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A COMPARATIVE STUDY OF THE LIFE HISTORY AND FORAGING BEHAVIOUR OF APHID HYPERPARASITOIDS

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Résumé

Les hyperparasitoïdes sont des guêpes parasitoïdes des parasitoïdes primaires. Au sein d'un écosystème, ils occupent le quatrième niveau trophique. Une meilleure connaissance de la biologie et du comportement des hyperparasitoïdes est indispensable pour comprendre leur relation avec les parasitoïdes et leur rôle dans les écosystèmes. Dans cette étude, quatre espèces d'hyperparasitoïdes de pucerons différant quant à leur phylogénie, mode de développement (koinobionte vs. idiobionte), stades d'hôte attaqués, et spécificité parasitaire ont été choisies: *Dendrocerus carpenteri* (Curtis) (Megaspilidae), *Asaphes suspensus* Walker (Pteromalidae), *Alloxysta victrix* (Westwood) (Alloxystidae) et *Syrphophagus aphidivorus* (Mayr) (Encyrtidae). Au laboratoire, j'ai comparé leurs paramètres d'histoire de vie et comportements de recherche par une approche comparative directe au sein du système trophique: pomme de terre (*Solanum tuberosum* L.); puceron de la pomme de terre, *Macrosiphum euphorbiae* (Thomas); parasitoïde hôte *Aphidius nigripes* Ashmead.

Les résultats ont révélé une grande variation interspécifique des paramètres d'histoire de vie des hyperparasitoïdes. Cette variation n'a pu être attribuée exclusivement à la dichotomie du mode de développement (koinobionte ou idiobionte), tel que démontré pour les parasitoïdes primaires. L'hyperparasitoïde *S. aphidivorus* est atypique, ayant la capacité d'attaquer soit la larve parasitoïde dans le puceron vivant, soit sa pupe après la momification du puceron. Les femelles préféraient cet hôte, lequel s'est également avéré le plus convenable au développement. Des tests d'olfactométrie et des observations comportementales ont révélé que les femelles hyperparasitoïdes en quête d'hôtes ne seraient pas attirées à distance par des odeurs. Toutefois, elles utilisent des stimuli de contact sur la plante afin de localiser leur hôte. À ce niveau, le miellat de puceron est apparu comme l'un des principaux stimuli utilisés par les femelles, lesquelles discriminaient entre le miellat de puceron et celui de cochenille, *Coccus hesperidum*, n'abritant pas d'hôtes potentiels. Par contre, les femelles hyperparasitoïdes n'ont pas distingué le miellat de pucerons sains non-parasités, et celui de pucerons parasités par *A. nigripes*.

Cette étude indique que plusieurs facteurs influencent simultanément l'histoire de vie des hyperparasitoïdes de pucerons. Leur subdivision habituelle en endoparasitoïdes koinobiontes de larves parasitoïdes dans les pucerons vivants, et ectoparasitoïdes idiobiontes de pupes de parasitoïdes dans les pucerons momifiés ne traduit pas toutes les différences interspécifiques observées. Des différences d'ordre phylogénique seraient également importantes, ces espèces provenant de taxons différents. A bien des égards, les paramètres de vie et le comportement des hyperparasitoïdes de pucerons diffèrent de ceux des parasitoïdes primaires de pucerons.

Abstract

Hyperparasitoids are parasitic wasps that attack primary parasitoids. They constitute the fourth trophic level in many ecosystems. A better understanding of hyperparasitoid biology and behaviour is needed to unravel the nature of parasitoid - hyperparasitoid interactions and their role in the functioning of communities and ecosystems. In this thesis, the life history traits and host searching behaviour of aphid hyperparasitoids are studied using a direct comparative approach. Four species were chosen that differ in development mode (koinobiont or idiobiont), host stage attacked and host range: *Dendrocerus carpenteri* (Curtis) (Megaspilidae), *Asaphes suspensus* Walker (Pteromalidae), *Alloxysta victrix* (Westwood) (Alloxystidae) et *Syrphophagus aphidivorus* (Mayr) (Encyrtidae) have been studied on the same potato (*Solanum tuberosum* L.), potato aphid (*Macrosiphum euphorbiae* (Thomas)) and primary parasitoid *(Aphidius nigripes* Ashmead) system.

The results revealed a large variation in life history traits between species, which could not be explained simply by dichotomy in development mode, as proposed for primary parasitoids. The hyperparasitoid *S. aphidivorus* is special because females can attack the parasitoid host in the still-living aphid, or in the mummified aphid. Female *S. aphidivorus* had a preference for aphid mummies, which also contain the most profitable host stage for hyperparasitoid development. Olfactometer tests and behavioural observations indicated that searching hyperparasitoid females were not attracted by olfactory cues. However, they clearly reacted to host-related contact cues while searching on a plant. Here, honeydew was one of the principal contact cues used by female hyperparasitoids to locate hosts. Females discriminated between honeydew from an aphid host and that from a non-host, the soft brown scale, *Coccus hesperidum*, but made no difference between honeydew from healthy, unparasitised aphids, and those parasitised by *A. nigripes*.

This study indicates that several factors probably act simultaneaously on life history strategies. The simple classification of aphid hyperparasitoids as koinobiont endoparasitoids of parasitoid larvae in living aphids, or idiobiont ectoparasitoids of parasitoid pupae in mummified aphids does not explain all observed interspecific differences. Lineage specific effects must also be important, as the species belong to

different taxa. Finally, in many aspects, the life history parameters and behaviour of aphid hyperparastoids differ from those reported for primary aphid parasitoids.

Avant-Propos

Chapter 3 has been submitted for publication in Ecological Entomology: Buitenhuis, R., Boivin, G., Vet, L.E.M., and Brodeur, J. Preference and performance of the hyperparasitoid *Syrphophagus aphidivorus* (Hymenoptera: Encyrtidae): Fitness consequences of selecting hosts in live aphid vs. aphid mummy

Chapter 5 has been accepted for publication in the *Journal of Chemical Ecology*: Buitenhuis, R., McNeil, J.M., Boivin, G. and Brodeur, J., 2004. The role of honeydew in host searching of aphid hyperparasitoids.

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To Mark, for his courage and his love

The enemy of my enemy is my friend?

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Chapter 1.

General Introduction

1.1. Introduction

Arthropod herbivores are considered as pests in many agricultural systems. They damage, by feeding or as vectors of plant diseases, plants that are meant for human use or consumption. In the war against pests, humans have developed various methods to prevent herbivores from doing extensive damage. The simplest method was killing all life that threatened our crops with pesticides. This worked …, for a while. We rapidly discovered that nature is well equipped to counteract the most destructive chemicals. A few resistant individuals survived that could reproduce and build up new populations of damaging insects that have become resistant to pesticides. An alternative method of pest control is to exploit what already exists in nature: Almost all arthropod herbivores have natural enemies that can be used in what is known as 'biological control'. For this we need to understand the relationship between the plant, the herbivores and their natural enemies, as well as the factors that can influence these interactions.

1.2. Multitrophic interactions

Information about multitrophic level interactions can provide an essential foundation for designing effective biological control (Lewis *et al*., 1997), and for improving the efficacy and understanding the suppression of herbivore populations in biological control. In the last two decades, the study of trophic interactions between organisms has evolved from simple plant-herbivore or prey-predator interactions to a more complex approach involving three or more trophic levels. This complex approach addresses the complexity of food webs much more realistically (Tscharntke and Hawkins, 2002). Research on multitrophic interactions aims to identify the forces that regulate populations. In general, interactions between trophic levels can constitute bottom-up forces (controlled by resources) or top-down forces (controlled by predators and antagonists). However, many multitrophic interactions are more complex than the linear bottom-up or top-down interactions. For instance, food webs can be characterised in two dimensions: vertically, in which they form a food chain of rarely more than four or five trophic levels, or horizontally, i.e. within one trophic level (Hassel and Waage, 1984). Furthermore, ecological interactions between two species are often (indirectly) mediated by a third species of the same or another trophic

level (Bronstein and Barbosa, 2002). This is found for example in intra-guild interactions, where two species that share a host or prey also engage in a trophic interaction with each other (Rosenheim, *et al*., 1995; Rosenheim 1998), or in apparent competition, where two species, that do not come into direct contact, interact because they share a natural enemy (Holt and Lawton, 1993), adding complexity to the study of these food webs.

In insects, tritrophic interactions between plants, herbivores and their natural enemies are among the most studied multitrophic interactions (e.g. Turlings *et al*., 1990; Vet and Dicke, 1992; Vet *et al*., 1995; Lewis *et al*., 1997). In this food chain, the first trophic level, represented by the plant, influences the herbivore (the second trophic level) by its quality and quantity as a food-source. On the third trophic level, natural enemies limit herbivore populations by mortality. However, interactions between herbivores and their host plants and between herbivores and their natural enemies can only be understood when considered all together within a tritrophic context (Price *et al*., 1980). For instance, the first trophic level (plant) can also influence the efficiency of the third trophic level (natural enemy) by providing shelter, mediating host/prey accessibility and availability, providing host/prey finding cues, influencing host/prey suitability, and providing supplemental food sources for natural enemies (Cortesero *et al*., 2000). Vice-versa, natural enemies may 'help' the plants, using the benefits named above, thus limiting the herbivore population more than would be otherwise possible.

To further understand fluctuations in predator or parasitoid populations and the level of herbivore suppression, not only tritrophic interactions have to be examined, but also the impact of higher-level natural enemies. Predatory and parasitic insects are attacked by their own suite of predators, parasitoids and pathogens (Rosenheim, 1998), which constitute the fourth trophic level. The impact of these higher trophic levels on natural enemies of herbivores has received relatively little attention. They may exert a significant negative effect on plant-fitness by removing parasitoids or predators of the herbivores (top-down regulation) (Luck *et al*., 1981). But not only top-down effects are to be expected. It has been shown recently that bottom-up forces may also play a role in mediating interactions involving plants, herbivores, parasitoids and hyperparasitoids. Harvey *et al*. (2003) have

demonstrated that qualitative differences in herbivore diet can differently affect the performance of interacting organisms across four trophic levels.

1.3. Hyperparasitoids

Hyperparasitoids are also called secondary insect parasitoids as they develop at the expense of insect primary parasitoids (Sullivan and Völkl, 1999). In other words, a hyperparasitoid attacks another insect that is itself parasitic on a host insect, which is often a herbivore, and is therefore part of the fourth trophic level. The great majority of hyperparasitoids are members of the order Hymenoptera, a few species belonging to the Diptera and the Coleoptera (Gordh, 1981; Sullivan, 1987). There exist several types of hyperparasitism. Obligate hyperparasitoids can develop only in or on a primary parasitoid. On the other hand, facultative hyperparasitoids can develop as either primary or secondary parasitoids (Sullivan, 1987). The immature hyperparasitoid can in turn be attacked by a conspecific or another species of hyperparasitoid. This is called tertiary parasitism, or if this tertiary parasitoid is itself the host, quaternary parasitism. These types are rare, and Gordh (1981) hypothesised that tertiary and quaternary hyperparasitism is too precarious to evolve as an obligate trophic strategy, as depletion of host resources causes a significant decrease in the size of tertiary and quaternary parasitoids (Kfir and Rosen, 1981, cited in Brodeur, 2000), which makes these types of hyperparasitism less profitable. The last type of hyperparasitism is heteronomy. Heteronomous species (or adelphoparasites) produce females as primary parasitoids and males as hyperparasitoids, often on the females of the same species or other primary parasitoids (Gordh, 1981; Hunter and Woolley, 2001). These species are also called autoparasitoids

Like parasitoids, larvae of endophagous hyperparasitoids feed inside the host, whereas ectophagous species feed externally. Koinobiont hyperparasitoid species allow their host to continue development after oviposition, and idiobionts attack non-growing or non-feeding host stages and/orarrest the development of the host by paralysis of killing during oviposition (Sullivan, 1987).

