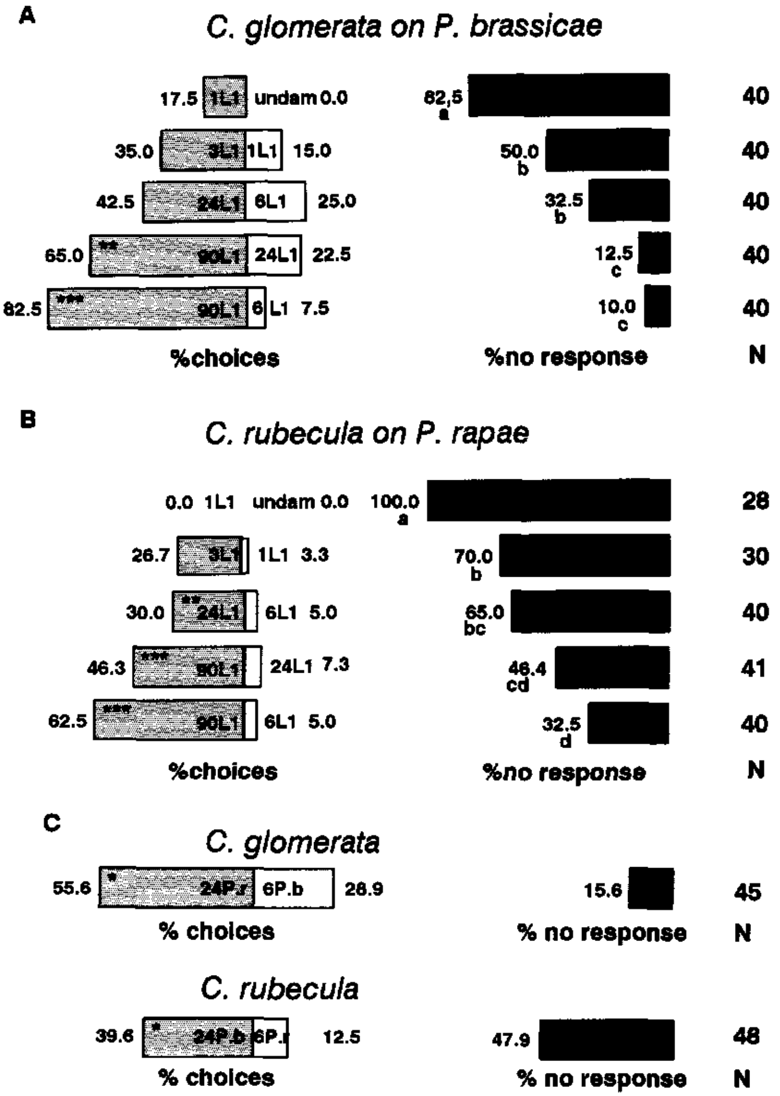

Fig. 1. Responses of naive (a) *C. glomerata* females to Brussels sprouts leaves infested by different numbers of first instar *P. brassicae* larvae, (b) *C. rubecula* females to Brussels sprouts leaves infested by different numbers of first instar *P. rapae* larvae, and (c) *C. glomerata* or *C. rubecula* females to Brussels sprouts leaves infested by different numbers of *P. brassicae* or *P. rapae* larvae. Asteriks indicate significant differences within a choice test *** P<0.001; ** P<0.01; * P<0.05. No-response bars with the same letters are not significantly different. N indicates number of females tested.

b. Experienced parasitoids. Both naive and experienced females of *C. glomerata* showed a significant preference for plants infested by three first instar *P. brassicae* larvae over plants infested by only one L1. It should be noted that naive females did not show a preference for three L1 over one L1 in experiment 1a (Fig 1a; P=0.057), but that they did show a preference here (Fig 2a; P=0.038). Preference for the higher host density in experienced females was more clear than in naive females (P=0.00073), and responsiveness of experienced females was significantly higher than that of the naive group (P<0.0001). Choice distributions did not differ between naive and experienced females (P=0.69) (Fig 2a). In contrast, *C. rubecula* females hardly responded to these low host densities. The responsiveness of *C. rubecula* in this test was very low, not allowing statistical analysis and reliable interpretation of results (Fig 2b).

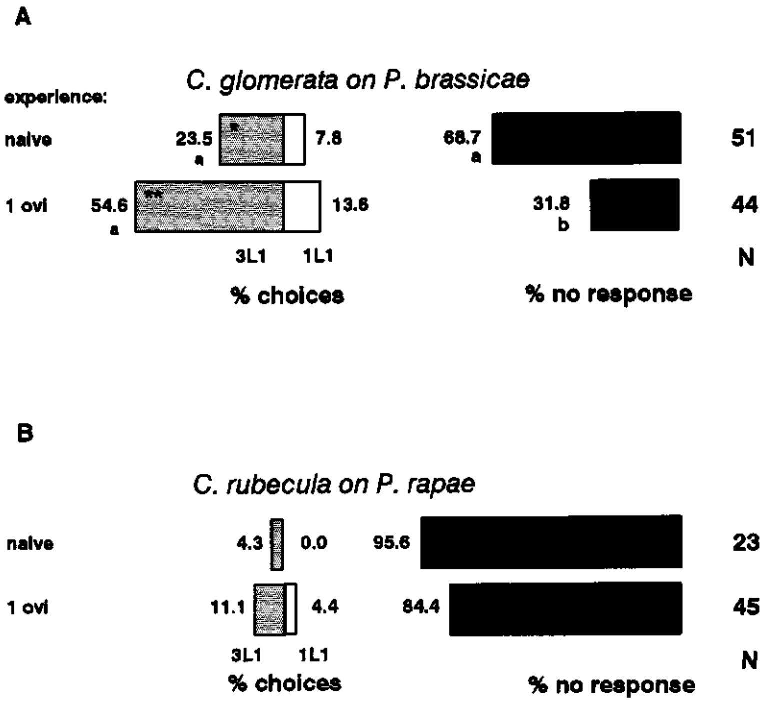

Fig. 2. Responses of naive or experienced females of (a) *C. glomerata* to Brussels sprouts infested by 3 first instar larvae or 1 first instar larva of *P. brassicae*, or (b) *C. rubecula* to Brussels sprouts infested by 3 first instar larvae or 1 first instar larva of *P. rapae*; Asteriks indicate significant differences within a choice test ** P<0.01, * P<0.05. N indicates the number of females tested.

Experiment 2. Multiple experience with different plant species infested by different host numbers.

Responsiveness of *C. glomerata* females that had two subsequent oviposition experiences was high. Of all groups more than 90% of the females made a choice for one of the odour sources offered in the test. In the two parts of the experiment responsiveness did not differ between *C. glomerata* groups with different preflight experiences (Fig 3a & 3b).

Experience with only high host densities, first on Brussels sprouts and subsequently on nasturtium, led to preferences for the *P. brassicae*-infested Brussels sprouts leaves ($P<0.05$), whereas experience in the reverse order resulted in an equal distribution over the two alternative odour sources ($P=0.18$) (Fig 3a). Choice distributions between the two groups were significantly different ($Chi^2=7.228$; $df=1$; $P=0.0072$).

GLIM analysis shows that there is a significant effect ($P<0.0001$) of the experienced high host density on the subsequent choices of *C. glomerata* females (table 1). This indicates that a significant difference exists in the chance of choosing *P. brassicae*-infested Brussels sprouts between an experience with high *P. brassicae* density on Brussels sprouts and high *P. brassicae* density on nasturtium. Furthermore, the order in which high or low host densities are experienced plays an important role. The first experienced host density has a significant effect ($P<0.0001$) on the subsequent landing decisions of the experienced *C. glomerata* females.

Table 1. GLIM analysis of choices for different *P. brassicae*-infested plants for *C. glomerata* females with different pre-flight treatments.

Model	Scaled Deviance	Deviance Change	df	P
null	38.484	-	3	
+ High density experience	16.181	22.30	2	< 0.0001
+ High density + First experience	0.034	16.15	1	< 0.0001

A first oviposition experience on a high host-density, followed by an oviposition experience on a low host-density, leads to a significant preference for the first learned odour (high on S + low on N, $P<0.0001$, and high on N + low on S, $P<0.0001$) (Fig 3b, two upper bars). Choice distributions of these first two *C. glomerata* groups differed significantly ($P<0.001$). On the other hand, the first experience on a low host-density, followed by an experience on a high host-density, does not lead to a preference for either of the offered odour sources (low on S + high N, P=0.31, and low on N + high on S, P=0.08) (Fig 3b, two lower bars). Choice distributions of the latter two *C. glomerata* groups did not differ significantly. Females with first experience on a high host density showed significantly different choice distributions than females with a first experience on a low host density. Overall choice distributions differed significantly (Chi^2=24.91; df=3; P<0.0001) (Fig. 3b).

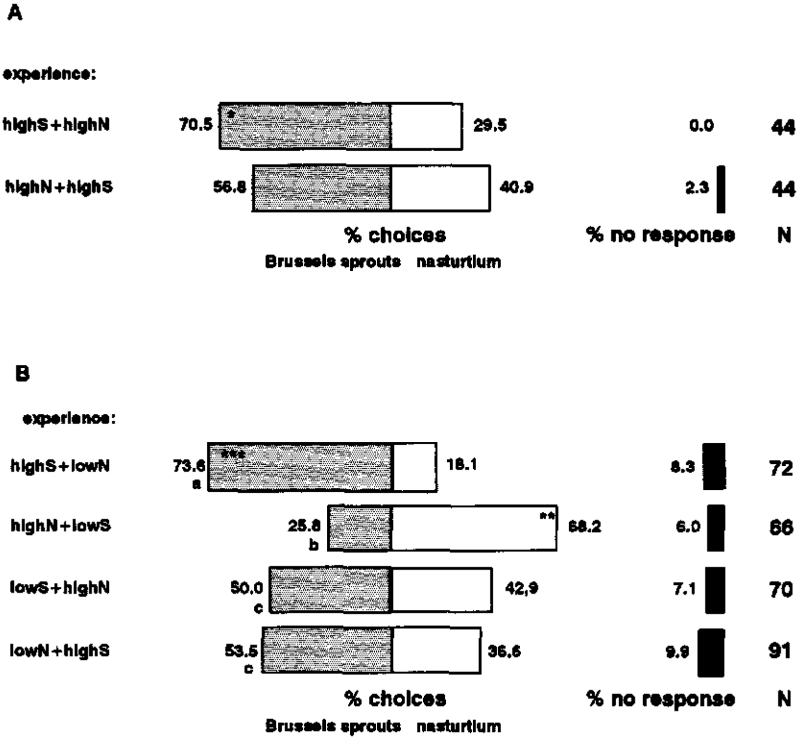

Fig. 3. Effect of experienced host densities on choices of *C. glomerata* for *P. brassicae*-infested Brussels sprouts or nasturtium leaves; (a) experience on high host densities alternatingly on Brussels sprouts or nasturtium; (b) experience on alternatingly high and low host densities on Brussels sprouts or nasturtium. Asteriks indicate significant differences within a choice test *** P<0.001; ** P<0.01; * P<0.05. Bars with same letters are not significantly different. N indicates number of females tested.

Experiment 3. Retention of learned odour preferences.

Naive *C. glomerata* females showed a clear preference for the *P. brassicae*-infested Brussels sprouts leaf (S) over the nasturtium leaf (N) (P<0.0001) (Fig 4). One day after experience with *P. brassicae*-infested nasturtium leaves more females compared to naive females chose infested nasturtium, although females did not show a preference for this plant-host complex (P=0.087). However, females that had an oviposition experience two days earlier preferred the nasturtium-*P. brassicae* complex (P<0.05). From day three onwards, preference for the experienced plant-host complex gradually waned, until preference of *C. glomerata* had switched back to the Brussels sprouts - *P. brassicae* complex. Responsiveness of females with an oviposition experience was in all cases significantly higher than that of naive females and did not differ among groups of experienced wasps.

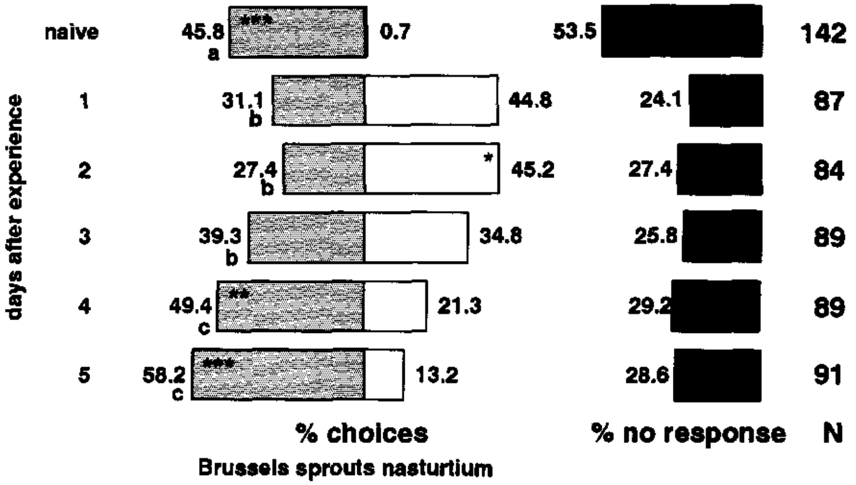

Fig. 4. Influence of time after experience with *P. brassicae*-infested nasturtium leaves on the choices of *C. glomerata* for *P. brassicae*-infested Brussels sprouts or nasturtium. Asteriks indicate significant differences within a choice test *** P<0.001; ** P<0.01; * P<0.05. Bars with same letters are not significantly different. N indicates number of females tested.

DISCUSSION

Experiment 1. Host densities

The results show that parasitoids are capable of assessing patch profitability from a distance, and that quantitative differences in infochemicals play a role in patch selection.

As expected, naive females of *C. glomerata* and *C. rubecula* were able to discriminate between infochemicals from plants with high and lower host densities offered in two-choice situations. Furthermore, responsiveness of female parasitoids to plant-host odours significantly increased with increasing host densities.

After one single oviposition experience on low *P. brassicae* densities, *C. glomerata* showed a more clear discriminative ability compared to naive *C. glomerata* females: i.e experience somewhat improved their ability to distinguish between odours from plants infested with one and three host larvae. Overall responsiveness to low host densities is much higher in experienced *C. glomerata* females than in naive *C. glomerata* females (cf. Fig 1a and Fig 2a). This may have been a priming effect, where experience increases the females' motivation to search for hosts. On the other hand, or additionally, it may have been an active foraging decision, based on estimation of low habitat profitability.

Although we expected *C. rubecula* to deal easily with low host densities, given the scattered distribution of *P. rapae* under natural circumstances (Root & Kareiva, 1984; Davies & Gilbert, 1985), responses of this parasitoid species were low. Even experience did not increase the proportion of females that fly and search for hosts. In earlier studies with the two *Cotesia* species, the effect of experience on the behaviour of *C. rubecula* was always very low compared to *C. glomerata* (Chapter 6). Our results with *C. rubecula* agree with those of Kaiser & Cardé (1992) who found that the proportion of naive *C. rubecula* females that located odour-sources increased by increasing the odour concentration and that oviposition experience did not increase the proportion of females that responded to high odour concentrations. However, in contrast to our results, Kaiser & Cardé (1992) did find that the proportion of *C. rubecula* females that located the odour source at low odour concentrations, increased substantially after oviposition experience on the plant-host complex.

Experiment 2. Multiple experiences with different plant species infested by different host numbers.