Hyperparasitism has a wide taxonomic distribution among insects. However, none of the parasitoid families consists exclusively of hyperparasitoids, although within families

hyperparasitism may follow phylogenetic lines (Brodeur, 2000). This suggests that hyperparasitism has evolved independently several times in different taxa (Gordh, 1981). Obligate hyperparasitism could have evolved in at least two ways: a) via facultative hyperparasitism as an opportunistic trade-off to use herbivore or parasitoid hosts, and/or if the hyperparasitic species frequently encounters already parasitised hosts; or b) by a host shift where a primary parasitoid of one host becomes a secondary parasitoid of another species. This host transfer is facilitated if the usual primary and new secondary hosts share physiological and/or ecological attributes (Sullivan and Völkl, 1999). One of the reasons why hyperparasitism may have evolved in a multitrophic context might be in order to avoid the sequestration of plant toxins in the host. Compared to the herbivore, the primary parasitoid may be a less toxic resource, especially after voiding the meconium (Vet, pers. comm).

1.4. Influence of hyperparasitism on primary parasitoid populations

Interactions between hyperparasitoids and primary parasitoids have been primarily studied in biological control situations because these systems are often less complex than natural ecosystems and the economical value of the crops justifies research done on pest control. Traditionally, hyperparasitoids have been thought to have a negative effect on primary parasitoid populations. There are several ways in which hyperparasitoids can influence primary parasitoid populations: directly through mortality, or indirectly by changing the behaviour of parasitoids or herbivores.

Theoretically, if a large fraction of a parasitoid population is attacked by hyperparasitoids, an increase in the herbivore's equilibrium density should be expected. If that fraction becomes large, the herbivore population may escape control by the primary parasitoid entirely (Luck *et al*., 1981). Mathematical models have given variable results. The majority of models predict an increase of the herbivore density (May and Hassell, 1981; Briggs, 1993). On the other hand, Beddington and Hammond (1977) predicted that in a stable host - primary parasitoid - hyperparasitoid system, hyperparasitism weakens biological control, but when the system is unstable, the presence of a hyperparasitoid may dampen the

oscillations and may enable a stable three-species equilibrium to be attained. This may benefit biological control, altering the system from one in which the pest exhibits periodic outbreaks to one of continuous sub-economic densities (Luck *et al*., 1981). It is not known how realistic these models are because of a lack of information on the biology and behaviour of hyperparasitoids. It is often assumed that hyperparasitoids and primary parasitoids have similar life histories and information about primary parasitoids is extrapolated to the next trophic level.

In the literature, high levels of hyperparasitism have often been reported. In an agro-ecosystem, the mortality of parasitoids due to hyperparasitism can even reach 100% (Höller *et al*., 1993). It is often assumed that as hyperparasitism increases, the greater the negative impact on herbivore control by primary parasitoids. Indeed, in several studies the low level of biological control by parasitoids has been repeatedly attributed to the high level of hyperparasitism (e.g. Burton and Starks, 1977; Bourchier and Nealis, 1992). However, in other cases, hyperparasitoids had little or no influence on biological control, even when the level of hyperparasitism was high (Farrell and Stufkens, 1990; Agricola and Fischer, 1991; van den Bosch *et al*., 1979; (Walker and Cameron, 1981; Wilson and Swincer, 1984; Hughes *et al*., 1987 cited in Mackauer and Völkl, 1993)). These differences have been explained based on the timing of hyperparasitoid attack during the season and synchronisation between primary parasitoid and hyperparasitoid. In conclusion, these studies have produced little definitive evidence regarding the impact of hyperparasitism on the regulating capacity of parasitoids in biological control. Luck *et al*. (1981) emphasised that percent mortality is not necessarily a good measure of a mortality's importance without knowing the levels of other sources of mortality, and the interactions between different sources of mortality.

Finally, experimental investigations (Burton and Starks, 1977; Shi, 1986 cited in Rosenheim, 1998) Goergen and Neuenschwander, 1992) led to the conclusion that hyperparasitoids disrupt the short-term regulation of herbivore hosts by primary parasitoids. The longer-term, multi-generation experiments needed to test the prediction that

hyperparasitoids stabilise the herbivore-parasitoid interaction have not been conducted (Rosenheim, 1998).

In addition to direct mortality, hyperparasitoids may have indirect effects on parasitoid populations. Höller *et al*. (1993) and Mackauer and Völkl (1993) state that hyperparasitoids can also influence biological control of herbivores indirectly by modifying the behaviour of primary parasitoids. It was demonstrated that when hyperparasitoids are present, primary parasitoid females could abandon patches of their herbivore host without having exploited the resource completely, to minimise the mortality risks of their progeny (Ayal and Green, 1993; Höller *et al*., 1993, 1994; Mackauer and Völkl, 1993; Weisser *et al*., 1994; Petersen, 2000). However, Völkl *et al*. (1995) found no evidence of an effect of adult hyperparasitoids on foraging behaviour or resource exploitation patterns of primary parasitoids within an aphid colony. Finally, hyperparasitoids might influence the herbivore. Boenish *et al*. (1997) and van Veen *et al*. (2001) demonstrated that the presence of females of different species of hyperparasitoids stimulated the reproduction of the aphids *Sitobion avenae* and *Acyrthosiphon pisum*, indicating some kind of communication between herbivores and hyperparasitoids. Increased reproduction of aphids in the presence of hyperparasitoids may be advantageous as their descendants will be less likely to be parasitised, especially if parasitoid wasps currently in the vicinity respond to incoming hyperparasitoids by dispersing away (van Veen *et al*., 2001)

Regardless of the impact of a hyperparasitoid on a parasitoid population, a hyperparasitoid cannot affect biological control if biological control does not exist in the first place (Luck *et al*., 1981). Therefore, the impact of primary parasitoids on herbivores in the absence of hyperparasitoids should be studied first. For example, Mackauer and Völkl (1993) suggested that the degree of aphid colony exploitation primarily results from the wasp's foraging efficiency and oviposition decisions, instead of hyperparasitism. Furthermore, aestivation, a high mortality due to other factors than hyperparasitism (e.g intra-guild predation) and dispersal can also result in low levels of primary parasitoid abundance (Höller *et al*., 1993).

In summary, our knowledge of the impact of hyperparasitism on primary parasitoid populations is limited and very fragmented. I agree with the conclusion of Rosenheim (1998), that limited experimental evidence supports the idea that hyperparasitism significantly disrupts the short-term regulation of herbivorous host populations by parasitoids, but critical multi-generation studies have yet to be conducted to assess the long-term effects. Moreover, accurate knowledge of the natural history of some important groups of hyperparasitoids is a prerequisite for improving our understanding of their origin, distinctive biological attributes, and role in community structure (Brodeur, 2000). However, major gaps exist in our knowledge of the mode of development (koinobiont or idiobiont), life-table characteristics, searching behaviour and competitive ability of hyperparasitoids.

1.5. Studied species

For this study, aphid hyperparasitoids were chosen as a model because they are the best known group of hyperparasitoids in terms of taxonomy, host associations, mode of development, behaviour and impact on primary parasitoid populations (Sullivan, 1987; Mackauer and Völkl, 1993). The potato – potato aphid – *Aphidius nigripes* system was used with four aphid hyperparasitoids: *Alloxysta victrix*, *Asaphes suspensus*, *Dendrocerus carpenteri* and *Syrphophagus aphidivorus*.

1.5.1. Host plant and aphid

The potato, *Solanum tuberosum* L. var. Norland was used as the host plant to rear the potato aphid, *Macrosiphum euphorbiae* (Thomas). This polyphagous aphid is a serious pest on potato crops and damages the plant directly by diverting photosynthate and indirectly by acting as plant virus disease vector. Also, the honeydew that is excreted as a waste product of aphid feeding causes mold to grow on the leaves (Shands *et al*., 1965; Lange and Bronson, 1981; Radcliffe, 1982).

Aphids live in colonies in shaded areas on leaves, stems and blossoms of plants. In nature, the potato aphid reproduces asexually during the summer, giving birth to female nymphs. At the end of summer, sexual forms are produced and aphids overwinter in the egg stage. In the laboratory potato aphid colonies are easily maintained asexually on potato plants.

Adults are usually without wings under controlled conditions. Winged adults develop in response to high population densities, decline of host plant quality and changes in environmental conditions (MacGillivray and Anderson, 1964).

1.5.2. Primary aphid parasitoid

The primary parasitoid *Aphidius nigripes* Ashmead belongs to the subfamily Aphidiinae (Hymenoptera: Braconidae). It is the dominant parasitoid of the potato aphid in North America (Walker *et al*., 1984). It is a solitary species and the females attack the aphid in the nymphal or adult stage (Cloutier *et al*, 1981). As it has a koinobiont development, the parasitised aphid continues to live and grow or reproduce, although reproduction is often diminished or repressed by aphidiine wasps (Stary, 1988). The larval instars feed on the aphid, destroying the remaining tissues and ultimately killing the host. Before completing its development, the parasitoid larva spins a cocoon inside or under the empty aphid skin. At this stage, the aphid skin becomes indurate and the typical "mummy" is formed (Stary, 1988). The pupal stage of the parasitoid develops within the mummy.

1.5.3. Aphid hyperparasitoids

The hyperparasitoids that are used in this study (Figure 1.1) were chosen as representatives of the four principal aphid hyperparasitoid families: *Asaphes suspensu*s Walker (Pteromalidae), *Dendrocerus carpenteri* (Curtis) (Megaspilidae), *Alloxysta victrix* (Westwood) (Alloxystidae), and *Syrphophagus aphidivorus* (Mayr) (Encyrtidae). These species were chosen because while they all naturally exploit *Aphidius* spp., they possess different biological attributes and host ranges, which are described in detail in the sections below. All are found attacking hosts in the chosen model system of potato, *M. euphorbiae*, *A. nigripes* (Shands, 1965; Brodeur and McNeil, 1994). I refer to Sullivan (1987) and Sullivan and Völkl (1999) for a more complete description of the biology of aphid hyperparasitoids and to Brodeur (2000) for a discussion on their host range.

1.5.3.1. Development mode

Aphid hyperparasitoids can be divided according to their development mode: *A. victrix* and *S. aphidivorus* are koinobionts, which means that after oviposition their living host continues its development (Sullivan, 1987). In contrast, *D. carpenteri* and *A. suspensus* are

idiobionts. During oviposition they kill or paralyse their host which stops its development (Bocchino and Sullivan, 1981; Höller *et al*., 1994). Koinobiosis is associated with endoparasitism (the egg is placed inside the host) and idiobiosis with ectoparasitism (the egg is placed on the surface of the host).

1.5.3.2. Host stages

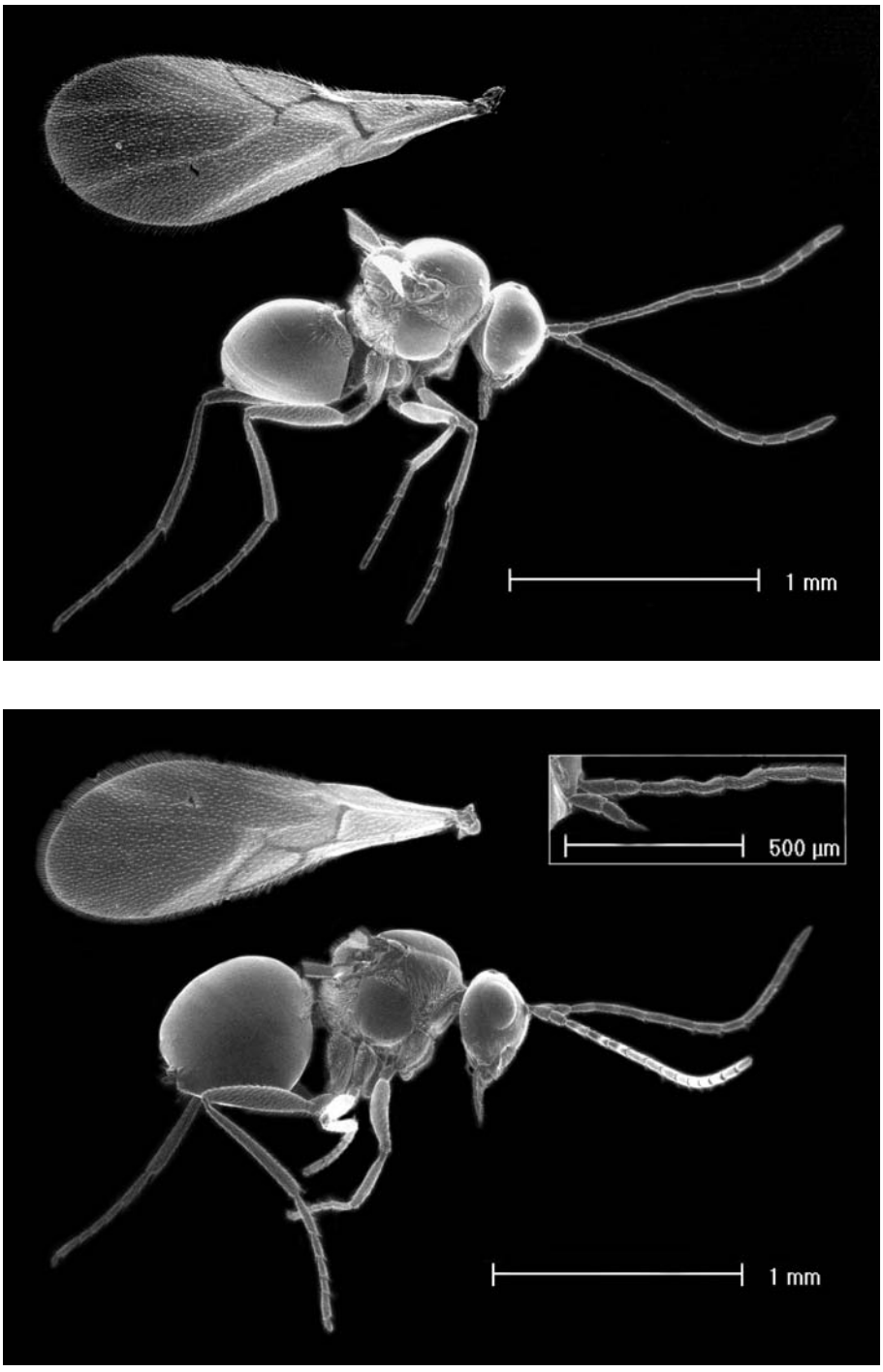
Aphid hyperparasitoids can attack the immature parasitoid within the aphid at different stages of its development. Mummy hyperparasitoids, such as *D. carpenteri* and *A. suspensus*, attack the parasitoid prepupa or pupa after it has killed the aphid and the mummy is formed (hereafter called aphid mummy) (Sullivan, 1987). Hyperparasitoids, such as *A. victrix*, attack parasitoid larva in live aphids before mummification (hereafter called parasitised aphid). *Alloxysta victrix* does not use a venom to paralyse its host, so after parasitisation the primary parasitoid larva continues to feed and grow. It is only after the aphid is mummified by the primary parasitoid larva, that the egg hatches and the hyperparasitoid larva starts to feed endophagously until it kills and completely consumes the host (Gutierrez and van den Bosch, 1970; Sullivan, 1987). Although *Syrphophagus aphidivorus* is an endophagous koinobiont hyperparasitoid like *A. victrix*, it has a dual oviposition behaviour that is atypical for aphid hyperparasitoids. It has the capacity to attack both parasitoid larva in a live aphid or parasitoid prepupa or pupa in a mummified aphid. Furthermore, it prefers to oviposit in mummified aphids (Kanuck and Sullivan, 1993), which is not found in other endophagous koinobiont hyperparasitoids of aphids.

1.5.3.3. Host range

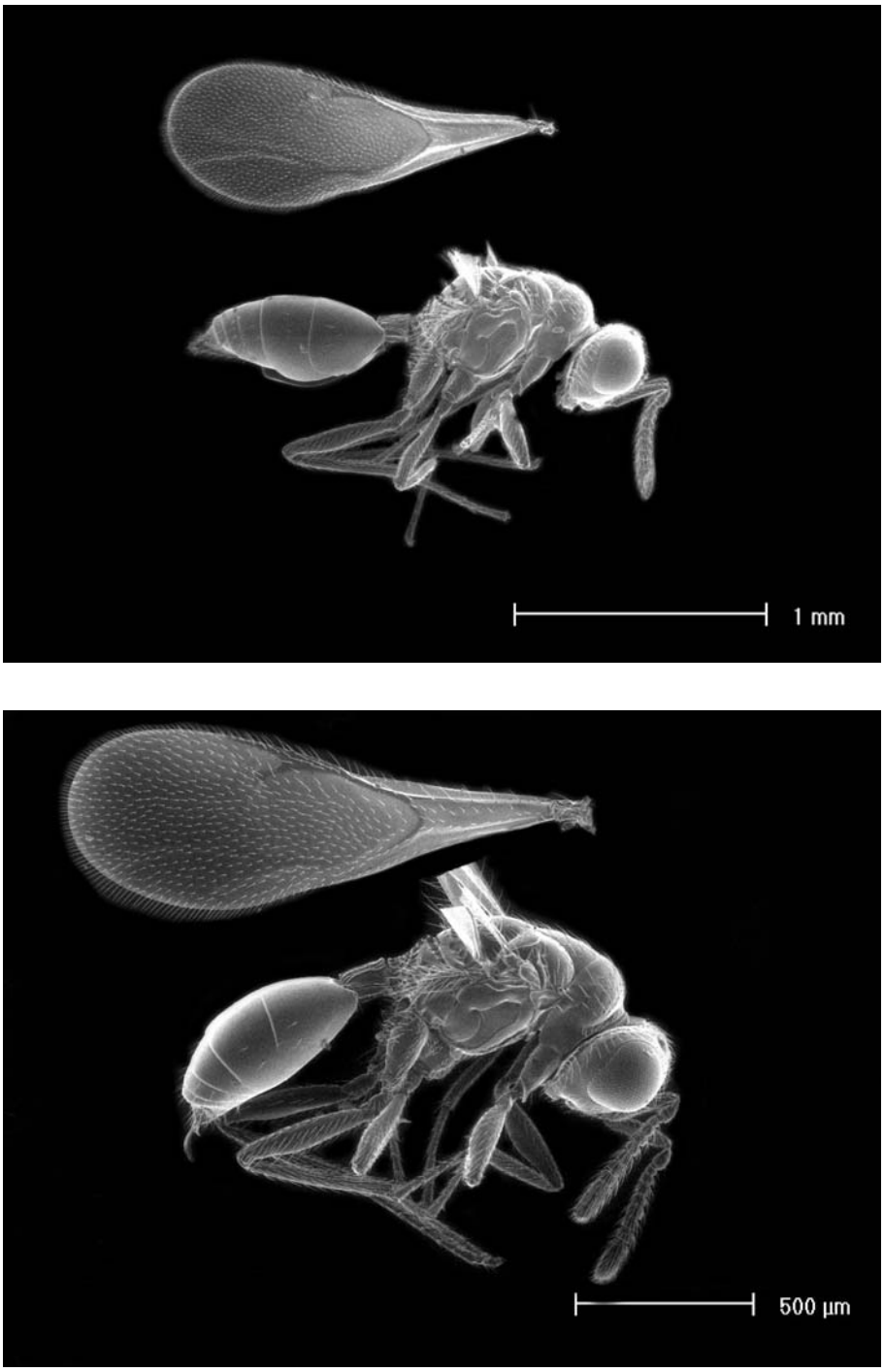
There are differences in host range between the four selected aphid hyperparasitoid species. *A. victrix* is considered to have the most restricted host range. Its potential host range includes several aphidiine parasitoid species (Höller *et al*., 1993), and some authors include aphelinid species (Andrews, 1978; Grasswitz and Reese, 1998), but *A. victrix* seems to prefer Aphidiinae (Gutierrez and van den Bosch, 1970; Andrews, 1978). *Dendrocerus carpenteri* and *A. suspensus* are generalist hyperparasitoids of four to five genera of aphidiine and aphelinid primary parasitoids (Fergusson, 1980; Sullivan, 1987; Höller *et al*., 1993; Chow and Mackauer, 1999). They can also be tertiary parasitoids of their own species (Bennet and Sullivan, 1978; Levene and Sullivan, 1983) or of other aphid

hyperparasitoids (Matejko and Sullivan, 1984; Carew and Sullivan, 1993). *Syrphophagus aphidivorus* attacks at least four genera of aphidiine and aphelinid primary parasitoids (Hoffer and Stary, 1970; Sullivan and van den Bosch, 1971; Mertins, 1985; Völkl and Barczak, 1990).

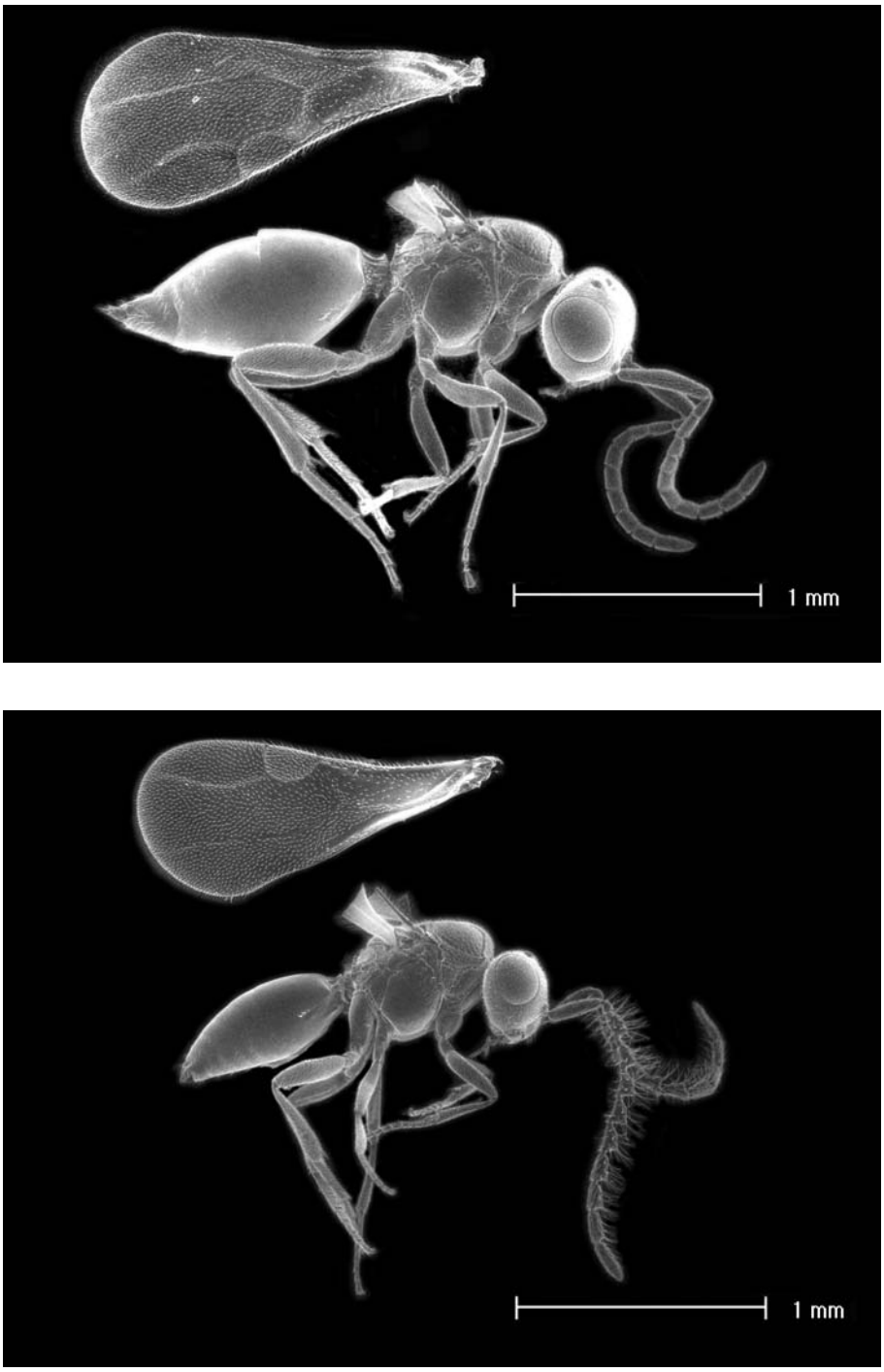
Figure 1-1 Scanning electron microscope pictures of the hyperparasitoids that are studied in this thesis. A: *Alloxysta victrix*; B: *Asaphes suspensus*; C: *Dendrocerus carpenteri*; D: *Syrphophagus aphidivorus*. Upper panel female, lower panel male.