Insects can be restricted in their capacities to memorise several different stimuli (Lewis, 1993) and multiple experiences may affect subsequent foraging decisions. Furthermore, learning of novel odours can interfere with the recall of previously learned odours, such that the insect has to relearn the first learned odour (Lewis, 1986; 1993), or that the order of learning is important for subsequent preferences (de Jong & Kaiser, 1992; Kaiser & de Jong, 1993). On the other hand, insects may learn to become more choosy after estimating the average value of the habitat (Vet et al., 1997).

Cotesia glomerata females are capable of adjusting their preference to profitable patches, although the encountered higher profitability is not the only cue in their host-searching decisions. Preferences for host-habitats with the highest host densities were expected in all treatments, but they were found for females with a first experience on high host densities. They seem to show a strong response to the first learned plant-host complex. A second experience with high profitability after a first experience with a low host density did alter the choice distribution of females, but could not lead to a preference for the patches with higher profitability. A first experience can induce a strong preference which is not easily overruled. The second experience may have a stronger effect on the parasitoid's behaviour when it is delayed to the time that the induced preference is waning (see experiment on retention). In honey bees memory of the first learned flower is erased, when a different rewarding flower is offered on a subsequent day at the same time of the day as the first one (Bogdany, 1978). For our study *C. glomerata* females had their second experience immediately after the first experience, with a maximum time elapse of 1 h. The second experience might have interfered with the first experience, but apparently did not totally overrule this experience. This assumption further indicates, that *C. glomerata* is able to memorise odours from more than one plant-host complex: females responded equally well to plant-host complexes on which they had their first experience with low host densities as to plant-host complexes on which they had their second experience with high host densities. If *C. glomerata*'s memory could hold the information of one plant-host complex only, a preference for either the first or the second learned odour was expected. Testing the effect of unrewarding experience in combination with rewarding experience may give further insight in the effect of the first experience on behaviour of parasitoids (Papaj et al., 1994).

Experiment 3. Retention of learned odour preferences.

In all groups of experienced *C. glomerata* females, responsiveness was very high, compared to responsiveness of naive females. It was already known that an oviposition experience enhances the motivation of parasitoids to search for hosts (Vet, 1988; Papaj & Vet, 1990; Steinberg et al., 1992; Turlings et al., 1993). Our data show that this motivation remains high. Apparently, the effect of priming did not wane.

Our data show that the delay of reinforcement has a significant effect on the foraging behaviour of *C. glomerata*. The learned response waned and with time the experienced females showed the same choice distribution as naive females. Vet (1988) and Poolman-Simons et al. (1992) also found that lack of repeated reinforcement obliterates the effect of associative learning of a substrate on subsequent choices of parasitoids. Wasps switch back to their innate preference for a certain substrate. Physiological constraints such as memory capacities may be involved (Poolman Simons et al., 1992). Furthermore, in variable and unpredictable environments waning of learned responses may be adaptive, when parasitoids associate the lack of reinforcement with the absence of the resource. In the meantime, novel odours can be learned. Experience with these odours can affect the behaviour, when the initially learned stimulus does not lead to host encounter. Parasitoids are likely to switch back to their innate preference.

In conclusion, this study illustrates plasticity in the host-searching behaviour of the generalist *C. glomerata*, and the almost complete absence of plasticity in the behaviour of the specialist *C. rubecula*. According to our hypothesis, naive females of both parasitoid species easily discriminate from a distance between plant-host complexes with different profitability on the basis of volatile cues and they prefer to fly to patches with the highest number of hosts. Experience plays a more important role in the patch selection behaviour of *C. glomerata* than in that of *C. rubecula*. In *C. glomerata* experience increased the ability of females to locate low-density patches, and to choose for the one with the highest number of hosts (i.e. the most profitable one). In *C. rubecula* experience did not affect the ability to assess patch profitability. Furthermore, after multiple experience with different plants with different host densities *C. glomerata* can adjust its preference to the most profitable one, although the first experience they get also has a significant effect. In accordance to what was found for several other parasitoid species, learned responses of *C. glomerata* wane after delay of reinforcement with the stimulus. This study illustrates the importance of quantitative differences in volatile cues in long-distance host- searching decisions. It further demonstrates how experience can modify the parasitoid's response to variation in resource availability.

III. PARASITOID PERFORMANCE AND HOST USE

9

EFFECTS OF HOST SPECIES ON SELECTED LIFE-HISTORY PARAMETERS IN A SPECIALIST AND A GENERALIST *Cotesia* PARASITOID.

ABSTRACT

Host specificity and host-selection by insect parasitoids are hypothesized to be correlated with suitability of the hosts for parasitoid development. The present study investigates the correlation between host suitability and the earlier studied host-finding behaviour of two closely related braconid larval parasitoid species, the generalist *Cotesia glomerata* and the specialist *C. rubecula* (Hymenoptera: Braconidae). We compared the capability of both parasitoid species to parasitize and develop in three *Pieris* host species, i.e. *P. brassicae* (L.), *P. rapae* (L.) and *P. napi* (L.) (Lepidoptera: Pieridae). We predicted that variation in parasitoid performance between host species is lower for the generalist than for the specialist. In laboratory experiments, we measured the effect of host species on the fitness parameters such as survival, development, sex ratio and size of parasitoid progeny. The results show that *C. glomerata* is capable of developing in the three host species, with significant differences in parasitoid survival, clutch size and adult weight among *Pieris* species. The host range for development was more restricted for *C. rubecula*. Although *C. rubecula* is physiologically able to develop in *P. brassicae* larvae, parasitoid fitness is negatively affected by this host species, compared to its most regular host, *P. rapae*. A comparison of the present data on host suitability with data from earlier studies on host-searching and host-acceptance behaviour leads to the conclusion that the host-foraging behaviour of both parasitoid species leads to selection of the most suitable host species for parasitoid development.

INTRODUCTION

Current optimality models assume that animals assess and select resources according to their relative impact on fitness. For instance, populations of herbivorous insects feeding on a range of host plants that differ in abundance and quality have been shown to prefer those that maximize larval performance and reproduction (Wicklund, 1975; Denno et al., 1990; Singer et al., 1994). However, several studies indicated an imperfect degree of concordance between host plant selection and insect fitness (Thompson, 1988, Courtney & Kibota, 1990; Horner & Abrahamson, 1992). The weak expression of host use by herbivores could arise from a variety of determinants such as plant attributes (architecture, phenology, reliability, chemical defense), impact of natural enemies and inability of insects to assess host suitability (reviewed by Fox & Lalonde, 1993). The relationship between host selection and host suitability for parasitoids has received less attention, although for some parasitoid-host systems it has been extensively examined (Smilowitz & Iwantsch, 1973; 1975; Iwantsch & Smilowitz, 1975; Janssen, 1989). However, endoparasitoids are likely to be more specialized in host use than herbivores. Typically, immature stages of parasitoids have intimate nutritional and physiological interactions with their hosts (Vinson 1975, Beckage 1985, Slansky, 1986; Beckage & Riddiford, 1983; Beckage & Templeton, 1986; Strand, 1986; Mackauer & Sequeira, 1993), and can regulate host behaviour (Brodeur & McNeil 1989; Brodeur & Vet, 1994). Assuming that consequences of choosing a poor host in the presence of suitable hosts, are highly detrimental for the developing offspring, any mechanisms resulting in increased capacities of females to discriminate against inferior hosts should have been favoured by natural selection. This has been shown for example for *Hyposoter exiguae*, a parasitoid of lepidopterous hosts by Smilowitz & Iwantsch (1975) and Iwantsch & Smilowitz (1975). Accordingly, recent studies have emphasized the relative importance of parasitoid foraging behaviour, as opposed to immunological and physiological constraints, in the evolution of parasitoid host range (Vet & Dicke 1992, Wiskerke & Vet 1994, Chapters 3, 5, 6, 7, 8 and 10). In this study, we compare the capacity of two closely related *Cotesia* wasps (Hymenoptera: Braconidae) to parasitize three *Pieris* host species (Lepidoptera: Pieride) and discuss how these performances are related to their host specificity. *Cotesia glomerata* (L.) is a gregarious endoparasitoid that commonly attacks caterpillars belonging to a few genera of the Pieridae (Feltwell 1982), whereas *C. rubecula* (Marshall) is solitary and specific to *P. rapae* (L.), although some individuals had been recovered from *P. brassicae* (L.) larvae in the field (Richards 1940). In Europe, both parasitoid species have overlapping niches and may coexist in the field. *Pieris brassicae* and *P. rapae* are occasional pests of cabbage crops, whereas *P. napi* (L.) usually feeds on wild crucifers and lives in more permanent shady and humid habitats (Richards, 1940; Terofal, 1965; Ohsaki & Sato, 1990; Tax, 1989). The foraging behaviours of *Cotesia* wasps are well-documented (see Chapters 3, 5, 6, 7, 8 and 10 and references therein) as well as some aspects of the suitability of different *Pieris* species to *C. glomerata* and *C. rubecula* (Sato 1976, Brodeur & Vet 1995). In chapter 5 the role of *Pieris* host species on ovipositional decisions by *C. glomerata* and *C. rubecula* was examined. This study showed that *C. glomerata* has a higher degree of behavioural plasticity towards acceptance of *Pieris* host species than *C. rubecula*. *Cotesia glomerata* females parasitized the three *Pieris* species and showed higher acceptance of first and second instars over third instar. Host acceptance by *C. rubecula* was higher for *P. rapae* and females did not distinguish among the host ages. In contrast, when foraging for *P. brassicae* and *P. napi*, *C. rubecula* females more readily attacked early first instars.

In this paper, we tested the hypothesis that the level of host acceptance is directly related to the degree of profitability of the host. From the above observations, we predict that variations in parasitoid performance between host species would be lower for the generalist *C. glomerata* than for the specialist *C. rubecula*. We determined in the laboratory the relative suitability of the three *Pieris* host species for *Cotesia* by measuring their effect on several important components of parasitoid fitness such as survival, development, sex ratio and size of parasitoid progeny.

MATERIALS AND METHODS

Study organisms. Parasitoid and butterfly cultures were established from individuals collected in Wageningen, The Netherlands, and maintained as described in Brodeur & Vet (1995). *Cotesia glomerata* and *C. rubecula* were reared on *P. brassicae* and *P. rapae*, respectively, and had been in culture for ca. 16 months. Early first and late first instar *Pieris* larvae were used for *C. rubecula* and *C. glomerata* respectively, since previous tests indicated that these were the stages the parasitoids preferred (Chapter 5). Synchronous cohorts were obtained by transferring and rearing neonate larvae on Brussels sprouts (*Brassica oleracea gemmifera* cv. Titurel) seedlings. All hosts were weighted 1-2 h prior to the test to standardize initial host size. The range of host weights used for each *Pieris* species are given in chapter 5. *Cotesia* females used in this study were standardized as followed. Following eclosion, males and females fed with honey were caged together to allow mating. Prior to each test, 3-5 days old naive females (no oviposition experience) were selected at random and kept individually in a petri dish of 5 cm in diameter and 1.5 cm in depth. Unless stated otherwise, parasitoid females were allowed to parasitize only once.

Measurement of parasitoid performance. The following procedure was used to parasitize and rear each host used in the experiment. *Pieris* hosts were individually placed on a leaf disk bearing host-derived products as well as leaf damage areas and offered to the wasps. *Cotesia glomerata* was restricted to a maximal oviposition time of 20 seconds to prevent unusual large clutch sizes (Ikawa & Okabe, 1985). Following parasitization, hosts were withdrawn from the petri dish and individually reared at 20 ± 1°C, 16L:8D in a petri dish containing wet cotton wool, a filter paper disk and Brussel sprouts leaf disks. Larvae were reared individually in this way to be able to follow host and parasitoid performance for each parasitized caterpillar. All larvae were examined daily for host survival and pupation and for parasitoid egression and emergence. Larvae were supplied with fresh food and petri dishes were cleaned every second day. The experiment lasted until host death, host pupation or adult parasitoid emergence. Parasitized larvae that died prior to host pupation or parasitoid egression were dissected to determine parasitoid development. Following larval parasitoid egression, all *Pieris* larvae were dissected to determine the number of *Cotesia* larvae which failed to egress. For each parasitized host, another larva of the same cohort was used as a control and reared under the same condition. Six replicates were carried out with 10 parasitized and 10 unparasitized larvae of the three host species. A second cohort of six additional replicates was done with *C. rubecula* parasitizing *P. brassicae* and *P. rapae*, as levels of successful parasitism were low on these two host species. *Pieris napi* was excluded from the second set of replicates as *C. rubecula* completely failed to develop in this host species. The second cohort comprised 6 groups of 20 parasitized larvae and one group of 20 unparasitized larvae. In this instance, each female was allowed to have a maximum of 6 ovipositions.

Five parameters were calculated to determine the effect of *Pieris* host species on the overall performance of *Cotesia*. Survivorship, development time, number of *C. glomerata* larvae per host, adult weight and sex ratio of the offspring were measured.

Percentage parasitism. Levels of *Cotesia* offspring survival (completion of development from egg to adult) were determined for the three *Pieris* host species by measuring the proportion of parasitized larvae yielding adult parasitoids. Parasitism was calculated after adjusting for 'natural' host mortality using the appropriate unparasitized control hosts. Successful parasitism is expressed as the proportion of parasitized larvae producing parasitoid cocoon masses or the proportion of adults emerging from parasitized hosts for *C. glomerata* and *C. rubecula*, respectively. Parasitoid mortality was further assessed from larval egression to adult emergence. We made a distinction between larvae that (i) failed to egress from the host, i.e. fully developed larvae that either remained within the host or were trapped in egression holes, (ii) failed to spin cocoons, often because of host bleeding, and (iii) died during pupation or failed to emerge from the cocoons. Contingency table and Chi-square tests were used to determine whether percentage parasitism differed between host species. Following a significant difference, the contingency table was subdivided using the STP method at the 0.05 level of significance (Sokal & Rohlf, 1981). Mortality parameters were analysed using Kruskall-Wallis and Chi-square tests for *C. glomerata* and *C. rubecula*, respectively.

Development time. We determined the effect of *Pieris* host species on egg-to-pupa, pupa-to-adult and total development time of *Cotesia*. Only parasitoids that survived to the adult stage were used to calculate developmental periods. Parasitoid egression and emergence were monitored daily. Data were analysed using a Kruskal-Wallis one-way ANOVA and a one-way ANOVA for *C. glomerata* and *C. rubecula*, respectively.

Clutch size. Following *C. glomerata* larval egression the number of parasitoid cocoons was determined and the host remainings dissected to look for non-egressed parasitoid larvae. Hereafter, clutch size of *C. glomerata* corresponds to the number of fully developed parasitoid larvae produced by parasitized larvae. Comparisons of clutch size between host species were made using a one-way ANOVA.

Adult weight and sex ratio. Following emergence *Cotesia* wasps were collected in petri dishes and sexed. They were then killed and dried for 4 days at 60°C after which they were individually weighed on a Cahn electrobalance. For *C. glomerata*, coefficients of variation were used to determine if, within a clutch, individuals of a given sex differ in dry weight (Sokal & Rohlf 1981). The relation between clutch size and adult weight was further examined using Pearson correlations, in this instance analyses were done from clutches that gave rise to males only. Differences in dry adult weight were tested using the Student's t test for *C. rubecula* and a two-way ANOVA followed by the Tukey-Kramer unplanned test for *C. glomerata*. Sex ratios, expressed as the proportion of males, were analysed using a G-test of independence and William's correction for continuity for *C. rubecula* and a Kruskal-Wallis one-way ANOVA for *C. glomerata*. In this instance, data from females which produced only males were excluded. Frequency data were arcsine transformed prior analysis. All ANOVA were conducted using PROC GLM, PROC TTEST was used for t-tests, PROC REG for linear regressions and PROC FREQ for chi-square test of independence (SAS, 1988).

RESULTS

Parasitization success.

Cotesia glomerata has the capacity to develop in the three *Pieris* species tested. The adjusted percentage of parasitized *Pieris* larvae from which *C. glomerata* larvae had egressed was highest on *P. brassicae*, intermediate on *P. rapae*, and lowest on *P. napi* (Table 1). However, once parasitoid larvae had successfully completed their development within the host, survivorship from egression to adult emergence was similar on the three *Pieris* hosts (Table 1).

Cotesia rubecula completely failed to develop in *P. napi*. None of the 60 parasitized larvae yielded a parasitoid cocoon and no parasitoid larvae were found in *P. napi* prepupae following dissection. Parasitism success of *C. rubecula* in *P. brassicae* and *P. rapae* was low (from 19.6 to 41.7%), especially for the first cohort (Table 2). The percentages of hosts successfully parasitized by *C. rubecula* did not differ between the two host

Table 1. Percentage parasitism, clutch size and mortality parameters (%), from parasitoid larval egression to adult emergence, of *Cotesia glomerata* parasitizing late first instar larvae of three *Pieris* hosts. For clutch size and mortality parameters, each value is a mean of six replicates. Values in parentheses are standard error means.

Pieris species	Parasitism[1]		Clutch size[2]		Parasitoid mortality (%)			
	x/N	%	Range	X	Larvae failing to egress	Larvae failing to spin cocoons	Pupal mortality	Total
P. brassicae	33/50	66.2 a[3]	4-42	27.8 a[4] (1.6)	2.7 (0.9)	8.8 (3.3)	25.0 (4.1)	31.8 a[5] (4.9)
P. rapae	18/39	46.2 ab	6-34	21.7 b (1.8)	8.1 (5.1)	3.0 (1.2)	36.1 (7.9)	39.7 a (7.5)
P. napi	13/38	34.2 b	16-37	28.3 a (1.5)	3.5 (1.4)	11.5 (6.8)	32.0 (7.8)	43.5 (7.9)
		Chi^2 = 7.31 $P < 0.05$		$F_{2,62} = 3.80$ $P = 0.03$				$H = 2.43$ $P = 0.30$

[1] Proportions of parasitized hosts that produced parasitoid cocoon masses, corrected for larval and prepupal mortality of unparasitized hosts.

[2] Numbers of fully developed parasitoid larvae egressing or failing to egress from the host.

[3] Means followed by different letters are significantly different (Contingency table).

[4] Means followed by different letters are significantly different (ANOVA)

[5] Means followed by the same letter are not significantly different (Kruskal-Wallis).

Table 2. Percentage parasitism and mortality parameters, from parasitoid larval egression to adult emergence, of *Cotesia rubecula* parasitizing early first instar larvae of two *Pieris* hosts.

Cohort	*Pieris* species	Parasitism[1]		Parasitoid mortality				
		x/N	%	Larvae failing to egress x/N	Larvae failing to spin cocoons x/N	Pupal mortality x/N	Total x/N	Total %
A	*P. brassicae*	10/37	27.0 a[2]	1/12	1/11	0/10	2/12	16.7 a[2]
	P. rapae	9/46	19.6 a	0/12	0/12	3/12	3/12	25.0 a
			$Chi^2 = 0.61$					$Chi^2 = 0.26$
B	*P. brassicae*	41/108	38.0 a	4/48	0/44	2/44	6/48	12.5 a
	P. rapae	45/108	41.7 a	1/48	0/47	1/47	2/48	4.2 a
			$Chi^2 = 0.30$					$Chi^2 = 2.18$

[1] Proportions of adult parasitoids emerging from parasitized hosts, corrected for larval and prepupal mortality of unparasitized hosts.

[2] Withing each cohort, means followed by the same letter are not significantly different (Chi^2, $P > 0.1$).

species. Accordingly, parasitoid mortality from larval egression to adult emergence was similar for both *Pieris* host species (Table 2).

Development time.

Data for males and females were pooled as there was no difference in developmental time beween sexes for *C. glomerata* (ANOVA, F1,99= 1.48, P= 0.22; pupal development). *Cotesia glomerata* egg-to-adult development time was almost identical for the three *Pieris* host species tested (Table 3). Similarly, no difference in the time period between oviposition and larval egression was found for *C. rubecula* parasitizing *P. brassicae* and *P. rapae* (Table 4). However, pupal development was shorter for parasitoids reared in *P. brassicae* than in *P. rapae*. The difference was only significant for males, female numbers probably being too low to be meaningfully analysed.

Clutch size.

For each *Pieris* host species tested, we observed a large range of *C. glomerata* clutch size (Table 1). The number of parasitoid larvae produced per clutch was significantly higher on *P. brassicae* and *P. napi* than on *P. rapae*.

Adult dry weight.

Within a *C. glomerata* clutch, individuals of a given sex (males or females) are of similar size. Coefficients of variation of parasitoid dry weights ranged from 11.3 to 14.8% for each of the 6 combinations tested (males and females emerging from three *Pieris* host species). Furthermore, in our study, adult weight was not significantly correlated with clutch size for males arising from parasitization of *P. brassicae* (r=0.152, P=0.62), *P. rapae* (r=0.213, P=0.79), *P. napi* (r=-0.565, P=0.32) or for pooled data (r=0.107, P=0.64). The effects of sex (ANOVA, F1,94=5.87, P=0.02) and host species (F2,94=4.07, P=0.02) were significant factors in determining *C. glomerata* adult weight (Table 1). Females were heavier than males and individuals of both sexes were smaller when developing in *P. napi* than in *P. brassicae* or *P. rapae*. *Cotesia rubecula* larvae developing in *P. rapae* produced heavier adults, both males and females, than those developing in *P. brassicae* (Table 4). Females were heavier than males, in *P. brassicae* and in *P. rapae*.

Sex ratio.

Sex ratio of offspring did not vary significantly as a result of *Pieris* spp. for both *Cotesia* species (Table 3 and 4). However, most emerging adults were males, this biased sex ratio being more evident for *C. rubecula*.

Table 3. Development time (days), adult dry weight (mg) and sex ratio of *Cotesia glomerata* parasitizing late first instar larvae of three *Pieris* hosts. Where performance differed significantly between sexes, means are given separately for males and females. Values in parentheses are standard error means.

	N(1)		Development time					Adult dry weight				Sex ratio(2)
Pieris species	Males & Females	Males only	Egg-Pupa		Pupa-Adult		Egg-Adult	Males		Females		(% Male)
			Range	X	Range	X	X	Range	X	Range	X	
P. brassicae	20	13	13-20	14.5 a (3) (0.3)	6.1-10.4	8.1 a(3) (0.2)	22.5 a(3) (0.4)	0.12-0.48	0.31 a(4) (0.02)	0.18-0.52	0.36 a(4) (0.02)	62.6 a(5) (4.8)
P. rapae	8	10	13-17	14.1 a (0.2)	7.0-10.0	8.2 a (0.2)	22.3 a (0.3)	0.20-0.40	0.32 a (0.02)	0.24-0.49	0.39 a (0.02)	60.0 a (9.5)
P. napi	7	6	14-17	14.6 a (0.3)	6.4-9.5	7.9 a (0.2)	22.4 a (0.2)	0.16-0.45	0.27 b (0.02)	0.48-0.38	0.29 b (0.03)	67.5 a (6.2)
				H=2.51 P=0.28		H=1.76 P=0.42	H=0.07 P=0.96					H=0.13 P=0.93

(1) Numbers of parasitized hosts that produced parasitoid cocoon masses containing males and females or males only.

(2) Only clutches with at least one female offspring were included in this analysis.

(3) Means within columns followed by the same letter are not significantly different (Kruskal-Wallis).

(4) Means within columns followed by different letters are significantly different (Two-way ANOVA followed by Tukey-Kramer tests, $P<0.05$, see text for F-values).

(5) Means within columns followed by the same letter are not significantly different (Kruskal-Wallis).

Table 4. Development time (days), adult dry weight (mg) and sex ratio of *Cotesia rubecula* developing in early first instar larvae of two *Pieris* hosts. Where performance differed significantly between sexes, means are given separately for males and females. Values in parentheses are standard error means.

	N(1)		Development time						Adult dry weight				Sex ratio(2)
Pieris species	Males	Females	Egg-Pupa		Pupa-Adult		Egg-Adult		Males		Females		(% Male)
			Range	X	Range	X	Males	Females	Range	X	Range	X	
P. brassicae	43	7	11-14	12.5 a(1)	8-10	8.7 a(1)	21.1 a	21.7 a	0.22-0.56	0.44 a (0.01)	0.48-0.62	0.55 a (0.02)	86.0 (4.8)
P. rapae	48	6	10-16	12.6 a	8-11	9.2 b	22.1 b	22.0 a	0.28-0.81	0.61 b (0.01)	0.60-0.91	0.77 b (0.01)	88.9 (9.5)
			F= P=	0.13 0.72		5.16 0.03	5.34 0.03	0.14 0.72		$T=9.05$ $P<0.001$		$T=4.15$ $P<0.002$	$Chi^2=$ P>

(1) Numbers of parasitized hosts that produced parasitoid cocoon masses containing males and females or males only.
(2) Only clutches with at least one female offspring were included in this analysis.
(3) Means within columns followed by the same letter are not significantly different (Kruskal-Wallis).
(4) Means within columns followed by different letters are significantly different (Two-way ANOVA followed by Tukey-Kramer tests, $P<0.05$, see text for F-values).
(5) Means within columns followed by the same letter are not significantly different (Kruskal-Wallis).

DISCUSSION

In this study we examined the relationship between performances of *Cotesia glomerata* and *C. rubecula* in different *Pieris* host species and host-specificity, host-searching behaviours and ovipositional decisions of both parasitoid species. We predicted that a smaller variation would be observed in parasitoid performance between different host species for *C. glomerata* than for *C. rubecula*, which is partially supported by the results.

Parasitization success, parasitoid survival, and developmental time

Differences in parasitization success between host species may help to explain host specificity and host ranges in insect parasitoids. The most striking difference between the generalist *C. glomerata* and the specialist *C. rubecula* is, that the generalist was capable of developing in all three host species offered, whereas the specialist failed to develop in *P. napi* larvae, confirming, to some extent, the parasitoids' specialist nature. The results of this study indicate a general lower suitability of *P. napi* as a host for the two *Cotesia* species compared to *P. brassicae* or *P. rapae*. Field data showed that this host species was hardly parasitized by *C. glomerata* and *C. rubecula* (Chapter 10).

Unsuitability may reside in the poor nutritional quality of the host for the parasitoid larvae, or in the parasitoid's ability to obtain certain nutrients from the host tissues at the proper time. In addition, other determinants host-suitability include hosts' defense capability, the presence of toxic factors or improper physiological state of the host (Vinson, 1988; Vinson & Iwantsch, 1980; Vinson & Barbosa, 1987). Which of these factors play a role in the unsuitability of *P. napi* for *C. rubecula* remains to be investigated.

Differences in host species quality did not affect parasitoid developmental time for *C. glomerata* and *C. rubecula*. Once parasitoid larvae had successfully completed their development within the host, survival from egression to adult emergence was equal for the three host species in *C. glomerata* and the two host species in *C. rubecula*. With respect to this parameters, *C. glomerata* and *C. rubecula* did not differ.

Host records and thus host selection lists are based largely on whether a particular insect serves as a host in nature. Numerous examples show that after induction of oviposition, parasitoids can be reared on unnatural hosts that are nutritionally suitable (Vinson & Iwantsch, 1980), which suggests that such potential hosts fall outside the host-selection process because of behavioural factors rather than host (un)suitability. This can be the case for the combinations *C. glomerata*-*P. napi* and *C. rubecula*-*P. brassicae*, that are rarely found in field situations in The Netherlands, although the hosts are suitable for parasitoid development. We forced *C. rubecula* to parasitize *P. brassicae* and *P. napi* larvae by placing them on *P. rapae*-infested leaf disks, so by contaminating the vicinity of hosts with kairomones of their preferred host (Vinson, 1976).

Sex ratio

Sex ratios were male biased in all *Cotesia-Pieris* associations tested, but did not differ between species. The observed adult sex ratio may differ from the primary sex ratio (i.e. at oviposition) if one sex (in most cases females) suffers greater mortality while immature (Godfray, 1994). Karowe & Schoonhoven (1992) experienced male biased sex ratios in their laboratory experiments as well. They stated that this was not due to differential survival of male larvae in their case, since overall larval survivorship within caterpillars that survived until parasitoid egression exceeded 95%. In our study, this percentage exceeded 91.9% (Table 1). Host larvae that were parasitized by *C. glomerata* females from field populations yielded parasitoid offspring with female biased sex ratios (Chapter 10).

Adult dry weight and fitness.

Size of emerging parasitoids is an important parameter in determining suitability of hosts for parasitoid development. Insight into the relationship between adult size and fitness is crucial in understanding the evolution of oviposition behaviour. Adult size of the parasitoid is correlated with host size or age, and host species, but small hosts at oviposition do not necessarily lead to small adult parasitoids. The parasitoid's developmental strategy and host regulation can differ between different host species (Harvey et al., 1994; Harvey & Thompson, 1995). Gregarious species frequently increase feeding by their host larvae, whereas solitary species often reduce feeding and growth of their hosts (Slansky, 1986).

It has been shown, that female fitness increases with adult size (King, 1987), since larger individuals have a higher potential fecundity (Itawa, 1996; Rosenheim & Rosen, 1991; Vet et al., 1994). Furthermore, longevity of larger individuals is greater, and searching may be more efficient in large females (Hurlbutt, 1987; Harvey et al., 1994; Visser, 1995), factors that can affect lifetime reproductive success.

Male fitness will be determined by the number of matings achieved, where male adult size will influence longevity, ability to locate females and intersexual competition for mates (Godfray, 1994). Some evidence exists for braconid wasps that larger males have greater mating success (Charnov et al., 1981).

Adult weight of *C. glomerata* progeny (male and female) developing in *P. napi* was smaller than that of offspring developing in *P. brassicae* or *P. rapae*. *Cotesia rubecula* offspring developing in the two remaining potential hosts showed significantly higher dry weights (both male and female) in *P. rapae* than in *P. brassicae*. This may indicate physiological incompatability of *C. rubecula* developing in *P. brassicae* or a more efficient host-regulation of *P. rapae* larvae by *C. rubecula* larvae (J. Harvey, pers. comm.).

These results indicate that there are some fitness penalties for parasitization of *P. napi* by *C. glomerata* and of *P. brassicae* by *C. rubecula*, and hence that these hosts can be considered as less suitable. Discrimination against these less suitable hosts during searching for hosts was observed in field parasitizations (Chapter 10) and in host-acceptance (Chapter 5).

Clutch size.

The number of eggs deposited in a host by female parasitoids affects her fitness, and thus differences in clutch sizes between host species play a role in determining host suitability. Optimality theory predicts clutch sizes are correlated with host quality (Charnov & Skinner, 1984; Iwasa et al., 1984). In idiobiont species, smaller hosts indeed receive smaller clutches (Godfray, 1994). Although it was also found for koinobionts (Vet et al, 1993; 1994), studies on gregarious *Cotesia* species show that future host quality can also be dependent on clutch size, with heavily parasitized hosts having higher consumption and/or food utilization efficiencies (Slansky, 1978; Sato et al., 1986 and references therein).

The number of *C. glomerata* larvae produced per clutch was smaller in *P. rapae* than in *P. brassicae* or *P. napi*. The difference in clutch size between *P. rapae* and *P. napi* contrasts with host acceptance by *C. glomerata*, since *P. rapae* larvae were preferred for oviposition over *P. napi* (Chapter 5). The smaller clutch size in *P. rapae* larvae cannot be explained by sexual asymmetry (e.g. all female brood, with smaller clutches as a result of larger female larvae), since sex ratio of *C. glomerata* broods in *P. rapae* were male biased, like in broods from *P. brassicae* and *P. napi*.