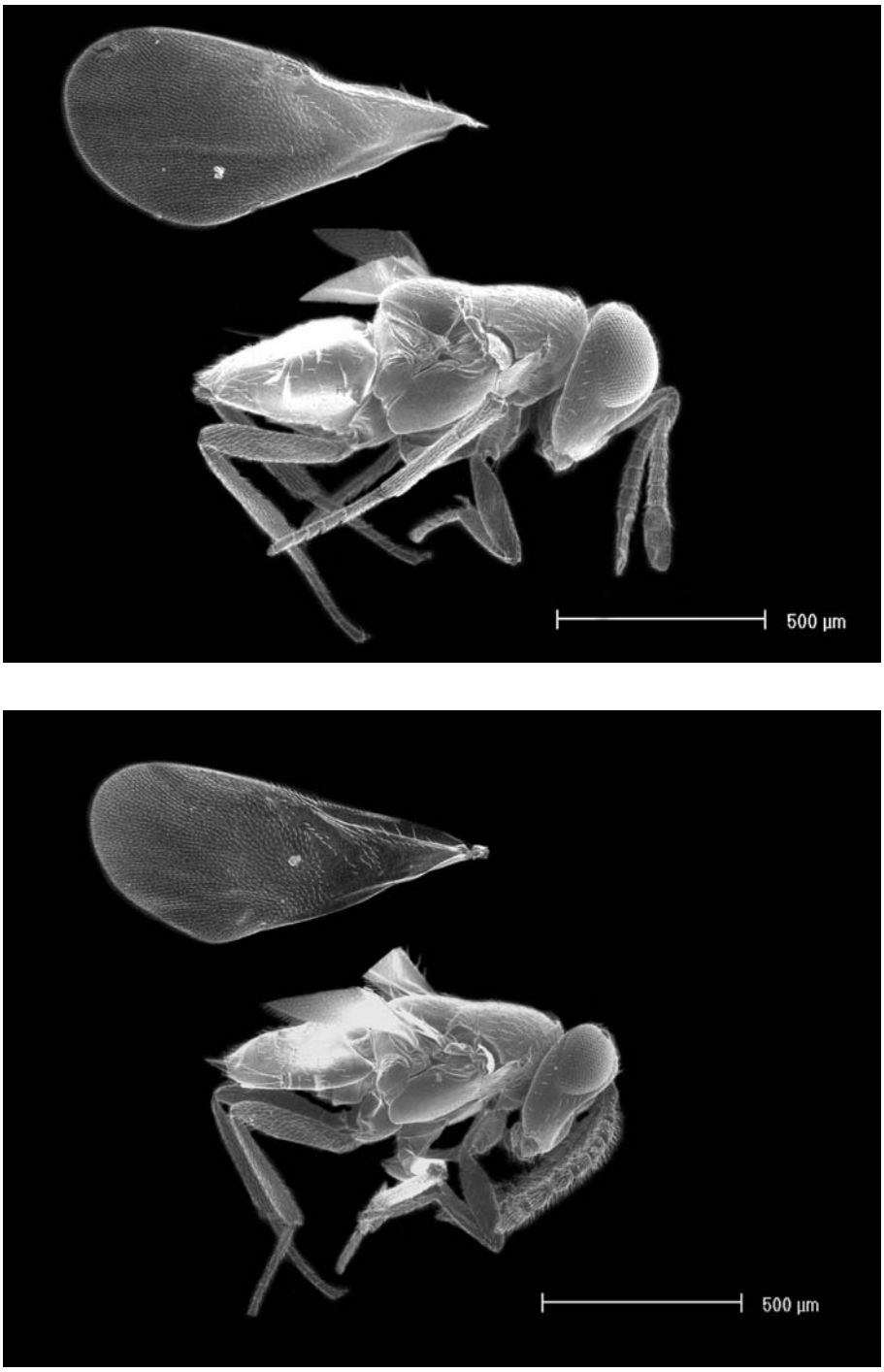
A. *Alloxysta victrix*, upper panel female, lower panel male.



B. *Asaphes suspensus*, upper panel female, lower panel male.



C. *Dendrocerus carpenteri*, upper panel female, lower panel male.



D. *Syrphophagus aphidivorus*, upper panel female, lower panel male.

1.6. General predictions

This thesis aims to a better understanding of hyperparasitoid biology and behaviour as a contribution to unravel the nature of parasitoid - hyperparasitoid interactions. The objective of this thesis is twofold, first to study the life history of hyperparasitoids and second to determine the stimuli that hyperparasitoids use in host location.

In parasitoids, development mode (koinobiont or idiobiont) has been emphasized as a major potential determinant of life histories (for a review, see Godfray, 1994; Quicke, 1997; Mayhew and Blackburn, 1999; Strand, 2000; Harvey and Strand, 2002). The dichotomous hypothesis states that natural selection operates on the life history strategies of these two categories of parasitoids to magnify their differences (Godfray, 1994). Koinobiont endoparasitoids allow their host to continue development. Therefore they are able to attack small hosts that have less efficient defenses against parasitism. Moreover, younger hosts are generally more abundant than the later stages (Price, 1974). However, many koinobionts are able to attack hosts ranging in size from a fraction of that of the ovipositing female wasp to many times her size at oviposition (Harvey and Strand, 2002). In order to obtain sufficient resources to complete development in nutritonally suboptimal (=small) hosts, koinobionts may have to greatly reduce the rate of growth, resulting in an extended development time. Furthermore, because young hosts often suffer high mortality, the balanced mortality hypothesis predicts a high fecundity (Price, 1974). This high fecundity can be achieved by reducing egg size, which is possible because eggs are laid in the host haemolymph and therefore require less yolk as sufficient proteins for oogenesis are uptaken from the host. The eggs and larvae of endoparasitoids will also need to cope with the immune system of the host and engage in subtile synchronisation with the living host, which causes many endoparasitic species to have a relatively narrow host range. Idiobiont ectoparasitoids have an oposite set of life history traits. After parasitisation the development of the host is usually stopped, meaning that idiobionts must attack more mature stages of hosts that are larger. Therefore, the development time of idiobiont parasitoids is predicted to be generally less than that shown by koinobiont . Idiobiont ectoparasitoids that develop externally on their host require large, yolky eggs which tends to reduce fecundity. Furthermore, they do not have to cope with the immune system of the

host, so that many species have comparatively large host ranges. Due to the innumerbale trade-offs and relationhipss between life history parameters, the above descriptions are only an impression of how development mode is observed to influence life history, and many causal relationships between parameters remain to be studied. However, observations on the life history data of 474 parasitoid Hymenoptera support the dichotomous hypothesis (Mayhew and Blackburn, 1999).

Although the same dichotomy (idiobiont – koinobiont) is also found in hyperparasitoids, it is not known if this is correlated to the same sets of life history traits as found in primary parasitoids. Because hyperparasitism evolved from parasitism (Gordh, 1981) and based on the many similarities between primary and secondary parasitoids, I predict that:

1. Similar to primary parasitoids, the life history parameters of hyperparasitoids are determined by development mode following the predictions of the dichotomous hypothesis.

Life history parameters are also influenced by the profitability of the host, for example the nutritional quality. Most aphid hyperparasitoid species attack either a host in the living aphid before mummification or in the aphid mummy. For them, the profitability of the host may vary between different parasitoid host species. In the case of *S. aphidivorus* however, a female can attack both parasitoid larvae in live aphids and parasitoid (pre-)pupae in aphid mummies, two very different stages of the same host species. It is unknown if these two host stages differ in profitability for the offspring of *S. aphidivorus*, but females seem to have a preference for the mummy host (Kanuck and Sullivan, 1992). Theoretical models predict that ovipositional decisions of parasitoid females should lead to the selection of the most profitable host for parasitoid development. Therefore the following prediction was formulated:

2. Female *S. aphidivorus* have a preference for pupae of primary parasitoids within aphid mummies, because these are the most profitable hosts for offspring fitness.

Both parasitoids and hyperparasitoids have to search for hosts to reproduce. Most searching strategies involve the use of cues (for example chemical, visual or tactile cues). In parasitoids, it has been shown that females zoom in from long to short distance cues, thereby slowly confining their search area, shifting from long range cues to short range cues. Within this gradual transition, we usually observe a shift from indirect, often unreliable cues, such as plant cues, to more direct, reliable cues, such as contact chemicals directly derived from the host itself. The resulting intensified search of the restricted area where any cue is perceived enhances the chance of locating the host (Vet *et al.*, 2002). The use of cues varies according to the host-specificity of the parasitoid. There is a continuum from intense and specific use of cues in specialists, to the absence of cue use in extreme generalistic species (Vet and Dicke, 1992). To find their host, hyperparasitoids potentially have many cues at their disposal from all trophic levels. However, we have little insight in which cues are actually being used by hyperparasitoids. Alloxystine aphid hyperparasitoids have narrower host ranges than the Pteromalidae, Megaspilidae (Brodeur, 2000) or Encyrtidae (Hoffer and Stary, 1970). It is therefore expected that they will differ in host searching strategies:

3. The relatively host specific alloxystid hyperparasitoid species will use general cues associated with aphids and specific cues from primary parasitoid females and/or host plant volatiles associated with their plant – aphid - host system. Ecto-hyperparasitoids with a broader host range than koinobionts will depend less on specific cues, and use only general cues associated with aphids and aphid mummies on different plant – aphid – host systems. The species with the dual oviposition behaviour, *S. aphidivorus*, is predicted to resemble the ecto-hyperparasitoids because of its broad host range and its preference for mummies.

One of the cues that aphid hyperparasitoids may use in host searching is aphid honeydew (Budenberg, 1990; Grasswitz, 1998). The composition of honeydew can vary with various factors, among which are aphid species (Hendrix *et al.*, 1992; Völkl *et al.*, 1999; Fisher and Shingleton, 2001) and parasitism of the aphid by braconid wasps (Cloutier and Mackauer 1979, Cloutier 1986, Rahbé *et al.*, 2002). Therefore, honeydew could be a direct and

reliable cue for hyperparasitoids if females have the capacity to discriminate between the different chemical compositions of honeydew. I predict that:

4. Foraging aphid hyperparasitoid females not only have the ability to detect honeydew but also show a preference for honeydew from aphid rather than non-aphid species and, more specifically, for honeydew from parasitised vs. unparasitised aphids.