An alternative explanations is, that encapsulation levels in *P. rapae* are higher than in *P. napi* (Brodeur & Vet, 1995), resulting in higher partial encapsulation of the brood in *P. rapae*.

Host-suitability versus host-searching behaviour

To examine host specificity of the two parasitoid species through a comparative approach, host-searching and host-selection behaviour was extensively studied in laboratory, wind tunnel and semi-field experiments (Wiskerke & Vet, 1994; chapters 3, 5, 6, 7, and 8) as well as in the field (Chapter 10). These studies revealed that *C. glomerata* learns to prefer *P. brassicae* over *P. rapae*, and that *C. rubecula* prefers *P. rapae*, but only after actual contact with hosts, leaf-damage and host-by products. The host-acceptance (Chapter 5) and host-suitability experiments demonstrated that the generalist *C. glomerata* readily accepts for oviposition and develops in all three host species, and that overall performance is best in *P. brassicae* larvae, although differences are not very large. The specialist *C. rubecula* strategies between parasitoid species and may provide insight in the coexistence of species that co-occur with overlapping niches. For the latter a comparative approach is essential.

We have employed the comparative method in extensive laboratory studies on the host-searching behaviour of two parasitoids of *Pieris* caterpillars: the generalist *Cotesia glomerata* and the specialist *C. rubecula* (Hymenoptera: Braconidae). We tested the hypothesis, that host-searching strategies of parasitoids depend on their degree of specialization on the host and the food plant level. Specialists are expected to show a more fixed host-searching behaviour, whereas generalists are likely to show flexible responses with varying circumstances in space and time (see Vet & Dicke, 1992). For the two *Cotesia* species mentioned, behavioural studies have been carried out in the laboratory to establish differences in host-searching strategies of both species (Wiskerke & Vet, 1994; chapter 2, 3, 6 and 8) with emphasis on chemically mediated orientation. These studies revealed that between the parasitoid species little difference exists in their responses to various types of infochemicals from plant-host complexes (Steinberg et al., 1993; chapter 2). The most apparent difference between *C. glomerata* and *C. rubecula* was found to be the involvement of learning during host-habitat location. In the generalist *C. glomerata* preference learning was important, while it was absent in the specialist *C. rubecula*. Experience with host feeding-damage or oviposition experience led to preference shifts in *C. glomerata* and learning modified the parasitoid's response to variation in resource availability. In *C. glomerata*, experience with host species led to a preference for *P. brassicae*. In contrast, in *C. rubecula* similar experiences merely led to increased responsiveness (chapter 6 and 8). Further laboratory studies were carried out to determine the relation between host-habitat location strategies and actual host-acceptance or host-suitability (chapter 5 and 9). The generalist nature of *C. glomerata* was reflected in host-acceptance and host suitability of the three host species, *P. brassicae*, *P. rapae* and *P. napi*, whereas *C. rubecula* showed the highest acceptance for various instars of *P. rapae* larvae and performed best in larvae of this host species.

The question remains, how the two *Cotesia* species perform under natural circumstances. Their hosts occur simultaneously in similar habitats (Terofal, 1965) and parasitoid sampling carried out between 1990 and 1993 revealed that both species always occurred together in the same habitats (Geervliet, Brodeur unpublished results). This implies that they share host resources.

Field studies on both parasitoid species ánd with more than one *Pieris* host present are scarce. Recently, Peters (1992) studied the occurrence of parasitoids of cabbage pests in Germany. He recovered *C. glomerata* from *P. brassicae* and *P. rapae*, whereas *C. rubecula* was found in *P. rapae* and in

Plutella xylostella. In most other study areas, only one parasitoid species is present (Australia, Japan, parts of the USA, Canada) and/or only one host is present (Australia, USA, Canada). Biever (1992) studied the distribution of *C. rubecula* in relation to that of *C. glomerata* in Washington State, USA, but the only host present there is *P. rapae*. In Japan oviposition patterns of *C. glomerata* were studied in three *Pieris* sp. hosts, *P. rapae crucivora*, *P. napi japonica* and *P. melete*, (Sato & Ohsaki, 1987; Ohsaki & Sato, 1990; 1994).

The aim of the present field study was to investigate whether parsitization of three *Pieris* species varies with parasitoid species and with food plant of the caterpillars. Since *C. glomerata* is a generalist, it is expected to be recovered from several host species, whereas the specialist *C. rubecula* is supposed to be found in *P. rapae* only. Furthermore, we expected to find differences in host-suitability on different food plants, like was found for *C. glomerata* in a laboratory study (Karowe and Schoonhoven, 1992) and we tested whether such differences were reflected in host-finding decisions by the parasitoids.

MATERIALS AND METHODS

Study plots. In August and September 1993 the experiments were conducted in Brussels sprouts plots in an experimental area of 'De Eng', Wageningen, The Netherlands, where no chemical crop protection took place. The site consisted of experimental fields with cabbage and corn crops. Adjacent to the north some allotment gardens were located, with mixed cropping (vegetables, e.g. cabbage, beans, peas, leek, tomatoes, sweet pepper, potatoes, onions and ornamental flowers, e.g. chrysanthemum, dahlia, nasturtium). The cabbage field for our experiment was located inside a corn field, and consisted of 12 plots of 10*10 m planted with Brussels sprouts (*Brassica oleracea* L. var *gemmifera* (DC.) Schulz. cv. 'Ottoline') with an interplant distance of 0.5 m. A path of 2 m wide separated the plots, and a strip of 3 m wide planted with clover surrounded the plot area (see fig 1). Meteorological data for August and September 1993, obtained from Meteoconsult Wageningen, and the period the test plants were present in the field are shown in figure 2.

Parasitoids. Preliminary studies in this area from 1990-1993 showed the presence of both *Cotesia glomerata* and *C. rubecula* populations. We were not able to determine parasitoid densities, and assume that they were homogeneously distributed over the experimental field.

Plants and Hosts. Red cabbage (*Brassica oleracea* L. var *rubra* (DC.) cv. 'Langedijker vroege rode'), Brussels sprouts (*Brassica oleracea* L. var *gemmifera* (DC.) Schulz. cv. 'Titurel') and nasturtium (*Tropaeolum majus* cv. Emperor of India) were reared in greenhouse compartments at 20-25°C, 50-70% RH and a L16:D8 photoregime. One week prior to the experiment, one plant of each species was placed during 4-6 h in a cage with butterflies of either *Pieris brassicae*, *P. rapae* or *P. napi* for oviposition. Plants with eggs were kept in climatic rooms at 20±2°C, 50-70% RH and a L16:D8 photoregime until hatching of the eggs. One day after hatching, first instar larvae were gently transferred to plants of the same type that were to be placed in the field. Plants with larvae were kept in the climatic room until the next day, to allow larvae to establish and cause initial feeding damage to the leaves. To mimic natural distribution patterns, *P. brassicae* larvae were transferred to the plants in 3 clusters, 1 cluster per leaf (150-200 L1 per plant), whereas *P. rapae* and *P. napi* were placed solitary (8-10 L1 per leaf). The numbers of caterpillars were chosen relatively high (± 150 L1 per plant) since preliminary experiments showed that host recovery was low due to considerable larval mortality (predation, desiccation, rainfall).

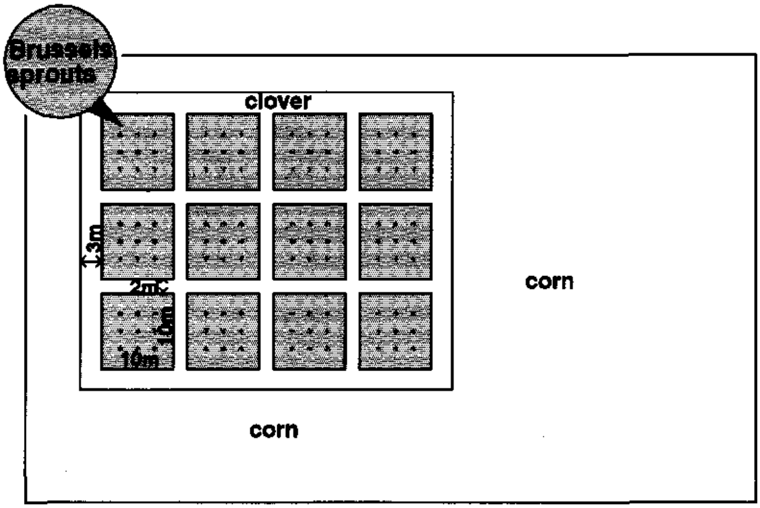

Fig. 1. Schematic drawing of the study plots. Asterisks(*) mark the position of the test plants in the field.

Method and Analysis. The experiments were conducted in August and September 1993. On day 1 the previously prepared host-infested plants were transported from the laboratory to the field. Per plot, nine plants (three plant types, three host species) were distributed in between the Brussels sprouts plants in a 3*3 configuration (see asterisk-marks in Fig 1), with a different distribution in each plot. Care was taken that plants were ± 1-1.5 m inside the border of the plot. Due to shortage of larvae, in August 1993 only 8 plots out of 12 could be supplied with the nine types of plant-host complexes. Because the two outer rows were assumed to be the most similar with respect to e.g. border effects, the four plots in the middle row were left empty. Due to a shortage of *P. napi* in September 1993, the combination nasturtium-*P. napi* and red cabbage-*P. napi* occurred only in six out of 12 plots.

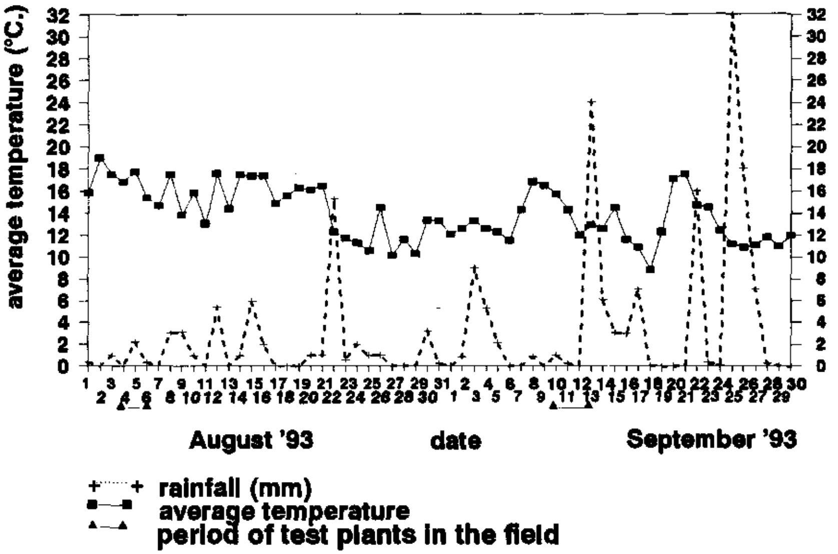

Fig. 2. Meteorologic data for the period August-September 1993. Daily rainfall (mm) is represented by the solid line with ■ marks, average daily temperature (°C.) is represented by dashed line with + marks. The periods test plants were present in the field are indicated by ▲-▲. Data provided by MeteoConsult Wageningen, The Netherlands.

To prevent plants from falling over they were attached to the soil with a plant stick through the plant pot. Plants were left in the field for two days, and when necessary they were supplied with water. On day 4 they were recollected from the field and transported to the laboratory. From each plant the number of recollected hosts was determined. Hosts were transferred to petridishes (9 cm i.d.) with wet cotton wool and leaf disks of the food plant they were collected from, and reared for two more days to enhance detectability of parasitoid eggs or larvae. Degree of parasitization was measured by dissecting per plant one half of the recovered hosts two days after collection from the field. For this analysis, larvae were transferred to clean petridishes and kept at -4°C until dissection, to stop development. An attempt was made to check for multiparasitism in the dissected larvae. But first of all, the time elapse between introduction in the field and recovery from the field was two whole days, in which effects of competition between larvae of two parasitoid species already will have crystallized (check name Trenscek, pers. comm.). Besides, the point in time of parasitization occurred was unknown. Secondly, it appeared to be hard to detect eventual *C. rubecula* larvae among *C. glomerata* larvae. Therefore we decided not to continue investigating multiparasitism and competition for the present study. Dissections earlier after parasitization and DNA-analyzing techniques are required to make a reliable examination of multiparasitism.

Parasitization was determined from the other half of the hosts, by rearing the caterpillars in petridishes as described above until host decease or parasitoid emergence. Every second day, hosts were supplied with fresh food and checked for mortality or parasitoid egression. Dead larvae were dissected to check for parasitization.

Data from dissected larvae and reared larvae were pooled to get overall parasitization results.

For parasitized larvae from the group that was reared, the following host suitability parameters were measured: clutch size, parasitoid mortality (i.e. parasitoid larvae that fail to egress, parasitoid larvae that fail to spin coocoons and parasitoid pupal mortality), parasitoid sex ratio (as proportion females of total offspring), and adult dry weight of female and male wasps. Data were analyzed using Mann-Whitney U tests (Siegel, 1988).

Direct observations Direct observations of behaviour of parasitoids from field populations were carried out during 10 days in a period of 6 weeks (July-August 1994). The Brussels sprouts plants in the field (*Brassica oleracea* L. var *gemmifera* (DC.) Schulz. cv. 'Titurel') were used as trap plants. One day prior to observations, laboratory reared *P. brassicae* and *P. rapae* first instar larvae were transferred from cabbage leaves to the plants in the field, to let larvae establish and to get feeding damage. Late first instar *P. brassicae* larvae were placed in clusters of 10-15 larvae on leaves, whereas ca 10 late first instar *P. rapae* larvae were placed solitary on the leaves to get similar quantities of feeding damage as in *P. brassicae*-infested leaves. Per plant two leaves were infested, one with *Pieris brassicae* and one with *P. rapae*. Two leaves close to eachother in the top of the plant were chosen, to facilitate observation of parasitoid behaviour. Overnight, the larvae were protected against parasitoids, predators and other disturbing factors by placing clip cages over the patches. The next day direct observations were made of the behaviour of parasitoids that were attracted to the infested leaves. Weather conditions were ideal for parasitiod host foraging: sunny, low wind speed, high temperatures (26-30° C.). We were seated at the border of the cabbage plot, close to the infested leaves. Parasitoids that approached the plant were easily identified as either *C. glomerata*, *C. rubecula* or none of these two. We observed the wasp by eye and recorded the behaviour, landings and ovipositions. Since we were not able to distinguish females that revisited the leaves from new visitors, we tried to catch away the females whenever possible after taking off.

RESULTS

Parasitization

In August 1993 the percentages parasitism were relatively high compared to those in September 1993, especially for the Brussels sprouts-*P. brassicae* complex and the Brussels sprouts-*P. rapae* complex. For both points in time a similar trend in host use and plant choice was found (Table 1.). *Cotesia glomerata* mainly parasitized *P. brassicae* larvae, whereas *C. rubecula* was almost exclusively found in *P. rapae* larvae. In both cases parasitized *P. napi* larvae were hardly observed. The highest degree of parasitization occurred on Brussels sprouts and on red cabbage, and hardly any parasitized hosts were found on nasturtium; in most plots the percentage parasitism on nasturtium was zero.

The number of *Pieris* larvae recovered was always highest on Brussels sprouts plants, and of the hosts the highest percentage recovered was of *P. brassicae* (Table 1; Approximately 1000 larvae per host species were put out in the field and the number recovered is represented by N). Losses in *P. rapae* or *P. napi* were relatively large, but from Brussels sprouts more larvae of these two species were recovered than from red cabbage and nasturtium.

Host and plant suitability. Suitability of hosts and plants cannot be compared for different plant-host combinations for *C. rubecula*, that only survived in *P. rapae* larvae (Table 3).

Because of low parasitization numbers by *C. glomerata* on *P. napi* and on nasturtium, host suitability parameters were only valuable for *C. glomerata* offspring from *P. brassicae* larvae on Brussels sprouts and red cabbage (Table 2). In August 1993, clutch size of *C. glomerata* was larger in *P. brassicae* larvae feeding on Brussels sprouts than in *P. brassicae* feeding on red cabbage ($P<0.001$), but sex ratio was more female biased on red cabbage than on Brussels sprouts ($P=0.0063$). Percentage mortality of the parasitoid larvae and male and female adult weight did not differ between parasitoids developing in *P. brassicae* feeding on Brussels sprouts or on red cabbage.

In September 1993 clutch size in *P. brassicae* on Brussels sprouts was also larger than clutch size in *P. brassicae* on red cabbage (P<0.001). Adult female weight was higher in *C. glomerata* offspring from *P. brassicae* on Brussels sprouts than on red cabbage (P=0.047). Mortality, sex ratio and adult male dryweight did not differ between *P. brassicae* from red cabbage and Brussels sprouts.

Table 1. Parasitization results based on dissected and reared larvae together. N = total number of larvae (dissected and reared)

		August 1993			September 1993		
		N	% of N paras. by *C. glomerata*[1]	% of N paras. by *C. rubecula*[1]	N	% of N paras. by *C. glomerata*[1]	% of N paras. by *C. rubecula*[1]
Brussels sprouts	*P. brassicae*	404	85.8±4.1	0.7±0.5	835	22.1±6.2	2.0±0.9
	P. rapae	94	26.3±7.1	51.9±8.3	274	0.0	7.2±3.7
	P. napi	115	12.2±3.8	4.0±1.7	91	1.0±1.0	0.0
red cabbage	*P. brassicae*	403	26.2±8.6	0.0	796	18.0±5.5	0.5±0.2
	P. rapae	35	0.0	7.5±7.5	227	0.5±0.5	1.3±0.9
	P. napi	18	4.2±4.2	0.0	112	0.0	1.0±1.0
nasturtium	*P. brassicae*	208	4.6±4.6	0.0	799	0.9±0.9	0.3±0.2
	P. rapae	12	0.0	25.0±16.0	207	0.0	0.0
	P. napi	22	8.3±8.3	0.0	94	0.0	0.0

[1] average ± S.E. per plot (n=8 in August; n=12 in September)

Table 2. Host-suitability data for *C. glomerata* offspring. N indicates the number of larvae.

C. glomerata August 1993									
		N reared	N parasitized	N prod. offspring	av. clutch size^{1,2}	av. % mortality^{1,2}	av. sex ratio^{1,2}	av. ♀ drywt (mg)^{2,3}	av. ♂ drywt (mg)^{2,4}
Brussels	*P. brassicae*	208	178	105	39 ± 2.1^{a}	32 ± 2^{a}	0.53 ± 0.02^{a}	0.27 ± 0.01^{a}	0.23 ± 0.01^{a}
sprouts	*P. rapae*	57	8	1	16	37	-	-	-
	P. napi	74	3	0					
red cabbage	*P. brassicae*	215	51	46	25 ± 1.9^{b}	28 ± 3^{a}	0.63 ± 0.03^{b}	0.28 ± 0.01^{a}	0.25 ± 0.01^{a}
	P. rapae	20	0						
	P. napi	12	0	1	21	19	0.76	0.14	0.17
nasturtium	*P. brassicae*	110	3	1	22	59	0.44	0.29	0.30
	P. rapae	7	1	0					
	P. napi	12	1	1	22	23	1.00	0.18	-

Table 2. (continued)

C. glomerata September 1993									
		N reared	N parasitized	N prod. offspring	av. clutch size^{1,2}	av. % mortality^{1,2}	av. sex ratio^{1,2}	av. ♀ drywt (mg)^{1,3}	av. ♂ drywt (mg)^{1,4}
Brussels	*P. brassicae*	432	92	78	30 ± 1.4^{a}	24 ± 3^{a}	0.77 ± 0.04^{a}	0.32 ± 0.01^{a}	0.26 ± 0.01^{a}
sprouts	*P. rapae*	145	0						
	P. napi	49	1	1	32	25	0.88	0.17	0.15
red cabbage	*P. brassicae*	436	88	64	23 ± 1.2^{b}	28 ± 3^{a}	0.81 ± 0.02^{a}	0.29 ± 0.01^{b}	0.26 ± 0.02^{a}
	P. rapae	120	1	0					
	P. napi	60	0						
nasturtium	*P. brassicae*	427	3	1	22	54	0.85	0.18	0.15
	P. rapae	119	0						
	P. napi	50	0						

[1] average ± SE per clutch.
[2] values with same letters within colums are not significantly different (Mann-Whitney U; $P < 0.05$)
[3] average ± SE of female offspring
[4] average ± SE of male offspring

Comparison of data from August and September reveals, that on Brussels sprouts clutch size and percentage mortality were lower in September than in August ($P<0.001$ for both parameters), but that sex ratio was significantly more female biased in September than in August ($P<0.001$). Female offspring was also heavier in September than in August ($P=0.0004$). On red cabbage clutch size and mortality did not differ between August and September, but again sex ratio was significantly more female biased in September than in August ($P<0.001$).

Direct observations

During the 10 observation days in the six week period in 1994, in total 38 *C. glomerata* individuals were observed and 6 *C. rubecula* females (Table 4). These numbers may give an indication of the relative parasitoid presence in the field. Females of *C. glomerata* landed as often on leaves infested by *P. rapae* as on leaves infested by *P. brassicae*, and 12 out of 15 females parasitized one or more *P. brassicae* larvae. The other 3 *C. glomerata* females left and did not land elsewhere in the observed area. Eight out of 23 females that made their first landing on *P. rapae* parasitized one or more individuals of this host species. Seven of the other females left the *P. rapae*-infested leaf in search for other leaves and 8 females left without parasitization and without a second landing. In all except one the second landing always occurred on a host-infested leaf different from the one of the first landing. One female landed for a second time on a *P. rapae*-infested leaf. Finally, more *C. glomerata* females parasitized *P. brassicae* than *P. rapae* larvae ($P=0.02$).

Few *C. rubecula* females were observed, but those that landed on a *P. rapae*-infested leaf, parasitized one or more larvae. After parasitization they would leave, and no second landing was observed for *C. rubecula* females. One female made a first landing on a *P. brassicae*-infested leaf, but did not parasitize any larva. Instead, she left, but did not land elsewhere in the observed area.

DISCUSSION

Parasitization and host suitability.

In this field experiment the expected dietary preferences were only partly confirmed. As expected, *C. rubecula* was recovered from *P. rapae* only, but in contrast to it's generalistic nature, viable *C. glomerata* offspring was also recovered from one host species (*P. brassicae*) only (Table 2). Parasitization of *P. rapae* and *P. napi* larvae in August and September (Table 1) did not yield *C. glomerata* offspring (Table 2). These parasitization results indicate at least a partial segregation of host resources by *C. glomerata* and

Table 3. Host-suitability data for *C. rubecula* offspring. N indicates the number of larvae.

C. rubecula August 1993								
		N reared	N parasitized	N prod. offspring	av. % mortality	sex ratio	av. ♀ drywt (mg)^1	av. ♂ drywt (mg)^2
Brussels sprouts	*P. brassicae*	208	3	0				
	P. rapae	57	26	14	14	0.75	0.32 ± 0.02	0.36 ± 0.02
	P. napi	74	2	0				
red cabbage	*P. brassicae*	215	0					
	P. rapae	20	2	2	50	1.00	0.24	-
	P. napi	12	0					
nasturtium	*P. brassicae*	110	0					
	P. rapae	7	2	1	100			
	P. napi	12	0	1	23	1.00	0.18	-

Table 3. (continued)

C. rubecula September 1993		N reared	N parasitized	N prod. offspring	% mortality	sex ratio	av. ♀ drywt (mg)[1]	av. ♂ drywt (mg)
Brussels	*P. brassicae*	432	1	0				
sprouts	*P. rapae*	145	11	6	40	1.00	0.50±0.04	-
	P. napi	49	0					
red cabbage	*P. brassicae*	436	0					
	P. rapae	120	2	2	0	0.50	0.39	0.37
	P. napi	60	1	0				
nasturtium	*P. brassicae*	427	3	0				
	P. rapae	119	0					
	P. napi	50	0					

[1] average ± S.E. of female offspring
[2] average ± S.E. of male offspring

C. rubecula. This may have evolved as a result of competition. In The Netherlands, competition is likely, since both species occur simultaneously with overlapping niches. Laing & Corrigan (1987) studied intrinsic competition between *C. glomerata* and *C. rubecula* in *P. rapae* and found that *C. rubecula* was superior to *C. glomerata* in this host species. Although reported as a generalist parasitoid, *C. glomerata* may be forced by *C. rubecula* to restrict its host range to *P. brassicae* larvae. Wind tunnel studies show that *C. glomerata* avoids patches of *P. rapae* larvae that are being visited by *C. rubecula* females and on which they are actively parasitizing hosts. In a wind tunnel set up (see chapter 2) naive *C. glomerata* females (N=46) were offered a choice between *P. rapae*-infested Brussels sprouts leaves on which *C. rubecula* females were present and on which parasitoids were absent. Of the 43 responding females, 34 chose the *P. rapae*-infested leaves without *C. rubecula* females. Whether olfactory, visual or mechanical cues (e.g. wing vibrations) play a role remains to be studied. This kind of competition avoidance was also found for *Leptopilina* species (Janssen et al., 1995a,b) and for predatory mites (J. Bruin, pers. comm). The specialist *C. rubecula* hardly parasitized *P. brassicae* larvae in our field study. As a result, *P. brassicae*, which is a suitable host for *C. glomerata*, is available for the latter parasitoid species. In the USA, *C. glomerata* was introduced from England in 1863 to control the imported *P. rapae* and established throughout most of North America. Through behavioural plasticity this generalist parasitoid is likely to adapt to varying host densities and distributions, like e.g. the solitary distribution of *P. rapae* (Chapter 8; Vos et al., 1996). However, Biever (1992) reported high levels of parasitism by *C. rubecula* in northern regions of Washington state and the absence of *C. glomerata* in this area. The latter species mainly occurred in the southern regions, where no *C. rubecula* was found. These data support the idea that *C. rubecula* replaces *C. glomerata* after establishment (Wilkinson, 1966). In the absence of alternative hosts such as *P. brassicae* and due to the fact that *C. glomerata* is an inferior competitor in *P. rapae* larvae, this is likely to happen. Wind tunnel studies on the two *Cotesia* species showed, that *C. glomerata* learns to concentrate on *P. brassicae*-infested Brussels sprouts plants (chapter 6). During host-habitat location *C. rubecula* does not distinguish between host species feeding on food plants (chapter 3), but host-acceptance studies (chapter 5), our direct observations in the field (this paper), a semi-field study (Wiskerke & Vet, 1994) and free-ranging wind tunnel experiments (chapter 7) showed that *C. rubecula* preferred to parasitize *P. rapae* larvae over *P. brassicae* larvae. Apparently, after landing kairomones play a role in distinguishing between host species (Mattiacci & Dicke, 1995a,b; Agelopoulos et al., 1995; chapter 7).

Low percentages parasitism on *P. napi* are in agreement with host-acceptance and host-suitability data of this herbivore species for the two

Table 4. Behavioural observations of *C. glomerata* and *C. rubecula* females on Brussels sprouts leaves in the field infested by *P. brassicae* or *P. rapae* first instar larvae.

	# observed	first landing[1]		second landing			# females ovipositing[2]			average # larvae parasitized per parasitoid female[3]	
		P. brassicae	*P. rapae*	*P. brassicae*	*P. rapae*	no second landing	*P. brassicae*	*P. rapae*	no ovipos.	*P. brassicae*	*P. rapae*
C. glomerata	38	15 a	23 a	8	4	26	19 a	8 b	11	3.4±0.5	1.2±0.2
C. rubecula	6	1	5	0	0	6	0	5	1	0	1.2±0.2

[1,2] values for *C. glomerata* with the same letter within row are not significantly different (Binomial test for probability; $P<0.05$). Not tested for *C. rubecula*.

[3] average ± S.E.

parasitoid species. Although it was a suitable host for *C. glomerata* (chapter 9), it was the least accepted host species compared to *P. brassicae* and *P. rapae* (chapter 5). *Cotesia rubecula* females were more specialized in their choice of hosts, and *P. napi* was hardly accepted (chapter 5) and those larvae that were parasitized did not give rise to parasitoid offspring (chapter 9).

The highest percentages parasitism were achieved on Brussels sprouts in *P. brassicae* and in *P. rapae* in August 1993. This preference of field populations of the two *Cotesia* species for herbivore-infested Brussels sprouts plants over the other food plants offered in the field may have been induced through earlier experience with hosts on this cabbage cultivar, as it was the standing crop. Wind tunnel experiments of chapter 6 showed that in *C. glomerata* females experience with *P. brassicae*-infested Brussels sprouts led to a preference for this plant-host complexes in subsequent flights.

Hardly any parasitization was observed on nasturtium, which indicates that this food plant may serve as an 'enemy free space' to *Pieris* spp. Wind tunnel experiments showed that this food plant hardly elicited attraction of naive females of either *C. glomerata* or *C. rubecula* (chapter 3), and although *C. glomerata* easily learned to switch to nasturtium after an oviposition reward on this food plant, responses waned with lack of reinforcement (chapter 8). Furthermore, wasps with prior experience on Brussels sprouts and an additional experience with nasturtium showed low responsiveness to nasturtium (chapter 8). Apparently, parasitoids hardly detect and/or visit nasturtium when placed in a cabbage field, where odour masking also may play a role too (Ohsaki & Sato, 1990;1994) and as a consequence, they are not able to get experience with this plant-host complex. The evidence for the exploitation of enemy-free space may be provided by fact that herbivore preference may be different from those of their natural enemies (Damman, 1987; Nordlund et al., 1988; Fox & Eisenbach, 1992). A study by Karowe & Schoonhoven (1992) showed that for unparasitized *P. brassicae* growth rate and pupal weight were lowest on nasturtium. In contrast, parasitoid larval developmental rate and adult longevity of *C. glomerata* were largest for wasps that were reared on *P. brassicae* feeding on nasturtium. Although less suitable for development, *P. brassicae* may select this food plant, to escape parasitism, since parasitoids are hardly attracted to nasturtium. Sato & Ohsaki (1987) and Ohsaki & Sato (1990, 1994) provided other evidence that *Pieris* larvae (*P. napi*) escape parasitism by feeding on cruciferous plants (*Arabis* sp.) that are less suitable as food plants for their development, and that are less frequented by their parasitoids as a result of being hidden or shaded by other food plants. Apparently, plant suitability for parasitoid development is not alway reflected in the host-finding decisions by parasitoids.

Recovery of larvae from plants differed between plant species and *Pieris* species. More larvae were recovered from Brussels sprouts than from red cabbage or nasturtium. From the latter two plant species larvae may have disappeared in search for other (better) food plants. Furthermore, of *P. brassicae* a higher percentage was recovered than of *P. rapae* and *P. napi*. Larvae of the latter two species are solitary feeders with a different feeding strategy than *P. brassicae*. Grouping by animals is commonly cited as a behavioural mechanism that offers protection from predation or disturbance (Mooring & Hart, 1992). Clusters of *P. brassicae* are embedded in a silk mat, which may protect them from falling of the leaf after mechanical disturbance. Also, *Pieris brassicae* is known for its strong defensive reaction when disturbed (Feltwell, 1982; chapter 5), which may protect them against invaders. However, the high parasitism percentages of *P. brassicae* by *C. glomerata* show that this protection is not very effective against this parasitoid species.