Although life history and host searching behaviour have already been studied to some extent in a few hyperparasitoid species, much of this information is fragmented or anecdotal and most of the emphasis has been put on working out the complex biology of individual species (Hawkins, 1994). Species are difficult to compare because data on different species often originates from different herbivore-primary parasitoid systems which can vary in quality, suitability and potential cues for host location. Furthermore, hyperparasitoids show a large interspecific variation in development mode, host stage and host range. Due to these facts, no firm conclusions on life history and host location of hyperparasitoids can be drawn based on literature data.

Contrary to previous studies, I have chosen an interspecific comparative approach. Comparative evidence brings generality, suggests hypotheses and places inter-specific patterns into context (Stearns, 1992). I used four hyperparasitoid species from the principal families that contain aphid hyperparasitoids, and reared them on the same aphid-primary parasitoid system. This made it possible to directly compare the results of different species and to find general patterns of the influence of development mode, host stage and host range on life history traits and host location behaviour. I expect to find that:

5. There are differences in life history and host location behaviour between hyperparasitoid species due to differences in development mode, host stage or host range.

In addition, the results are compared to similar data on primary parasitoids. In contrast to hyperparasitoids, hymenopteran primary parasitoids have been studied extensively (e.g

Godfray, 1994; Quicke, 1997). It is an intriguing question how much of the theory on primary parasitoids can be applied to hyperparasitoids. Although the degree of similarity between primary and secondary parasitoids is obvious because of their common evolutionary origins and life-history strategies, hyperparasitoids are likely to possess specific biological attributes enabling them to exploit resources from the third trophic level (Brodeur, 2000). It is therefore predicted that:

6. Hyperparasitoids have developed specific biological attributes enabling them to exploit resources from the third trophic level as compared to primary parasitoids.

1.7. Objectives

In order to test the above predictions on the life history and host searching behaviour of aphid hyperparasitoids, the following specific objectives were formulated:

Life history of aphid hyperparasitoids:

1 Measure the life history characteristics of the four aphid hyperparasitoid species that differ in development mode (two koinobiont endohyperparasitoids and two idiobiont ectohyperparasitoids) on the same plant-aphid-primary parasitoid system and determine the influence of development mode on life history traits (dichotomous hypothesis).

2 Investigate the dual oviposition behaviour of the aphid hyperparasitoid species *Syrphophagus aphidivorus*. Determine the profitability of parasitoid larvae in live aphids and parasitoid pupae in aphid mummies, and relate to the preference of the females for each host.

Host searching behaviour of aphid hyperparasitoids:

3 Examine the use of cues (both airborne volatile, and contact cues on a plant) from different trophic levels in host search of four aphid hyperparasitoids. Host range is a potential determinant in the foraging behaviour of insects (Vet and Dicke, 1992). As the studied hyperparasitoids differ in host range, the influence of host range on the use of cues is determined.

4 Determine the role of honeydew cues in host search of four aphid hyperparasitoids.

1.8. Description of the chapters

In chapter 2, the life history traits of four aphid hyperparasitoids are measured in the laboratory. It is investigated if the predicted dichotomy in life history traits between koinobiont and idiobiont applies to these hyperparasitoids, similar to what was found in primary parasitoids (Mayhew and Blackburn, 1999). Furthermore, the influence of other ecological factors, like host stage and host range, on life history traits is determined.

Chapter 3 aims to elucidate the dual oviposition behaviour of the encyrtid hyperparasitoid *Syrphophagus aphidivorus*. Female preference for either a parasitoid larva in the live aphid or a pupa in an aphid mummy is reinvestigated and correlated with the fitness of the offspring.

The host location behaviour in four species of aphid hyperparasitoids is studied in chapter 4. The influence of volatile and contact infochemicals from all trophic levels was tested in an olfactometer and while a female was searching on a plant, respectively. The influence of host stage, host range and mode of development are discussed and the results are compared to primary parasitoids.

In chapter 5, research is focussed on the role of one of the cues in chapter 3, honeydew, in host search of the four species of aphid hyperparasitoids. It is tested if female hyperparasitoids can distinguish between aphid and non-aphid honeydew, as only aphids may contain hosts. Furthermore it is tested if they can distinguish if honeydew comes from healthy unparasitised aphids, or from parasitised aphids hosting a suitable host. The response of the four species of hyperparasitoids is compared with respect to their respective biological attributes.

1.9. References

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Chapter 2.

Life history variation in aphid hyperparasitoids:

Is development mode a major determinant?

2.1. Abstract

In primary parasitoids, development mode has been identified as a major determinant of life histories. The dichotomous hypothesis contrasts the life history traits of koinobiont endoparasitoids with those of idiobiont ectoparasitoids. In this study we examined if the dichotomous hypothesis can also be applied to hyperparasitoids, or if the hyperparasitic strategy demands different adaptations that will confound the dichotomous hypothesis. We compared life history parameters of two koinobiont endoparasitic species (*Alloxysta victrix* (Westwood) and *Syrphophagus aphidivorus* (Mayr)) and two idiobiont ectoparasitic species (*Asaphes suspensus* (Nees) and *Dendrocerus carpenteri* (Curtis)) of aphid hyperparasitoids from four different families that attack either the parasitoid larva in the aphid before it is killed and mummified by the primary parasitoid, or the parasitoid prepupa or pupa in the dead aphid mummy.

The variation in life history traits in aphid hyperparasitoids cannot be explained by development mode alone. The data for the idiobiont ectohyperparasitoids mostly confirm the dichotomous hypothesis. The koinobiont endohyperparasitoids, however, have a long adult lifespan and a low fecundity, contrary to the predictions of the dichotomous hypothesis. These traits are best explained by synovigeny in these species. It is likely that several factors, including development mode, timing of egg production, host range and host stage, act together and are selected to optimise fitness. In addition, lineage specific effects might also determine life history traits.

Keywords: parasitoid, dichotomous hypothesis, koinobiont, idiobiont, host range, host stage, phylogeny.

2.2. Résumé

Chez les parasitoïdes primaires, le mode de développement à été reconnu comme facteur déterminant de l'histoire de vie. L'hypothèse de la dichotomie contraste les caractéristiques de l'histoire de vie des endoparasitoïdes koinobiontes et des ectoparasitoïdes idiobiontes. Dans cette étude nous avons examiné si l'hypothèse de dichotomie peut aussi être appliquée aux hyperparasitoïdes ou si l'hyperparasitisme exige des adaptations qui la confondent. Nous avons comparé les paramètres de l'histoire de vie de deux endo-hyperparasitoïdes koinobiontes (*Alloxysta victrix* (Westwood) et *Syrphophagus aphidivorus* (Mayr)) et deux ecto-hyperparasitoïdes idiobiontes (*Asaphes suspensus* (Nees) et *Dendrocerus carpenteri* (Curtis)), provenant de quatre familles différentes. Ces espèces attaquent soit la larve du parasitoïde dans le puceron avant qu'il soit tué, soit la prépupe ou la pupe du parasitoïde dans la momie de puceron.

La variation observée des caractéristiques d'histoire de vie des hyperparasitoïdes de puceron n'a pu être expliquée par le mode de développement seul. Les résultats pour les ecto-hyperparasitoïdes idiobiontes étaient en accord avec l'hypothèse de dichotomie pour la plupart des paramètres. Par contre, les endo-hyperparasitoïdes koinobiontes avaient une vie adulte plus longue et une fécondité plus basse, que prédit par l'hypothèse. Ces caractéristiques sont mieux expliquées par la synovigénie chez ces espèces. Il est probable que divers facteurs, incluant le mode de développement, la précocité de l'ovogénèse, la spécificité parasitaire et le stade d'hôte, agissent ensemble et sont sélectionnés pour optimiser le fitness. En plus, des effets d'ordre phylogénétique peuvent aussi déterminer les caractéristiques d'histoire de vie.

2.3. Introduction

Life histories across a range of organisms have been studied extensively to identify what determines the values of life history traits (Stearns, 1992; Roff, 1992). Within the parasitic Hymenoptera, development mode (koinobiont or idiobiont) was proposed as the major organiser of life history patterns in parasitoids (reviewed by Godfray, 1994; Quicke, 1997; Mayhew and Blackburn, 1999; Strand, 2000). Koinobiont parasitoids allow further development of the host after parasitism, and are mostly endoparasitic. This enables them to attack exposed, early instar hosts that have less efficient defences against parasitism.Koinobiosis implies a slow or delayed larval development, small eggs and high fecundity, and a short adult lifespan. Idiobiont parasitoids attack non-growing host stages such as eggs or pupae or arrest host development via the injection of venoms or other biochemical factors preceding oviposition. It is to their advantage to attack large, mature host stages as their host represents a fixed amount of resources. Therefore, idiobiont parasitoids are expected to possess a different (and potentially opposite) set of life history characteristics when compared to koinobionts. The dichotomous hypothesis states that natural selection operates on the life history strategies of these two categories of parasitoids to magnify the differences (Godfray, 1994). The first test of the dichotomous hypothesis was performed by Mayhew and Blackburn (1999) on life history data of 474 species of parasitoid Hymenoptera. Only partial support for the dichotomy hypothesis was found, as life history traits were not all correlated to development mode. Still, they concluded that the support was sufficient to retain development mode as the cental element of any comprehensive theory of parasitoid life histories, especially for parasitoids of larval hosts. Development mode probably reflects the evolutionary history of parasitoid lineages while other parameters, like fecundity, are not as strongly correlated to life history traits (Mayhew and Blackburn, 1999). Other factors have been proposed to explain the same variation in life history of parasitoids, such as the degree of pro-ovigeny or synovigeny (Jervis *et al*., 2001). These factors appear to be linked to parasitoid development mode so the effects of development mode and ovigeny are potentially difficult to separate.

Hyperparasitoids (or secondary parasitoids) parasitise the immature stages of primary parasitoids and therefore belong to the fourth trophic level in many ecosystems.

Hyperparasitism has a wide taxonomic distribution, suggesting diverse evolutionary origins (Gordh, 1981). Among the Hymenoptera, hyperparasitism occurs in 7 of 11 parasitoid superfamilies. It is mostly found in the superfamilies Ceraphronoidea, Chalcidoidea, Ichneumonoidea, and Trigonalyoidea (Brodeur, 2000). Because of their common evolutionary origins, hymenopterous parasitoids and hyperparasitoids share many biological characteristics. Like parasitoids, ectoparasitism is generally associated with idiobiont development and endoparasitic hyperparasitoids are mostly koinobiont. Therefore these developmental traits might also be used to explore interspecific variation in life history traits in hyperparasitoids. In this study, we examined if the dichotomous hypothesis can also be applied to hyperparasitoids, or if being a hyperparasitoid demands specific adaptations that confound the dichotomous hypothesis. Based on the great similarities in development mode between primary and secondary parasitoids, we predicted that we will find the same dichotomy in life history parameters according to development mode in hyperparasitoids.

The most intensive studies of hyperparasitism have been conducted on the Hymenoptera that attack immature parasitoids developing in Homopteran hosts. Aphid hyperparasitoids are the best known group of hyperparasitoids in terms of taxonomy, host association, development mode, behaviour and impact on primary parasitoid populations (Sullivan, 1987, Mackauer and Völkl, 1993). However, a detailed and accurate comparison among species to determine the influence of development mode on life history variation is not yet possible. Much of the published information is incomplete or anecdotal and often originates from different aphid-primary parasitoid systems whose host and host plant species can vary in suitability. Therefore, in our experiments, we adopted a comparative approach in which we compared life history traits of four different aphid hyperparasitoids reared on the same primary parasitoid host species. This permits us to generalise, suggests hypotheses and places intra-specific patterns into context (Stearns, 1992). We chose one species from each Hymenoptera family that contains aphid hyperparasitoids, with the exception of the Eulophidae (Table 2-1): Two idiobiont ecto-hyperparasitoids, *Asaphes suspensus* (Pteromalidae) and *Dendrocerus carpenteri* (Megaspilidae), and two koinobiont endo-

hyperparasitoids, *Alloxysta victrix* (Charipidae) and *Syrphophagus aphidivorus* (Encyrtidae).