Although *C. glomerata* clutch size was higher on Brussels sprouts than on red cabbage, both in August and September 1993, red cabbage was a suitable food plant for parasitoid development (table 2). Clutch size depends on the female's decisions during host acceptance (Godfray, 1994) and as in host-habitat location, females may have differential ovipositional responses on different food plants. Again, previous experience with hosts on Brussels sprouts may have played a role in clutch size decisions.

The most striking difference in host-suitability parameters between August and September is the higher proportion of females in September from *P. brassicae* on the two cabbage varieties.

Why would the percentage of females increase at the end of the season? Probably with decreasing daylength parasitoids start entering diapause, in the pupal stage of the hosts, and no new host cycle is to be expected. At the start of the next season, parasitoid densities are initially very low and so more sibmating is expected and stronger Local Mate Competition conditions will exist. Hence, females are expected to produce more daughters with few sons sufficient to mate their sisters. After emerging in spring, females can mate and immediately start their search for hosts. During the rest of the season, parasitoid densities are high, likely with weaker Local Mate Competition conditions, so higher percentages of males are expected.

Furthermore, females from *P. brassicae* on Brussels sprouts are heavier in September than in August. Probably the lower clutch sizes in hosts of the same size as in August account for heavier offspring, since more food resources are available for the parasitoid larvae.

Direct observations

The direct observations reveal that both *C. glomerata* and *C. rubecula* are able to distinguish between host species. Not so much in the host-habitat location process, although the number of observations was low, but in the host acceptance phase, where both parasitoid species have clear preferences for either *P. brassicae* or *P. rapae* larvae. These results are in agreement with earlier wind tunnel studies (Wiskerke & Vet, 1994; chapter 7 and 8) and with the results of the field parasitizations as described above.

In conclusion, these results give complementary insight in the host foraging by two closely related parasitoid species. The *Cotesia* species appear to coexist through niche segregation and share host resources, since *C. glomerata* is mainly recovered from *P. brassicae* and *C. rubecula* from *P. rapae*. Although we expected to find *C. glomerata* in higher numbers in other host species, based on the literature host records and host-suitability data, in The Netherlands specialization on *P. brassicae* may have occurred as a result of *C. rubecula* outcompeting *C. glomerata* in *P. rapae* larvae.

11

SUMMARIZING DISCUSSION.

Parasitoids have evolved and operate in a multitrophic system in which they occupy the third trophic level, their hosts the second and the food substrate of the hosts the first trophic level. Parasitoid females often use chemical stimuli as information from their environment while searching for hosts. These infochemicals can be directly or indirectly associated to their hosts or they can be derived from the food of the host. The efficiency of the host-searching strategy of parasitoids is based on genotypic and phenotypic factors. The degree of specialization sets the degree of specificity of information needed during searching for hosts. Specific information on the identity of hosts present in a certain habitat is supposed to be of less importance to parasitoids that attack a wide variety of host, whereas specialist will need more specific information. Parasitoids can modify their searching behaviour through learning, in response to different environmental conditions, thereby enhancing their searching efficiency. It is hypothesized that the extent to which they can or do adjust their host-searching strategy is related to their degree of dietary specialization: learning is expected to play a greater role in the searching behaviour of generalist species than that of specialist species.

A comparative approach of species is an important tool to determine correlations between behavioural traits and ecological factors indicative of adaptation. Comparison of phylogenetically related species that differ in some ecological characteristics has been applied successfully by ecologists to interpret functional differences in host-searching behaviour of parasitoids. A similar approach was used in the present study to test the hypothesis that infochemical use by parasitoids is related to their degree of dietary specialization.

The actors that have been used in this thesis consist of two congeneric and sympatric parasitoid species that differ in their diet breadth, *Cotesia glomerata* and *C. rubecula*. The gregarious *C. glomerata* is a generalist larval endoparasitoid, whose potential hosts belong to a few genera of *Pieris* species. The solitary *C. rubecula* is known primarily as a specialist on *P. rapae*. The parasitoids occur in the same type of habitat, where they have partly overlapping niches. Their long-distance host-searching behaviour is characterized by responses to olfactory and visual stimuli from damaged plants.

The three major objectives of this study were to determine 1) the specificity of the infochemicals used by *C. glomerata* and *C. rubecula*, 2) the influence of learning on the searching behaviour of these two parasitoids and 3) the fitness consequences of their host-searching decisions through a correlation of host-searching and host suitability.

Specificity of stimuli

The specificity of the stimuli used by the *Cotesia* species was investigated in behavioural studies, at long distance from the odour sources (chapter 2 and 3) and at short distances from the host after landing on the leaf (chapter 5 and 7), and in chemical analyses of volatile blends of caterpillar-infested and uninfested food plants (chapter 4).

In **Chapter 2** I describe the role of volatile stimuli in the long-range host-searching behaviour of the specialist parasitoid *Cotesia rubecula*. Components from the Brussels sprouts/*P. rapae* plant-host-complex were compared for their attractiveness in dual choice tests in a windtunnel. Stimuli from damaged cabbage plants (either mechanical damage or *P. rapae*-damage) were more attractive to *C. rubecula* than host-related stimuli. Parasitoids preferred leaves from the plant-host-complex over artificially damaged leaves. Undamaged cabbage plants were the least attractive to the foraging females. These results indicate that in-flight searching behaviour of *C. rubecula* is guided by plant-derived information in a similar way as was found for *C. glomerata* (Steinberg et al., 1993). In both species specific host-derived cues play a minor role at longer distances.

In **chapter 3** the host-searching behaviour of the generalist *C. glomerata* and the specialist *C. rubecula* are compared with respect to their innate preferences for plant-host complexes. The wasps are still 'naive', without foraging experience as adults with plants or hosts. The specialist *C. rubecula* was hypothesized to show more pronounced preferences for different plant-herbivore complexes than the generalist *C. glomerata*. Both parasitoid species preferred herbivore-damaged plants over non-damaged plants, and they did show preferences for certain infested cabbage cultivars over other cultivars and over other plant species. Neither naive *C. rubecula* nor naive *C. glomerata* discriminated between plants infested by different caterpillar species, nor between plants infested by host- and non-host species. **Chapter 7** revealed that naive *C. glomerata* and *C. rubecula* females do show differences in search times on certain types of host-damaged leaves, but that they do not show differential search times on leaves of a certain food plant with damage of different *Pieris* caterpillars. The innate preferences of the two parasitoid species appear to be similar. In naive wasps preferences were clear at the plant level of the tritrophic system, whereas they seem to lack specificity at the herbivore level.

Plants that are infested by herbivores emit volatile cues that can be used by the natural enemies of the herbivores in their search for hosts. Based on results from the behavioural studies in chapter 2 and 3 differences in the composition of the volatile blends from intact and host-infested food plants of *Pieris* was investigated (**chapter 4**). Chemical analysis of the headspace samples of red cabbage, white cabbage and nasturtium plants, that were infested by *P. brassicae* or *P. rapae* larvae or that were intact, revealed that herbivore-infested plants emit the largest number of compounds and in the highest amounts. The plant species affected the volatile blend more than did the herbivore species. Volatile blends differed less between plant varieties than between different plant species: host-infested nasturtium differed considerably from the cabbage varieties in a qualitative way, whereas headspace compositions of red and white cabbage varieties were comparable to that of the food plant Brussels sprouts as determined earlier in our laboratory (Blaakmeer et al. 1994; Mattiacci et al., 1994). Differences in volatile blends between plants infested by *P. brassicae* or by *P. rapae* were mainly of quantitative nature. These chemical data add to the insight in infochemical use by *Cotesia* species during their search for hosts, in which specificity (i.e. qualitative differences) of stimuli at the herbivore level are of minor importance.

In **chapter 5** we examined the extent to which host acceptance behaviour as related to host species, age, and defensive behaviour might explain the differences in host use that exist between the two parasitic wasps. Host acceptance by *C. rubecula* was high for *P. rapae* and females did not distinguish among the 6 host ages (early and late first, second and third instar) of this host species. In contrast, when foraging for *P. brassicae* and *P. napi*, acceptance was lower and *C. rubecula* females preferred to attack the early first instar. *Cotesia glomerata* showed a higher degree of behavioural plasticity towards acceptance of *Pieris* species and different host ages, than did *C. rubecula*. *Cotesia glomerata* females parasitized larvae of the three *Pieris* species and showed higher acceptance of first and second instars over third instar. Oviposition success was also influenced by host defensive behaviour. The frequency and the effectiveness of defensive behaviour rose with increasing age of the host, *P. brassicae* being the most aggressive *Pieris* species. Furthermore, the mean duration of *C. glomerata* oviposition was significantly reduced by the defesive reactions of *P. brassicae*. This is likely to affect parasitoid fitness as clutch size is positively correlated to oviposition time in *C. glomerata*. Acceptance frequencies corresponded well to field reports of *Pieris-Cotesia* associations and to patterns of parasitoid larval performance, suggesting that the acceptance phase can be used as a reliable indicator of *Cotesia* host-specificity.

Variability in response as a result of learning

Preference learning was studied and compared for *C. glomerata* and *C. rubecula* in long-distance behavioural studies (chapter 6) and by studying host-location behaviour after landing (chapter 7). The use of infochemicals for the assessment of patch profitability is described in chapter 8.

The effect of experience on the host-searching behaviour of *C. glomerata* and *C. rubecula* was compared and at this point clear differences between the two species became evident (**chapter 6**). The generalist *Cotesia glomerata* showed preference learning at the herbivore *and* at the plant level. After multiple experiences with host larvae, they showed a clear preference for volatiles from *P. brassicae*-infested Brussels sprouts leaves over *P. rapae*-infested Brussels sprouts leaves in two-choice tests. *Cotesia glomerata* was apparently capable of learning some subtle difference between plants infested with *P. brassicae* and *P. rapae*, in spite of the lack of clear qualitative differences in plant volatiles. Experience with different *P. brassicae*-infested cabbage varieties or nasturtium in most cases led to preferences for the experienced plant-host complex. Visual cues may have played a role in the learning process, but it certainly is not the only factor causing preferences. Evidence exists that *C. glomerata* learns odours from plant-host complexes in the absence of visual differences and develops a preference for the experienced odour (A. Zwaan, J. Geervliet, L. Vet & J. van Loon, unpublished results). Visual stimuli are not necessary for discrimination between odours.

In contrast, females of the specialist *C. rubecula* did not show preference learning at the herbivore level nor at the plant level. Multiple experience with *P. rapae* did not lead to a preference for this host species in a choice situation with *P. brassicae* and *P. rapae*, and experience with different *P. rapae*-infested cabbage varieties did not change the parasitoid's preferences.

These results strongly support the hypothesis that learning plays a more important role in host-searching behaviour of a generalist than in that of a specialist parasitoid. The behaviour of the generalist *C. glomerata* was more easily changed by experience than that of the specialist *C. rubecula*.

After arriving in a potential host-microhabitat, parasitoids enter the host-location phase, and by responding mainly to host-derived cues they may be able to estimate the suitability of the patch they are sampling. Through multiple visits they may obtain insight in the average profitability of the habitat. These host-derived cues can be recognized by the naive parasitoid or they can be learned, to discriminate between different plant complexes in future searching. The host-location behaviour of *C. glomerata* and *C. rubecula* was studied in a free-ranging wind tunnel experiment (**chapter 7**). Individual females were given the opportunity to sample a set of Brussels sprouts plants, bearing *P. brassicae-* or *P. rapae*-infested leaves and uninfested leaves, and get experienced with the availability of hosts. Number of visits and residence times were host-specific for both parasitoid species. In *C. glomerata* a higher number of visits and longer residence times on *P. brassicae*-infested leaves compared to the other leaf types, were accompanied by a higher number of ovipositions in *P. brassicae* larvae than in *P. rapae* larvae. For *C. rubecula* more visits and longer residence times were observed on *P. rapae*-infested leaves, but no ovipositional preferences were found.

How naive and experienced *C. glomerata* and *C. rubecula* females deal with differences in patch profitability in host-habitat location behaviour is discussed in **chapter 8**. Naive females of both parasitoid species discriminated between odours from plants with high and low densities. Responsiveness of naive females to odours from host-infested leaves increased with increases in the total number of hosts present. Experience with low host-densities increased responsiveness to these low host densities in *C. glomerata* females, which indicates an increase in sensitivity to low host densities. These results demonstrated that quantitative differences in infochemicals play an important role in host-habitat location by these parasitoids. Furthermore, it was shown that *C. glomerata* uses experience with different host densities for subsequent host-searching decisions and that the order of the experienced host densities influenced the behaviour to a great extent. The first experience a female gets, has a significant effect on subsequent decisions. High host densities on a certain leaf type that was the first one the wasps experienced, led to preferences for this specific plant-host complex composition. This result shows that experience can modify the parasitoid's response to variation in resource availability. For *C. rubecula* this has not been studied.

Host suitability

Host specificity and host selection by insect parasitoids are hypothesized to be correlated with suitability of the hosts for parasitoid development. In **chapter 9** the subject of research was the correlation between host suitability and the earlier studied host-searching behaviour (chapters 2, 3, 5, 7 & 8). The capabilities of the generalist *C. glomerata* and the specialist *C. rubecula* to parasitize and develop in *P. brassicae*, *P. rapae* and *P. napi* were compared, with the prediction that variation in parasitoid performance among host species is lower for the generalist than for the specialist.

The effect of host species on the fitness parameters such as survival, development, sex ratio and size of parasitoid progeny were measured, and the results show that *C. glomerata* is capable of developing in the three host species but differences in suitability between host species were found, in favor of *P. brassicae*. *Cotesia rubecula* appeared to be restricted to *P. rapae*. Although *C. rubecula* is physiologically able to develop in *P. brassicae* larvae, parasitoid fitness is negatively affected by this host species. Larvae of *P. napi* were unsuitable for development of *C. rubecula*, since parasitization did not lead to viable offspring. Comparison of the host suitability data with the host-searching and host-acceptance behaviour leads to the conclusion that the host-searching behaviour of both parasitoid species is attuned to encounters with the most suitable host species for parasitoid development.

Field populations

The generalist *C. glomerata* and the specialist *C. rubecula* co-occur in cabbage fields in The Netherlands. To investigate the ultimate performance of the two *Cotesia* species under more natural circumstances, field experiments with natural populations are needed. In **Chapter 10** a field study to investigate whether parasitization of the three *Pieris* species varies with parasitoid species and with food plant of the caterpillars is reported. Both parasitoid species had clear preferences for certain plant-host complexes in the field. *Cotesia glomerata* was mainly recovered from the gregarious *P. brassicae*. Although suitable for development of *C. glomerata* larvae, *P. rapae* and *P. napi* larvae were only occasionally parasitized by this parasitoid. For this generalist parasitoid species a broader host use was expected, but competition with *C. rubecula* in *P. rapae* probably plays a role in the actual host use by *C. glomerata*. Laboratory studies revealed that in the case of multiparasitism in *P. rapae*, the generalist *C. glomerata* is outcompeted by the specialist *C. rubecula* (Laing & Corrigan, 1982) and wind tunnel studies showed, that *C. glomerata* wasps avoid plants infested by *P. rapae* on which *C. rubecula* females are parasitizing larvae. As predicted, the specialist *C. rubecula* restricted its host use to *P. rapae*.

High levels of parasitization occurred on the cabbage varieties Brussels sprouts and red cabbage, but on nasturtium larvae of the three host species were rarely parasitized. Suitability data for *C. glomerata* from *P. brassicae* larvae on Brussels sprouts and red cabbage revealed that both food plants are suitable for development of this parasitoid species. The results are in concordance with the results of the behavioural studies described in chapter 3, 6 and 7. Furthermore, both *Cotesia* species appear to coexist by niche segregation, possibly through an avoidance of competition in *P. rapae* by *C. glomerata*.

Coexistence and niche segregation

The generalist *C. glomerata* appeared to be focussed towards the gregarious host *P. brassicae* in its final host selection. This may be the cause of ecological factors rather than physiological factors or constrained infochemical use. Local populations may experience different selection pressures. Populations from different regions may each be adapted to utilize specific resources and a generalist species can actually consist of specialist local populations (Fox & Morrow, 1981; Futuyma & Peterson, 1985). Studies with females from the Dutch population have demonstrated, that *C. glomerata* shows an area restricted search when searching for hosts, which can be considered as an adaptation to the clustered distribution of *P. brassicae* larvae (Wiskerke & Vet, 1994). Furthermore, observations from the field have shown that the main host of *C. glomerata* is *P. brassicae* (Feltwell, 1982; chapter 10). This adaptation may have arisen from ecological constraints when utilizing other host species than *P. brassicae*, for example competition with *C. rubecula* in *P. rapae*. Laboratory studies on competition between *C. glomerata* and *C. rubecula* in *P. rapae* larvae revealed that *C. glomerata* is the inferior competitor in most cases (Laing & Corrigan, 1987). In the Netherlands, competition with *C. rubecula* for *P. rapae* is likely to occur. Firstly, since both parasitoid species occur in the same habitats, with partly overlapping niches. And secondly, because *P. rapae* is much more abundant than *P. brassicae* (van Swaay, 1995). In those cases where *P. brassicae* is present, larvae are heavily parasitized by *C. glomerata*, whereas the parasitoid is hardly ever recovered from *P. rapae* larvae. As long as *P. brassicae* is present in Dutch ecosystems, coexistence of the two species appears to be possible.

In the USA, where *P. rapae* is the only host species available for *Cotesia* species, *C. glomerata* (and not *C. rubecula*!) was successfully introduced in 1863 to combat this pest insect (Biever, 1992; Le Masurier & Waage, 1993). The parasitoid established very well, and Le Masurier & Waage

(1993) stated, that natural selection is expected to have increased the adaptation of the introduced *C. glomerata* population to *P. rapae*. Indication for this adaptation to *P. rapae* was the fact that the American *C. glomerata* population attacked *P. rapae* at a significantly higher rate than did the British strain (le Masurier & Waage, 1993). The host-searching behaviour of the American *C. glomerata* population may have changed to exploit more efficiently the more sparse, uniformly distributed *P. rapae* in the USA (Courtney, 1986; le Masurier & Waage, 1993). This is presently being tested by comparing the patch leaving rules of the Dutch and American populations of *C. glomerata* (Vet, 1995). *Cotesia glomerata* established through most of North America, but was not recovered in British Columbia. It was concluded that *C. rubecula* had probably replaced *C. glomerata* (Wilkinson, 1966). Field recovery data (Biever, 1992) support this idea. In areas where *C. rubecula* was found, no *C. glomerata* was reported, and *C. glomerata* was recovered only in areas south of those where *C. rubecula* had established. The failure of *C. rubecula* to disperse further south is ascribed to its diapause (Nealis, 1985; Biever, 1992).

The question arises, whether both species coexist in The Netherlands through niche segregation only. Is *C. glomerata* prevented from 'extinction' by the availability of *P. brassicae* in The Netherlands, or does this parasitoid species have adopted other behavioural traits to coexist with *C. rubecula*? Is it able to switch its behavioural strategy when *P. brassicae* disappears? To answer these questions, the actual occurrence of competition between *C. glomerata* and *C. rubecula* in *P. rapae* in the field should be investigated. With parasitization experiments and DNA identification techniques, occurrence of multiparasitism in this host species could be determined and quantified. This may lead to an estimation of the chance of competition for host resources and of the selection pressure it puts on *C. glomerata* for competition avoidance.

Furthermore, it has often been argued that the superior dispersal capabilities of poorer competitors are important in explaining coexistence (the fugitive species hypothesis) (Pschorn-Walcher & Zwölfer, 1968; Zwölfer, 1971; Schröder, 1974). They have reasoned that the coexistence of parasitoids on a number of herbivores in forests can be explained by counterbalance competition and have classified the parasitoids of these insects as either intrinsically or extrinsically superior competitors. Extrinsically superior competitors are supposed to show higher dispersal and host finding rates. This could be true for *C. glomerata* as well. Wind tunnel studies (chapter 8) revealed that naive *C. glomerata* females respond to and learn to concentrate on low host densities, which might be an indication that it is able to search efficiently for the solitarily distributed *P. rapae* larvae. To be able to come to firmer conclusions, the dispersal rates and energy reserves should be measured and compared for *C. glomerata* and *C. rubecula* under field conditions and in laboratory studies (e.g. using flight simulators, caloric measurements).

It would be interesting to investigate the response of the American *C. glomerata* strain to their replacement by *C. rubecula*. Possibly extinction of *C. glomerata* will not continue further south, so that both species are seperated by a border (a physiological border for *C. rubecula*, which is an ecological border for *C. glomerata*). On the other hand, if the fugitive species hypothesis holds for *C. glomerata*, the species could adopt a different host searching strategy. Price (1980) argued that parasitoids in general should have the potential to undergo rapid adaptation to locally abundant host populations. With higher dispersal rates and higher sensitivity to low host densities, *C. glomerata* may be able to locate *P. rapae* before *C. rubecula* does. This might lead to coexistence of the two parasitoid species again in *P. rapae* in the British Columbian region.

The comparison of host-searching behaviour of a generalist and a specialist parasitoid species

Comparative studies, wherein phylogenetically close species are used to search for correlations between species characteristics and ecological factors, are important in delineating the adaptive value of behavioural traits. To design ecologically relevant bioassays with highly comparable stimuli it is essential to use species with highly comparable and preferably partly overlapping niches. This sort of comparison helps drawing unambiguous conclusions that differences in behaviour are indeed correlated to the ecological factor under study and not to some other ecological or phylogenetic factor (Vet et al., 1995). The comparison of the host-searching behaviour of the generalist *C. glomerata* and the specialist *C. rubecula* described in this thesis aimed to understand the adaptive value of inter- and intraspecific differences in the host-searching behaviour by parasitoids, and to investigate whether dietary specialization is reflected in the way these species use infochemicals.

Similarities in behaviour.

When inexperienced, *C. glomerata* and *C. rubecula* showed comparable behaviour, since they used the same type of stimuli and no evidence was obtained that the specialist needed a higher specificity of stimuli in host-searching behaviour (chapters 2, 3 and 7). The chemical analyses revealed that cabbage shows a more general response to herbivory, mainly by increasing the amount of volatiles upon damage (chapter 4; Mattiacci et al., 1994; Blaakmeer et al., 1994; Agelopoulos & Keller, 1994c) rather than by emitting qualitatively different blends. This is contrary to what was found in many other plant species (Dicke et al., 1990b; Turlings et al., 1990, 1993; Dicke, 1994; 1996). The fact that cabbage shows a more general response to feeding damage helps to explain the results of the behavioural studies. Apparently, parasitoids do not have the opportunity to use highly specific stimuli (i.e. qualitative differences in chemical composition of odour blends), and as a result they rely on quantitative differences in infochemicals as was found in chapters 2 and 8.

Differences in behaviour.

Differences in the effect of experience of *C. glomerata* and *C. rubecula* clearly leads to behavioural differences between these parasitoid species. Searching behaviour of *C. glomerata* is flexible. Preference learning for plant species and cultivars could easily be demonstrated, and experience with hosts led to a preference for *P. brassicae*. The strength of the preflight experience needed to induce this preference was higher compared to preference learning for plant species (chapter 6). Furthermore, experience with differences in patch profitability leads to efficient foraging decisions in *C. glomerata* (chapter 8). In contrast, behaviour of the specialist *C. rubecula* is more fixed to the Brussels sprouts-*P. rapae* complex and is not easily influenced by learning. Preference learning for host species could not be shown in two-choice wind tunnel experiments, and experience with other plant-host complexes did not lead to a change in preference for the Brussels sprouts-*P. rapae* complex (chapter 6). However, in the 'free-ranging' wind tunnel experiment (chapter 7), the informational state of *both* parasitoid species might have played a role in the outcome of the experiment. Besides the presence of both visual and chemical stimuli and contact with host-derived stimuli, females of *C. glomerata* and *C. rubecula* have likely gained experience with host availability by sampling the host-searching arena, resulting in an estimation of patch quality and an adjustment of their behavioural decisions. All this may lead to the observed preferences for *P. brassicae* in *C. glomerata* and for *P. rapae* in *C. rubecula*. Similar processes may play a role in the host-searching behaviour of *C. glomerata* and *C. rubecula* in the field (chapter 10). The fact that *C. glomerata* seems restricted in its host use to *P. brassicae* (chapter 10) may have to do with competition with *C. rubecula* in *P. rapae* larvae. In case of multiparasitism of *P. rapae* by *C. rubecula* and *C. glomerata*, the latter is the inferior competitor in most cases (Laing & Corrigan, 1987). Perhaps, *C. glomerata* actively avoids competition with *C. rubecula* for *P. rapae*. Wind tunnel studies showed that *C. glomerata* avoids visiting *P. rapae*-infested Brussels sprouts plants on which *C. rubecula* is parasitizing *P. rapae* larvae (chapter 10).

Differences in host searching decisions between the two *Cotesia* species become clear at the host acceptance level (chapter 5), where the generalist *C. glomerata* showed a more flexible behaviour than the specialist *C. rubecula* in accepting different hosts for oviposition. The specialist was more restricted to *P. rapae* larvae, but enlarged its host-range by accepting more larval instars of this host for oviposition. *Cotesia glomerata* and *C. rubecula* may use host-specific cues in this host acceptance phase, since evidence exists that parasitoids show specific responses to the host-related kairomones (from host faeces) (Agelopoulos & Keller, 1994c; Agelopoulos et al., 1995). The 'free-ranging' wind tunnel experiment (chapter 7) demonstrated that *C. glomerata* and *C. rubecula* use a complete set of information sources, probably including visual stimuli as well (Wäckers, 1994; Verdegaal, Vet & Wäckers, in prep.). Herbivore-induced plant-derived information may be used as an indication of damage (Vinson, 1981; Steinberg et al., 1993; Agelopoulos & Keller, 1994; chapter 2), while faeces at shorter distances may be used as the host species distinguishing cue (Weseloh, 1981; Agelopoulos et al., 1995).

For both parasitoid species the host-searching decisions are in accordance with the suitability of the hosts they parasitize for the development of their offspring (chapter 9). Host specificity is thought to be determined by the physiological capacity of the immatures to exploit the host, and the behavioural ability of adults to find the host resources. Females of the generalist *C. glomerata* were flexible in accepting different host species for oviposition (chapter 5) and their learning behaviour allows for flexible host-searching decisions (chapters 6, 7 & 8), although their final host preference turned out to be for *P. brassicae*. The host suitability experiments (chapter 9) revealed the suitability of all three *Pieris* hosts tested for development of *C. glomerata* larvae, with minor advantages for development in *P. brassicae*. Females of *C. rubecula* restricted their host search and host-acceptance to *P. rapae*, which is in concordance with the suitability of *Pieris* hosts: fitness penalties for development in *P. brassicae* were found and *P. napi* was completely unsuitable as a host.

Conclusion.

In conclusion, considering the aims of this thesis, the relative importance of infochemicals from the first and the second trophic level is similar for both parasitoid species. The use of these infochemicals differs for the two species.

This generalist species was expected to show flexibility in its behaviour by preference learning, which was clearly demonstrated. For the specialist *C. rubecula* no influence of learning processes on the parasitoid's behaviour was found, which was expected.

The generalist nature of *C. glomerata* is reflected in its host-acceptance behaviour and the suitability of different *Pieris* hosts for development of its offspring. However, its final host-selection seems to be focussed towards *P. brassicae*, both in laboratory studies and in the field. *Cotesia rubecula* develops most successfully in *P. rapae* larvae, and the parasitoid's behaviour appears to be adapted to search for this host.

In this study the adaptiveness of the host-searching behaviour of *C. glomerata* and *C. rubecula* has become obvious.

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SAMENVATTING

Sluipwespen zijn van groot belang voor natuurlijke en agrarische ecosystemen, daar zij de populatiedichtheden van hun gastheren kunnen beïnvloeden of reguleren. Tijdens het larvale stadium voeden ze zich in of op het lichaam van een gastheer, meestal een insekt, die daaraan uiteindelijk sterft. Onderzoek aan sluipwespen heeft inmiddels een grote hoeveelheid informatie opgeleverd over het gedrag en de oecologie van veel soorten. Er wordt veel aandacht geschonken aan fundamentele aspekten van de biologie van sluipwespen, enerzijds om theorieën over de evolutie van het gastheerzoekgedrag van sluipwespen te kunnen testen, anderzijds vanwege het grote belang dat kennis van de fundamentele biologie voor de toegepaste entomologie heeft.

Gedrags-oecologie houdt zich bezig met evolutionaire vragen over de interaktie tussen het gedrag van een dier en de omgeving waarin het dier zich bevindt. Onderzoek richt zich op de gevolgen van variatie in het gedrag op de fitness van een dier. Sluipwespen zijn zeer geschikte kandidaten voor onderzoek aan dergelijke evolutionaire vragen over gedrag. Hun voortplantingsstrategie is direct gekoppeld aan hun fitness en het niet vinden van een geschikte gastheer of van het maken van verkeerde beslissingen tijdens het parasiteringsproces heeft grote gevolgen voor de fitness.

Sluipwespen zijn geëvolueerd en funktioneren in multitrofe systemen, waarin zij zelf het derde trofische niveau innemen. Hun gastheren vormen het tweede en het substraat waarop hun gastheren zich voeden (planten bijvoorbeeld) vormt het eerste trofische niveau. Tijdens het zoeken naar gastheren gebruiken sluipwespvrouwtjes vaak chemische stimuli als informatiebron. Deze chemische informatiestoffen kunnen zowel direct als indirect afkomstig zijn van de gastheer, dan wel van het voedsel van de gastheer. De mate van specialisatie van de sluipwesp kan bepalen in hoeverre stimuli specifiek moeten zijn voor het vinden van gastheren. Specifieke informatie over de aanwezige gastheren wordt geacht minder belangrijk te zijn voor sluipwespen die een breed scala aan gastheren kunnen parasiteren, terwijl specialisten eerder specifieke informatie nodig zullen hebben.

De zoekstrategie van sluipwespen wordt bepaald door zowel genotypische als fenotypische faktoren. De efficientie waarmee zij naar gastheren zoeken, kunnen ze vergroten door hun zoekgedrag aan te passen. Ze leren te reageren op veranderingen of verschillen in de omstandigheden waarin ze moeten funktioneren. De mate waarin sluipwespen hun gedrag kunnen aanpassen, kan gerelateerd zijn aan de mate van specialisatie van de soort: leren wordt geacht een grotere rol te spelen in het gastheerzoekgedrag van generalisten dan van specialisten.

Vergelijkend onderzoek, waarin fylogenetisch verwante soorten worden gebruikt om te bepalen of er correlaties bestaan tussen gedragskenmerken en ecologisch faktoren, is van belang voor het vaststellen van de adaptieve waarde van gedragskenmerken. Voor het testen van stimuli in oecologisch relevante biotoetsen is het van belang gebruik te maken van fylogenetisch verwante soorten met vergelijkbare niches. Op deze wijze kunnen eenduidige conclusies getrokken worden dat de gevonden verschillen in zoekstrategieën gecorreleerd zijn met de bestudeerde oecologische faktor waarin de soorten verschillen en niet met een fylogenetische faktor.