Besides variations in development modes, these four species also differ in host range, from oligophages like *A. victrix* to generalists like *A. suspensus*, as described by Höller *et al.* (1993) (Table 2-1). Furthermore, they attack their immature host at different stages of development. Either they attack the parasitoid larva in the still living aphid, or they attack the parasitoid (pre-)pupa in the dead, mummified aphid (Table 2-1). These are ecological factors that have to be taken into account in the comparison because they also may influence life history traits, and may confound the results that are expected based on the dichotomous hypothesis.

We compared in the laboratory the most important life history parameters of the four hyperparasitoid species (survival, developmental time, size, longevity, fecundity, immature mortality and sex ratio) and the intrinsic rate of natural population increase (r_m) of each species. In the discussion, we also used data from the literature on related aphid hyperparasitoids.

2.3.1. Description of species

For this study the same potato – potato aphid – *Aphidius nigripes* system was used as the basic food web. The potato aphid (*Macrosiphum euphorbiae* Thomas) is a common pest on potatoes in North America (Shands, 1965; Radcliffe, 1982). These aphids live in colonies on leafs and stems. In nature, the potato aphid reproduces asexually during the summer, giving birth to female nymphs. At the end of summer, sexual forms are produced, which migrate to their primary host plant, roses, and aphids overwinter in the egg stage on these plants. In the laboratory the colonies are easily maintained asexually. The primary parasitoid *Aphidius nigripes* Ashmead (Hymenoptera: Braconidae) is the dominant parasitoid of the potato aphid in North America (Walker *et al*., 1984). It is a solitary koinobiont species and the females attack the aphid in the nymphal or adult stages (Cloutier *et al*, 2000). The parasitoid larval instars feed within the aphid, ultimately killing their host. Before completing its development, the mature larva (also called prepupa) spins a cocoon

inside the empty aphid cuticle and the typical aphid "mummy" is formed (Stary, 1988). The parasitoid pupa develops within the mummy.

All tested hyperparasitoid species are solitary, recorded from both Europe and North America, and are all found naturally attacking *A. nigripes* in the field (Shands, 1965; Brodeur and McNeil, 1994).

Asaphes suspensus (Nees) (Hymenoptera: Pteromalidae) is an idiobiont, ectophagous hyperparasitoid of aphidiine and aphelinid parasitoids, attacking the host (pre-)pupa after it has killed the aphid and the mummy is formed (Sullivan, 1987). It can be a tertiary parasitoid on its own species (Levene and Sullivan, 1983) or on other hyperparasitoids (Carew and Sullivan, 1993). The female *A. suspensus* envenoms its host, which deteriorates to a blackened mass on which the hyperparasitoid larva feeds (Bocchino and Sullivan, 1981). Hosts can also be used for destructive host feeding, for which the female constructs a feeding tube to feed on the host heamolymph (Levine and Sullivan, 1983; Christiansen-Weniger, 1992).

Similar to *A. suspensus*, *Dendrocerus carpenteri* (Curtis) (Hymenoptera: Megaspididae) is a solitary, idiobiont, ectophagous hyperparasitoid of aphidiine and aphelinid wasps inside mummified aphids (Fergusson, 1980; Sullivan, 1987; Chow and Mackauer, 1999). It can also be a tertiary parasitoid its own species (Bennet and Sullivan, 1978) or other hyperparasitoids (Matejko and Sullivan, 1984). Females inject their host with juvenile hormone (Höller *et al*., 1994), which arrests its development (Bocchino and Sullivan, 1981). The larva feeds externally. No host feeding is reported for this species.

Alloxysta victrix (Westwood) (Hymenoptera: Charipidae) is a koinobiont endophagous hyperparasitoid that attacks parasitoid larvae in aphids before mummification (Sullivan, 1987; Gutierrez and van den Bosch, 1970). It is only after the aphid is mummified by the primary parasitoid larva, that the hyperparasitoid egg hatches and the larva starts to feed endophagously until it kills and completely consumes the host (Sullivan, 1987). The potential host range is broad (Grasswitz and Reese, 1998), but *A. victrix* seems to prefer Aphidiinae (Gutierrez and van den Bosch, 1970; Andrews, 1978).

Syrphophagus aphidivorus (Mayr) (Hymenoptera: Encyrtidae) is also a koinobiont, endophagous hyperparasitoid. But compared to other aphid hyperparasitoids this species is atypical because it has a dual host-stage relationship and oviposition behaviour. The female hyperparasitoid attacks either the primary parasitoid larva in an aphid when the aphid is still alive, or the parasitoid prepupa or pupa after the mummy has been formed (Sullivan, 1987). However, choice experiments indicated that mummies are preferred (Kanuck and Sullivan, 1992; Buitenhuis *et al*., submitted) and data suggest that it is also from these mummies that the parasitoid gains the highest fitness (Buitenhuis *et al*., submitted). When a parasitoid larva is attacked in a live aphid, the egg of the hyperparasitoid hatches only following mummification of the aphid by the host prepupa (Kanuck and Sullivan, 1992). The larva of *S. aphidovorus* feeds endophagously until it kills its host. Females can feed on the heamolymph that escapes from the puncture hole made by the ovipositor after oviposition (Griswold, 1929; Kanuck and Sullivan, 1992). It attacks aphidiine and aphelinid primary parasitoids (Hoffer and Stary, 1970; Sullivan and van den Bosch, 1971; Mertins, 1985; Völkl and Barczak, 1990). It might be expected that this species shows some characteristics in life history that are intermediate between hyperparasitoids that attack live parasitised aphids or those that attack aphid mummies.

2.4. Materials and Methods

2.4.1. Insect colonies

Laboratory cultures of the potato aphid (*Macrosiphum euphorbiae* (Thomas)), the primary parasitoid *Aphidius nigripes* Ashmead and *A. victrix*, *A. suspensus*, *D. carpenteri* and *S. aphidivorus*) were established and maintained at 20 ± 1°C, 75 ± 10% RH under a 16L:8D photoperiod. The hyperparasitoid species originated from laboratory colonies on different aphid – parasitoid systems and were therefore held in the laboratory for more than 5 generations to adapt to the potato aphid – *A. nigripes* system. *Asaphes suspensus* came from Québec City, Canada, *D. carpenteri* from Burnaby, Canada, *A. victrix* from Newport, England and *S. aphidivorus* from Bayreuth, Germany.

Aphidius nigripes was reared on aphid colonies feeding on potato seedlings cv. 'Norland' following the techniques of Brodeur and McNeil (1994). All hyperparasitoids were maintained by weekly exposing potato plants, infested with aphid mummies (for *A. suspensus*, *D. carpenteri* and *S. aphidivorus*) or parasitised aphids (for *A. victrix*) to the hyperparasitoid females.

2.4.2. Hosts used in the experiments

Hosts for *S. aphidivorus, D. carpenteri* and *A. suspensus* were *A. nigripes* prepupae in newly (0-24 h) mummified aphids ("mummy" host), while *A. victrix* was given 3rd instar *A. nigripes* larvae in living aphids as hosts ("parasitised aphid" host). In several aphid parasitoid-hyperparasitoid systems, these two parasitoid developmental stages have been shown to be the most suitable for hyperparasitoids attacking their host either when the aphid is alive (Kanuck and Sullivan, 1992; Grasswitz and Reese 1998) or once the latter has mummified (Chow and Mackauer 1999). To obtain *A. nigripes* hosts of both stages, third-instar potato aphid nymphs were parasitised by 3 to 5-d-old mated *A. nigripes* females during 24 h. Parasitised aphids were then reared at 20 ± 1ºC, 75 ± 10% RH, and a 16L:8D photoperiod. Based on embryonic and larval developmental times of *A. nigripes* at 20ºC (Paré *et al*. 1979), 3rd instars in living aphids and prepupae in newly mummified aphids were obtained 5 and 8 days following parasitisation, respectively.

All experiments were carried out in a climate chamber at 20 ± 1°C, 75 ± 10% RH and a 16L:8D photoperiod. *Asaphes suspensus*, *D. carpenteri* and *S. aphidivorus* were tested at the same time. For logistic reasons, *A. victrix* was tested 5 months later.

2.4.3. Development time and longevity

Approximately 400 hosts (parasitised aphids for *A. victrix*, and mummies for *A. suspensus*, *D. carpenteri* and *S. aphidivorus*) on potato plants were exposed to parasitism by twenty 1 to 7 days old, mated female hyperparasitoids for 4 hours. Afterwards, mummies were collected from the plants and put in individual gelatine capsules to complete hyperparasitoid development. The parasitised aphids were left on the plants until mummification after which mummies were also collected and put in individual gelatine capsules. Temperature in gelatine capsules was checked regularly using thermocouples (OMEGA HH23). The hosts were monitored every 8 hours for hyperparasitoid emergence and the sex of each adult was recorded. Hyperparasitoids that had not emerged 10 days after peak emergence were excluded from the analysis.

To measure hyperparasitoid longevity, newly emerged hyperparasitoids from the development time experiment ($n > 30$ per hyperparasitoid species and sex) were kept isolated in small, ventilated cylindrical cages, (5 cm in diameter and 10 cm in height) with a supply of 40% sugar water, replaced every 3-4 days. The hyperparasitoids did not have access to hosts and were checked daily for mortality.

2.4.4. Fecundity, immature mortality and sex ratio

In a preliminary experiment, we determined the maximum daily number of hosts that each hyperparasitoid species could parasitise. Based on these results, the number of hosts that was provided daily to a female in the experiment was set to 30 mummies for *D. carpenteri, A. suspensus*, 70 mummies for *S. aphidivorus* and 30 parasitised aphids for *A. victrix*. These numbers insured that hosts were available ad libitum.

The females were obtained as in the development time experiment, isolated in cages in a climate chamber and provided with 40% sugar water (see longevity experiment). The first 5 days of the test, 2 males were present for mating. Every day, until death, females were

given new hosts which were available for 24 h. The mummies were glued with non-toxic white glue (Lepage Bondfast®) on a potato leaf that was held by the petiole in a glass vial containing wet cotton wool and inserted into the cage. The parasitised aphids were transferred on a similar leaf with a paintbrush. After exposure to a hyperparasitoid female, mummies were put individually in gelatine capsules. Parasitised aphids were left on the leaf until mummification and then put in capsules. All capsules were held in a climate chamber until emergence. Mummies from which nothing had emerged were dissected (40X magnification) 10 days after hyperparasitoid peak emergence from a daily cohort to determine if they contained a primary parasitoid or a hyperparasitoid. Dead hyperparasitoids were classified in three categories: larva/prepupa, pupa or unemerged adult.

Realised fecundity (number of offspring that reach the adult stage), potential fecundity (all offspring, including those that died before adult emergence), pre-oviposition period (time from emergence to first oviposition), oviposition period (period during which females laid eggs) and post-oviposition period (time after last oviposition until death) were calculated. The secondary sex ratio (proportion of males) was determined at adult emergence. Mortality was expressed as the proportion of hyperparasitised hosts that contained a dead hyperparasitoid larva/prepupa, pupa or unemerged adult. Females that escaped or died by accident were excluded from the analysis. Fifteen females were tested per species.

2.4.5. Adult body size

Cohorts of parasitised aphid hosts and mummy hosts were produced and parasitised as described above (see Hosts used in the experiments). To avoid hyperparasitoid adult size being affected by mummy size, mummies of similar weight (0.9-1.1 mg) were selected. Less than 24 h after emergence, hyperparasitoids were killed by freezing at -20ºC, dried for 4 days at 60ºC and individually weighed on a Mettler Toledo UMT microbalance. In addition, measurements of the head width and front wing length (from humerus to apex) were made to the nearest 0.01 mm using a stereomicroscope (40X magnification) equipped with an ocular micrometer. At least 20 individuals were measured per species per sex.