Ook voor het in dit proefschrift beschreven onderzoek is een dergelijke benadering toegepast, waarbij de hypothese getoetst is, dat het gebruik van chemische informatiestoffen door sluipwespen afhangt van hun mate van specialisatie op hun gastheer of het substraat waarop hun gastheer leeft.

De acteurs die in dit onderzoek optreden, zijn twee nauwverwante sluipwespsoorten, die verschillen in de breedte van hun gastheerspectrum. *Cotesia glomerata* is een generalist, die eipakketten afzet in rupsen van verschillende *Pieris* soorten, aldus een gregaire larvale endoparasitoid. *Cotesia rubecula* staat bekend als specialist op *Pieris rapae*, en is solitair: één eitje per rups. Deze sluipwespsoorten komen voor in habitats die erg op elkaar lijken, en bezetten deels overlappende niches. Hun zoekgedrag op langere afstand wordt gekenmerkt door responsen op geur en visuele prikkels van beschadigde planten.

De drie belangrijkste doelstellingen van dit onderzoek waren het vaststellen van 1) de specificiteit van de chemische informatiestoffen die *C. glomerata* en *C. rubecula* gebruiken tijdens het zoeken naar gastheren, 2) de invloed van leren op het zoekgedrag van deze twee sluipwespsoorten, en 3) de gevolgen voor de fitness van hun beslissingen tijdens het zoeken naar gastheren.

Specificiteit van de stimuli

De specificiteit van de stimuli die de twee *Cotesia* soorten gebruiken tijdens het zoeken naar gastheren is onderzocht in gedragsstudies, zowel op afstand van de geurbronnen (hoofdstuk 2 en 3) als in de buurt van de gastheer, na landen op het blad (hoofdstuk 5 en 7), en in chemische analyses van de vluchtige stoffen die afgegeven worden door intacte planten en planten met vraatschade door *Pieris* rupsen (hoofdstuk 4).

In **hoofdstuk 2** wordt de rol van vluchtige stimuli beschreven voor het lange-afstand zoekgedrag van de specialist *Cotesia rubecula*. Responsen van *C. rubecula* vrouwtjes op stimuli van verschillende onderdelen van het Spruitkool/*P. rapae* waardplant-gastheer complex werden vergeleken in twee-keuze toetsen in een windtunnel. Stimuli afkomstig van beschadigde spruitkool bladeren (mechanisch beschadigd of met vraatschade van *P. rapae* rupsen) leidden tot een hogere respons bij de sluipwespen dan gastheer-gerelateerde stimuli. Daarnaast vertoonden sluipwesp vrouwtjes een voorkeur voor bladeren van het waardplant-gastheer complex dan voor bladeren met kunstmatige schade. Onbeschadigde bladeren waren het minst aantrekkelijk voor de sluipwespen. Deze resultaten zijn een aanwijzing dat *C. rubecula* tijdens het zoeken naar gastheren op lange afstand voornamelijk gebruik maakt van chemische informatie afkomstig van de plant, hetgeen eerder ook is gevonden voor de generalist, *C. glomerata*. Voor beide soorten zijn chemische stimuli afkomstig van de gastheer van minder belang in deze fase van het zoekproces.

In **hoofdstuk 3** worden aangeboren voorkeuren voor chemische informatiestoffen afkomstig van verschillende waardplant-gastheer complexen vergeleken voor de generalist *C. glomerata* en de specialist *C. rubecula*. De sluipwespen waren naïef, dat wil zeggen, ze hadden als volwassen individu nog geen *Pieris* rupsen of planten ervaren. Het gedrag van de specialist werd geacht sterkere voorkeuren te vertonen dan dat van de generalist. Beide sluipwespsoorten prefereerden planten met *Pieris*-vraatschade boven onbeschadigde planten, en er bestonden voorkeuren voor bepaalde koolvariëteiten boven andere en boven een andere waardplantsoort, namelijk oostindische kers. Naïeve *C. rubecula* en *C. glomerata* vrouwtjes maakten geen onderscheid tussen chemische stimuli afkomstig van planten waarop zich gastheren van verschillende *Pieris* soorten voedden. Evenmin maakten zij onderscheid tussen stimuli afkomstig van planten met gastheren en niet-gastheren. **Hoofdstuk 7** laat zien, dat zowel voor naïeve *C. glomerata* als voor *C. rubecula* vrouwtjes zoektijden verschillen tussen door gastheren beschadigde bladeren van verschillende voedselplanten. Op bladeren van dezelfde voedselplant beschadigd door verschillende gastheersoorten werden geen verschillen in zoektijden gevonden voor beide sluipwespsoorten. De aangeboren voorkeuren van beide sluipwespsoorten vertonen grote overeenkomsten. Naïeve vrouwtjes vertoonden voorkeuren op het plant-niveau van het tritrofe systeem, terwijl specificiteit van chemische informatiestoffen op gastheer-niveau lijkt te ontbreken.

Nadat sluipwespen beland zijn in een potentiële gastheer-microhabitat komen ze in de gastheer-localisatie fase, en door voornamelijk te reageren op stimuli afkomstig van gastheren, kunnen ze een inschatting maken van de geschiktheid van de plek waarin ze zoeken. Via het meervoudig bezoeken van verschillende plekken, kunnen ze de relatieve profijtelijkheid van de habitat leren. De stimuli die met de gastheer geassocieerd zijn, kunnen door de naïeve sluipwesp herkend worden, of ze kunnen geleerd worden door ervaring, om zo onderscheid te maken tussen verschillende waardplant-gastheer complexen in toekomstige zoekprocessen. Het gastheer-localisatie gedrag van *C. glomerata* en *C. rubecula* werd onderzocht in windtunnel experimenten, waar vrouwtjes de kans kregen naar eigen believen naar gastheren te zoeken (**hoofdstuk 7**) op spruitkoolplanten met door *P. brassicae* en *P. rapae* beschadigde bladeren en onbeschadigde bladeren. Zodoende konden ze ervaring opdoen met de beschikbaarheid van gastheren. Het aantal bezoeken en verblijfstijden bleken specifiek voor beide sluipwespsoorten. *Cotesia glomerata* bezocht vaker bladeren met *P. brassicae* en besteedde daar meer tijd in vergelijking met de andere bladtypen, en dit ging samen met een groter aantal geparasiteerde *P. brassicae* rupsen dan *P. rapae* rupsen. Bij *C. rubecula* werden meer bezoeken en langere verblijfstijden waargenomen op bladeren met *P. rapae* dan op andere bladtypen.

Hoe naïeve en ervaren *C. glomerata* en *C. rubecula* vrouwtjes omgaan met verschillen in profijtelijkheid van gastheer plekken tijdens de gastheer localisatie wordt beschreven in **hoofdstuk 8**. Naïeve vrouwtjes van beide sluipwespsoorten maakten onderscheid tussen chemische stimuli afkomstig van planten met hoge en lage gastheerdichtheden. Responsen van naïeve vrouwtjes op geuren van door gastheren beschadigd spruitkoolblad nam toe met een toename van het aantal gastheren op het blad. Ervaring met lage gastheerdichtheden veroorzaakte bij *C. glomerata* een verhoging van de responsiviteit op deze lage gastheerdichtheden, hetgeen wijst op een toename van de gevoeligheid voor lage gastheerdichtheden. Deze resultaten tonen aan, dat kwantitatieve verschillen in chemische informatiestoffen een belangrijke rol spelen in het habitat-localisatie gedrag. Daarnaast werd duidelijk, dat *C. glomerata* ervaring met verschillende gastheerdichtheden gebruikt bij volgende zoekbeslissingen en dat de volgorde waarin gastheerdichtheden waargenomen worden het gedrag sterk beïnvloedt. De eerste ervaring die een vrouwtje krijgt is van essentieel belang voor volgende beslissingen, en kan niet zonder meer overschaduwd worden door een ervaring op een meer profijtelijke plek. Voorkeuren ontstaan onder invloed van ervaring wanneer de eerste ervaring plaats vindt op een plek met hoge gastheerdichtheden. De resultaten beschreven in dit hoofdstuk laten zien, dat ervaring de respons van vrouwtjes op variatie in beschikbaarheid van gastheren kan veranderen. Voor *C. rubecula* is dit niet onderzocht, omdat er voor deze soort in eerdere experimenten geen leren kon worden aangetoond.

Gastheergeschiktheid

Gastheerspecificiteit en gastheerselectie door sluipwespen worden verondersteld gecorreleerd te zijn met de geschiktheid van de gastheer voor de ontwikkeling van sluipwesplarven. In **hoofdstuk 9** is de correlatie tussen gastheergeschiktheid en het eerder beschreven zoekgedrag (hoofdstuk 2, 3, 5, 7 en 8) bestudeerd. De bekwaamheid van *C. glomerata* en *C. rubecula* om de drie gastheren *P. brassicae, P. rapae* en *P. napi* te parasiteren en zich daarin te ontwikkelen werd onderzocht en vergeleken, met de voorspelling dat de prestatie van de generalist in de drie gastheren minder variatie vertoont dan die van de specialist. Het effect van de gastheersoort op de fitness-bepalende parameters overleving, ontwikkeling, sex ratio en grootte van de nakomelingen werd gemeten. De resultaten laten zien dat *C. glomerata* in staat is zich succesvol te ontwikkelen in alle drie gastheersoorten. De verschillen in gastheergeschiktheid tussen de soorten onderling waren gering, met *P. brassicae* als meest geschikte gastheer. *Cotesia rubecula* bleek beperkt tot *P. rapae*. Hoewel deze sluipwespsoort fysiologisch gezien in staat is zich te ontwikkelen in *P. brassicae* rupsen, wordt fitness negatief beïnvloed door deze gastheersoort. Larven van *P. napi* waren ongeschikt voor *C. rubecula*, omdat parasitering niet leidde to productie van nakomelingen. Na vergelijking van deze resultaten met het gastheer-zoekgedrag en de gastheer-acceptatie kan geconcludeerd worden dat het gastheer-zoekgedrag van beide sluipwespsoorten leidt tot ontmoetingen met de gastheersoort die het meest geschikt is voor de ontwikkeling van de sluipwespen.

Veldpopulaties

De generalist *C. glomerata* en de specialist *C. rubecula* komen in koolvelden in Nederland naast elkaar voor. Om het uiteindelijke funktioneren van de soorten onder meer natuurlijke omstandigheden te onderzoeken, zijn veldexperimenten met natuurlijke populaties essentieel. In **hoofdstuk 10** wordt een veldexperiment beschreven, dat is uitgevoerd om vast te stellen of parasitering varieert met gastheersoort en voedselplant van de gastheer. Beide sluipwespsoorten hadden duidelijke voorkeuren voor bepaalde waardplant-gastheer complexen in het veld. *Cotesia glomerata* werd voornamelijk teruggevonden in *P. brassicae*. Hoewel *P. rapae* en *P. napi* geschikt zijn voor ontwikkeling van deze sluipwesp (hoofdstuk 9), werden van deze gastheersoorten nauwelijks door *C. glomerata* geparasiteerde individuen teruggevonden. Voor de generalist was een breder gastheerspectrum in het veld verwacht, maar competitie met *C. rubecula* in *P. rapae* speelt mogelijk een rol in het uiteindelijke gastheerspectrum van de generalist. Laboratoriumonderzoek heeft laten zien dat de generalist de concurrentie verliest van de specialist in geval van multiparasitering van *P. rapae* door *C. glomerata* en *C. rubecula*. Experimenten in de windtunnel hebben laten zien, dat *C. glomerata* planten links laat liggen waarop *C. rubecula P. rapae* parasiteert. Zoals verwacht, beperkt *C. rubecula* zich ook in het veld tot *P. rapae*.

Op spruitkool en rode kool werden hoge parasiteringspercentages gevonden, terwijl op oostindische kers nauwelijks geparasiteerde rupsen werden aangetroffen. Rode kool en spruitkool bleken even geschikt voor de ontwikkeling van *C. glomerata* in *P. brassicae*. De resultaten van het veldonderzoek ondersteunen de resultaten van de gedragsstudies beschreven in hoofdstuk 3, 6 en 7. Daarnaast lijken beide *Cotesia* soorten te kunnen coexisteren door niche-segregatie, wellicht door het ontwijken van competitie in *P. rapae* door *C. glomerata*.

Conclusie

Afsluitend kan de conclusie getrokken worden, dat het relatieve belang van chemische informatie van het eerste en tweede trofische niveau voor beide sluipwespsoorten gelijk is. De wijze waarop de twee soorten met deze informatie omgaan is echter verschillend.

Het gedrag van de generalist *C. glomerata* werd verondersteld plasticiteit te vertonen door leerprocessen, hetgeen duidelijk is aangetoond. Voor de specialist *C. rubecula* kon geen invloed van leerprocessen op het gedrag aangetoond worden, hetgeen verwacht werd.

De generalistische levenswijze van *C. glomerata* wordt weerspiegeld is het gastheer-acceptatie gedrag en de gastheer-geschiktheid van verschillende *Pieris* soorten voor de ontwikkeling van nakomelingen. Echter, de uiteindelijke gastheerselectie lijkt te zijn toegespitst op *P. brassicae*, zowel in laboratorium experimenten als in het veld. De specialist *C. rubecula* ontwikkelt zich succesvol in *P. rapae* en het gedrag van deze sluipwespsoort lijkt te zijn aangepast voor het zoeken naar deze gastheer.

De adaptieve waarde van het zoekgedrag van beide soorten is hiermee duidelijk geworden.

NAWOORD

Na het vastleggen van mijn bevindingen van vier jaar onderzoek rest mij nog het schrijven van dit laatste, belangrijke stukje tekst, waarin ik een persoonlijk woord wil richten tot al diegenen die bijgedragen hebben aan de voltooiing van mijn onderzoek en dit proefschrift.

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Jacqueline,
Zuilichem, 28 maart 1997

CURRICULUM VITAE

In 1984 voltooide ik, Jacqueline Brigitte Françoise Geervliet (3 juni 1965, Meerssen) mijn Gymnasium ß opleiding aan het Sint-Maartenscollege in Maastricht. In dat zelfde jaar kwam ik naar Wageningen voor een studie Planteziektenkunde aan de toenmalige Landbouwhogeschool. Twee van de drie doctoraalvakken tijdens mijn studie waren gericht op biologische bestrijding: voor het eerste onderzocht ik het effect van de baculovirussen (insektevirussen) in combinatie met de bacterie *Bacillus thuringiensis* op de mortaliteit van rupsen van de Floridamot. Het tweede doctoraalvak richtte zich op de werking van nematode-parasitaire schimmels. Mijn derde doctoraalvak was gewijd aan gedragsoecologisch onderzoek aan malariamuggen. Tijdens mijn stage in Costa Rica bij het Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) in Turrialba heb ik gewerkt aan een ziekte in koffiezaailingen. In augustus 1990 behaalde ik mijn doctoraalexamen Planteziektekunde (cum laude). Van september 1990 tot juni 1991 was ik werkzaam bij het Instituut voor Planteziektenkundig Onderzoek (IPO) te Wageningen, waar ik als toegevoegd onderzoeker werkte aan de indiening van een aanvraag voor de toelating van baculovirussen als biologisch bestrijdingsmiddel. Na honorering van een project voor een Braio-plaats aan de Landbouwuniversiteit werd ik aangesteld als assistent-in-opleiding bij de vakgroep Entomologie van 1991 tot 1995. Het onderzoek dat ik voor dit project heb uitgevoerd staat beschreven in dit proefschrift.
Sinds 1996 ben ik werkzaam bij Vikwezu b.v., een bedrijf gespecialiseerd in de teelt van siervis.