2.4.6. Intrinsic rate of increase (r_m)

The r_m was estimated for each hyperparasitoid species by repeated iteration of the Birch formula (Birch, 1948):

$$\sum_x e^{-r_m x} l_x m_x = 1$$

where x is female age, l_x is the fraction of females surviving to age x and m_x is the age-specific fertility that records the number of living females born per female per day, calculated from the daily sex ratio data measured in the fecundity experiment.

2.4.7. Statistical analysis

Differences in development time between hyperparasitoid species and between males and females were tested with a two-way ANOVA following the Poisson distribution (GENMOD). The differences in longevity and size between between species and between males and females were tested with a standard twoway ANOVA. Immature mortality percentages and sex ratios were arcsin-transformed prior to analysis with a standard ANOVA. Potential and realised fecundity data were square-root transformed and analysed with an ANCOVA. All species are synovigenic and produce eggs throughout their life (Sullivan, 1987). This means that total fecundity is correlated with the duration of the period that females lay eggs. Oviposition period was therefore used as a covariable, Oviposition period and pre- and postoviposition periods were square-root transformed prior to analysis with a standard ANOVA. All means were separated by Fisher's protected LSD with Bonferroni adjustment of the significance level ($\alpha=0.05/k$; k=number of comparisons). All data were analysed using SAS (SAS, 1999).

2.5. Results

Values of the life history parameters and intrinsic rates of increase of the four hyperparasitoid species are summarised in Table 2-2.

2.5.1. Development time

The egg to adult development time ranged from 2 to 3 weeks. There were significant differences between species and between sexes (GENMOD species $\chi^2_3 = 2194.61$, $P < 0.0001$, sex $\chi^2_1 = 48.67$, $P < 0.0001$, interaction $\chi^2_3 = 49.01$, $P < 0.0001$). The interaction was significant, which means that the differences in developmental time between the sexes were not the same for all species. Among the female hyperparasitoids, *D. carpenteri* had the shortest development time, followed by *S. aphidivorus* and *A. victrix*. The development time of *A. suspensus* females was the longest of all species. The same pattern was observed for males, except that the difference between *A. victrix* and *S. aphidivorus* was not significant. Except for *S. aphidivorus*, males emerged before the females (protandry). Because of the few successful emergences of *A. victrix* males, the results regarding their development time are only indicative.

2.5.2. Immature mortality

There were significant differences in total immature mortality (ANOVA $F_{3,47} = 2.95$, $P = 0.0420$). Total immature mortality was about 10% in *A. suspensus*, *D. carpenteri* and *S. aphidivorus*, and significantly higher (20%) in *A. victrix*. Larval and pupal mortality was similar in all species (ANOVA, larva: $F_{3,47} = 2.28$, $P = 0.0819$; pupa: $F_{3,47} = 0.67$, $P = 0.5709$). The significant differences were only found in adult mortality (ANOVA $F_{3,47} = 14.00$, $P < 0.0001$). *A. victrix* had a significantly higher adult mortality.

2.5.3. Longevity

There were significant differences in longevity between species and sexes (Two way ANOVA species $F_{3,443} = 77.11$, $P < 0.0001$, sex $F_{1,443} = 72.94$, $P < 0.0001$, interaction $F_{3,443} = 10.15$, $P < 0.0001$). The significant interaction shows that differences between sexes were not the same for all species. Under the experimental conditions, aphid hyperparasitoids could live over 2 months, depending on species. *Syrphophagus aphidivorus* and *D.*

carpenteri had the shortest longevity, followed by *A. victrix*. *Asaphes suspensus* lived almost twice as long as the other three species. For all species, except *A. victrix*, male longevity was shorter than for the females.

2.5.4. Fecundity

Significant differences in realised and potential fecundity were observed (ANCOVA, realised fecundity $F_{3,39} = 36.63$, $P < 0.0001$; potential fecundity $F_{3,39} = 39.12$, $P < 0.0001$). *Asaphes suspensus* had the highest realised and potential fecundity, followed by *S. aphidivorus*. *Dendrocerus carpenteri* and *A. victrix* had the lowest fecundities The covariable (oviposition period) was significant (realised fecundity $F_{1,39} = 26.46$, $P < 0.0001$; potential fecundity $F_{1,39} = 29.81$, $P < 0.0001$), which means that the fecundity is dependent on the length of the oviposition period. The fecundity curve was bell-shaped, with a longer tail to the right (Figure 2-1). Maximum daily fecundity (mean ± SE) was 4.0 ± 1.3 for *A. victrix*, 23.6 ± 1.5 for *A. suspensus*, 12.7 ± 0.9 for *D. carpenteri* and 41.8 ± 2.8 for *S. aphidivorus*. *Dendrocerus capenteri* and *S. aphidivorus* started oviposition from the first day on, while *A. suspensus* and *A. victrix* had a pre-oviposition period of one and two days respectively. Although these differences are significant (ANOVA, $F_{3,40} = 30.97$, $P < 0.0001$), they are negligible as compared to the long life of the hyperparasitoids. The oviposition period lasted two months for *A. suspensus*, and one month for *A. victrix*, *S. aphidivorus* and *D. carpenteri*, these differences being significant (ANOVA, $F_{3,40} = 20.72$, $P < 0.0001$). *Alloxysta victrix* generally died two days after laying the last egg, while the other species had a post-oviposition period of two to three weeks, with *D. carpenteri* living the longest after stopping oviposion, interspecific differences being significant (ANOVA, $F_{3,40} = 3.11$, $P < 0.0369$).

2.5.5. Sex ratio

The females *A. victrix* had the lowest lifetime sex ratio, followed by *D. carpenteri*. *S. aphidivorus* and *A. suspensus* had the highest lifetime sex ratio's, differences between species being significant (ANOVA, $F_{3,42} = 27.93$, $P < 0.0001$). The daily sex ratio showed an upward curve for all species, levelling off at 100% during the later half of life, especially for *S. aphidivorus* and *A. suspensus* (Figure 2-2). To prevent a bias due to sperm depletion,

we calculated also the mean sex ratio for the first 10 days of the fertile period. During this period, we assume that the female still has enough sperm to fertilise her eggs. Although this calculation lowered the sex ratio's per species (*A. victrix* 12.5%, *S. aphidivorus* 51.2%, *D. carpenteri* 27.1% and *A. suspensus* 52.2%), the interspecific differences were still significant as for the lifetime sex ratio (ANOVA, $F_{3,42} = 28.94$, $P < 0.0001$)

2.5.6. Intrinsic rate of increase

A. victrix had the smallest r_m, followed by *D. carpenteri* and *A. suspensus*. *S. aphidivorus* has the largest intrinsic rate of increase (Table 2-2).

2.5.7. Adult body size

The dry weight, head width and wing length, were significantly different between species and, females were significantly larger than males (Two way ANOVA, dry weight: species $F_{3,305} = 134.33$, $P < 0.0001$, sex $F_{1,305} = 28.27$, $P < 0.0001$, interaction $F_{3,305} = 0.04$, $P = 0.9896$; head width: species $F_{3,304} = 789.17$, $P < 0.0001$, sex $F_{1,304} = 34.55$, interaction $F_{3,304} = 12.25$, $P < 0.0001$; wing length: species $F_{3,303} = 1408.15$, $P < 0.0001$, sex $F_{1,303} = 117.40$, $P < 0.0001$, interaction $F_{3,303} = 3.54$, $P = 0.0150$). The significant interaction means that differences between species were not the same in males and females. Within a species, males were always significantly smaller than females. In the comparison between species, *Syrphophagus aphidivorus* males and females were much smaller than the other hyperparasitoid species for all size measurements and *D. carpenteri* was generally the largest. Remarkable was the long wing length for *A. victrix* and the relatively short wing length for *D. carpenteri*.

2.6. Discussion

According to the dichotomous hypothesis (Godfray, 1994; Quicke, 1997; Mayhew and Blackburn, 1999), variation in life history traits can be explained by a dichotomy in development mode. However, our results based on direct comparison of four species (Table 2-2) suggest that the variation in life history traits in aphid hyperparasitoids cannot be explained by development mode alone. Some of their traits support the hypothesis, eg. koinobiosis in the tested hyperparasitoids is associated with endoparasitism, no or temporary paralysis and slow or delayed development, while idiobiosis is associated with a generally different set of traits. However, no such grouping between koinobionts and idiobionts was possible based on the life history parameters that we measured in this study. The species *A. suspensus* was clearly different from the other three species in longevity. Furthermore *A. suspensus* and *S. aphidivorus* both had a high fecundity and a high proportion of male offspring as opposed to *A. victrix* and *D. carpenteri*, which had lower fecundities, and more female offspring. For none of the life history parameters measured could the koinobiont species *A. victrix* and *S. aphidivorus* be separated as a group from the idiobiont species *D. carpenteri* and *A. suspensus*. It is clear that other factors, besides development mode, influence the life history traits of aphid hyperparasitoids.

Among the obtained results three things should be explained. First, in *A. victrix*, the mortality during the last (unemerged adult) stage of development was higher than in the other species (Table 2-2). In addition, the intrinsic rate of increase calculated based on all life history parameters of this species was the lowest of all species. Because few data are available for *A. victrix*, we do not know if these values are normal for this species or if the *Aphidius nigripes – Macrosiphum euphorbiae* system might be less suitable for the development of this hyperparasitoid species. Furthermore, this species was not tested at the same time as the other three species, which might have influenced the results. Second, the observed pattern of sex ratio is most likely associated with sperm availability. During the second half of their life, females were observed to lay only male offspring. The amount of sperm acquired during the five days that males were present might not have been enough for females to produce an optimal sex ratio as females might have run out of viable sperm long before the end of their oviposition period. It is possible that in these species females

mate several times during their lifetime to replenish their sperm supply like Brodeur and McNeil (1994) proposed for the aphid hyperparasitoid *Asaphes vulgaris*. Finally, when we compare our results with the parameters reported in the literature, it appears that our results are equivalent or higher than those of other studies (Spencer, 1926 (cited in Schooler, 1996); Gutierrez and van den Bosch, 1970; Walker and Cameron, 1981; Christiansen-Weniger, 1992; Völkl and Kranz, 1995; Chow and Mackauer, 1996; Grasswitz and Reese, 1998). The differences are possibly due to the size of the host in our rearing system, compared to the hosts used in the other studies (various *Aphidius* species on *Acyrthosiphon pisum* (Harris), *Sitobion avenae* (F.), *Myzus persicae* (Sulzer) or *Uroleucon jaceae* L.). It shows that for a comparison between species it is important to rear the hyperparasitoids on the same parasitoid-aphid-plant system.

In table 2-3 the life history parameters of the four hyperparasitoids are compared to those of (primary) parasitoids in general in the context of the dichotomous hypothesis (Quicke, 1997; Mayhew and Blackburn, 1999). The expected koinobiont life history characteristics are listed on the left and those of idiobiont parasitoids to the right, as predicted by the dichotomous hypothesis. In the middle, the hyperparasitoids (on genus level to have access to more data) are compared to this model. The data were measured in this study or found in the literature. For continuous variables we compared the data on a scale between the extreme values that are known for parasitoids.

The data for the two idiobiont ectohyperparasitoids are mainly in agreement with the hypothesis. The most important exceptions are the long development time and high fecundity of *A. suspensus*. These traits are also found in another *Asaphes* species (e.g. *A. vulgaris*, Brodeur and McNeil, 1994). In contrast to *D. carpenteri*, *Asaphes* species can host-feed, which provides the essential nutrients to produce large eggs. For *D. carpenteri*, the nature of the yolk bodies and the origin of the substances used to form them are not known. The low fecundity of this species is perhaps related to the difficulty in obtaining resources required to produce yolk-rich eggs with external nutrients limited to carbohydrates, honeydew or pollen (Le Ralec, 1995). The long development time of

Asaphes species might be correlated to their long lifespan. Further research has to point out if these two traits are correlated and their function in the biology of these species.

Compared to the idiobiont ectohyperparasitoids, the data for the two koinobiont endohyperparasitoids diverge much more from the predictions of the hypothesis and the results of Mayhew and Blackburn (1999). The most striking differences are the long adult lifespan and the high occurrence of egg production (low ovigeny index) in these species (Table 2-3). Furthermore, *A. victrix* has a very low oviposition rate (eggs/day) (mean daily fecundity 2.4 offspring, maximum 4.0 offspring per day), which is contrary to the predictions, although other species within the *Alloxysta* genus have somewhat higher oviposition rates (Chua, 1979; Singh and Srivastava, 1987; Mackauer and Völkl, 1993). The other endohyperparasitoid, *S. aphidivorus*, has the highest oviposition rate of the four studied species (mean daily fecundity 20.0 offspring, maximum 41.8 offspring per day), but these oviposition rates are still low compared to other koinobiont (primary) parasitoids (40-140 offspring per day; Aphidiidae (Force and Messenger, 1964)). Further inconsistencies with the dichotomous hypothesis for *S. aphidivorus* are that it is a generalist, has large eggs and is capable of host feeding. This species is able to hyperparasitise mummies and still living parasitised aphids. It strongly prefers mummies, and has higher fitness on this host (Kanuck and Sullivan, 1992; Buitenhuis *et al*., submitted). So, although it is a koinobiont endohyperparasitoid, it shares more life history characteristics with idiobiont ectohyperparasitoids.

In general, it is likely that many endoparasitic koinobionts should have higher ovigeny indices than ectoparasitic idiobionts. However, in our study we observed a high level of egg production in the koinobiont hyperparasitoids, and therefore should assign them a low ovigeny index (Jervis *et al*., 2001). This might explain the divergence of the results from the predictions of the dichotomous hypothesis. It appears that the low ovigeny value in these species has more influence on life history than development mode as this is correlated to long adult lifespan, large eggs and host feeding (Jervis *et al*., 2001). There is some evidence that natural selection adjusts egg production characteristics to approach the expected rate of host encounter (Jervis *et a*l., 2001). A correlation may exist between

synovigeny and a greater degree of host dispersion (Quicke, 1997). Parasitised aphids and mummies are not abundant hosts, because aphidiid wasps that oviposit in aphid colonies usually lay only a few eggs per colony and show high dispersal (Dettner *et al*., 1997). However, the actual availability of hosts for hyperparasitoids has still to be elucidated.

Other factors, besides development mode, that could potentially influence life history characteristics in the hyperparasitoids of this study are host stage and host range. Because the two host stages differ in many aspects (for example morphology, olfactory cues, abundance, number of competitive species) hyperparasitism of living parasitised aphids vs. aphid mummies does not necessarily demand the same adaptations in life history. If either host stage or host range would have been the major organisers of the evolution of life history traits in aphid hyperparasitoids, we would expect *A. victrix* to be different from the other species, because it attacks only the parasitised aphid before mummification, and also has a narrower host range than the other three species. It is restricted to *Aphidius* hosts and is considered to be more specialised than *A. suspensus* and *D. carpenteri* that attack several genera within the Aphidiidae, as well as an Aphelinidae (Höller *et al*., 1993). For *S. aphidivorus* the known host associations involve at least four primary parasitoid genera from the Aphidiidae and the Aphelinidae. However, based on the measured life history traits, *A. victrix* could not be placed apart from the other species. It is the closest to *D. carpenteri* in longevity and fecundity, while this species attacks uniquely mummified aphids, and has a large host range. In addition, we observed great differences between the species that attack mummified aphids. Both *A. suspensus* and *D. carpenteri* parasitise mummies of various parasitoids in various aphid species, and appear to occupy the same habitats and to have a large host range. However, we observed that *A. suspensus* lives much longer, has a higher fecundity and a longer fertile period, and has a longer development time than *D. carpenteri* (Table 2-2). Therefore, both host stage and host range cannot explain the differences in life history traits in these aphid hyperparasitoids.

Although the dichotomous hypothesis explains many parasitoid life history traits, it is unlikely that life history traits are determined exclusively by the dichotomy in development mode. It is clear that the variation in life history in aphid hyperparasitoids cannot be

explained by single factors like development mode, synovigeny, host stage or host range. Probably all factors act at the same time on life history evolution. Darwin (1859) supposed that a balanced interpretation of an evolutionary pattern requires two components: adaptation and lineage specific effects. The effect of adaptation is that life history traits are adapted to each other and to local environmental conditions. At the same time, some life history traits are fixed at high taxonomic levels (lineage specific effects). Following this reasoning, the differences between species might also be partly determined by their different phylogenetic origins. Hyperparasitism has a wide taxonomic distribution, indicating that it has evolved independently several times in the Hymenoptera (Gordh, 1981). It is likely because the expression of hyperparasitism is phylogenetically spotty, that different development modes occur among hyperparasitoids and that hyperparasitoid wasps have different ovipositional strategies. The different species have probably evolved within the phylogenetic constraints of their origin to exploit the same resource. All the above mentioned factors act together and are selected to optimise fitness gain during the life of an individual.

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2.8. References

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Table 2-1 Selected aphid hyperparasitoids and their taxonomic position (family within Hymenoptera), parasitic relationships of the larva (endo/ectoparasitoid, development mode and host stage), and host range. Host range is indicated as the number of aphid parasitoid genera that are parasitised.

Species	**Family**	**Endo/ecto parasitoid**	**Development mode**	**Host stage**	**Host range**
Asaphes suspensus	Pteromalidae	Ecto-	Idiobiont	(Pre-)pupa in aphid mummy	5
Dendrocerus carpenteri	Megaspilidae	Ecto-	Idiobiont	(Pre-)pupa in aphid mummy	4
Alloxysta victrix	Charipidae	Endo-	Koinobiont	Larva in live aphid	1
Syrphophagus aphidivorus	Encyrtidae	Endo-	Koinobiont	Larva in live aphid or (pre-)pupa in aphid mummy	4

Table 2-2 Life history parameters and intrinsic rates of increase for four aphid hyperparasitoids (means with standard error between brackets) reared on the *Aphidius nigripes*, *Macrosiphum euphorbiae*, potato system. Within rows, means followed by the same letter a are not significantly different based on Bonferroni–adjusted PLSD test. For differences between species, lower case letters are used for females and upper case letters for males (ANOVA/ANCOVA see text for F and p-values). N is the number of individuals tested.

	Alloxysta victrix		*Syrphophagus aphidivorus*		*Dendrocerus carpenteri*		*Asaphes suspensus*	
	♀	♂	♀	♂	♀	♂	♀	♂
Developmental strategy	Endo parasitic Koinobiont		Endo parasitic Koinobiont		Ecto parasitic Idiobiont		Ecto parasitic Idiobiont	
Development time (days) (egg-adult)	19.5 (0.7) *c* N=14	17.8 (0.8) *B* N=4	17.7 (0.1) *b* N=172	17.4 (0.1) *B* N=96	15.7 (0.2) *a* N=136	14.6 (0.03) *A* N=129	21.4 (0.6) *d* N=21	20.5 (0.3) *C* N=19
Larval mortality (%)	3.0 (0.8) *a*		4.5 (0.7) *a*		5.2 (1.2) *a*		6.6 (1.6) *a*	
Pupal mortality (%)	5.0 (1.7) *a*		3.0 (0.4) *a*		3.5 (1.2) *a*		2.0 (0.2) *a*	
Unemerged adult mortality (%)	12.4 (2.3) *b*		1.4 (0.2) *a*		2.5 (0.5) *a*		2.5 (0.7) *a*	
Total immature mort. %	20.4 (3.4) *b* N=11		8.9 (0.7) *a* N=12		11.3 (2.4) *a* N=13		11.1 (1.8) *a* N=15	
Longevity (days)	43.5 (1.5) *b* N=69	41.7 (1.1) *B* N=62	37.4 (3.2) *a* N=50	22.2 (1.8) *A* N=33	39.6 (1.6) *ab* N=56	25.7 (1.2) *A* N=30	70.3 (2.0) *c* N=100	47.5 (2.2) *C* N=51
Realised fecundity	86 (12.3) *a*		577 (52.5) *b*		154 (11.6) *a*		834 (138) *c*	
Potential fecundity	108 (16.0) *a*		629 (56.7) *b*		175 (12.5) *a*		924 (139) *c*	
Pre-ovipos. period (days)	2.1 (0.5) *b*		0 (0) *a*		0.1 (0.1) *a*		1.1 (0.1) *b*	
Oviposition period (days)	32 (2.7) *a*		26 (1.2) *a*		24 (2.2) *a*		59 (7.0) *b*	
Post-ovipos. period (days)	2.4 (0.6) *a* N=9		17.9 (4.5) *ab* N=11		19.1 (3.5) *b* N=15		16.4 (7.5) a*b* N=9	
Sex ratio (% males)	25.0 *a* N=11		70.2 *c* N=11		39.5 *b* N=13		76.7 *c* N=7	
Dry weight (µg)	102.2 (2.4) *bc*	91.4 (2.1) *B*	55.7 (1.1) *a*	44.9 (1.7) *A*	108.9 (3.1) *c*	96.4 (3.5) *B*	98.5 (3.7) *b*	87.0 (3.9) *B*
Head width (µm)	426.1 (2.4) *a*	402.9 (5.2) *B*	423.5 (1.5) *a*	389.5 (5.5) *A*	549.5 (9.1) *c*	566.9 (3.2) *D*	500.9 (5.7) *b*	481.6 (4.9) *C*
Wing length (µm)	1770.3 (12.8) *d* N=33	1669.2 (11.8) *D* N=42	1091.9 (3.2) *a* N=85	1032.6 (7.7) *A* N=19	1440.0 (23.5) *b* N=65	1405.5 (7.9) *B* N=29	1570.0 (21.3) *c* N=22	1455.8 (17.8) *C* N=19
Intrinsic rate of increase (r_m) (d^{-1})	0.1180		0.2194		0.1712		0.1844	

Table 2-3 Comparison of four aphid hyperparasitoid genera, relative to their conditions as predicted by the koinobiont/idiobiont dichotomous hypothesis (Quicke, 1997; Mayhew and Blackburn, 1999). Matching of Alloxysta and Syrphophagous is to predicted koinobiont traits, and *Asaphes* and *Dendrocerus* matching is to idiobionts.

Predicted for koinobionts	*Alloxysta*	*Syrphophagus*	*Asaphes*	*Dendrocerus*	Predicted for idiobionts
Specialist	Yes	No	Yes	Yes	Generalist
Host exposed	Yes	Yes	Yes	Yes	Concealed host
Host stage attacked smaller than wasp	Yes	Yes	No/yes	No/yes	Host stage attacked larger than wasp
No or temporary paralysis	Yes	Yes	Yes	Yes	Paralysis
Slow or delayed development[4]	Yes	Yes	No	Yes	Rapid development[4]
Small eggs[4]	Yes[1]	No[1]	Yes[1]	Yes[1]	Large eggs[4]
High oviposition rate[4]	No	Intermediate	Yes	Yes	Low oviposition rate[4]
High fecundity[4]	No	Yes	No	Yes	Low fecundity[3]
Pro-ovigeny	No	No	Yes	Yes	Synovigeny
Host feeding uncommon	?	No	Yes	No	Host feeding common
No oösorption	?	?	Yes[2]	No[2]	Oösorption
Short adult lifespan[4]	No	No	Yes	Yes	Long adult lifespan[3]
Large adult size	Yes	No	No	No	Small adult size
Less or no sexual dimorphism	No?	No?	Yes	Yes	Sexual dimorphism
Do not choose sex to match host size	?	?	Yes[3]	Yes	Choose sex to match host size

[1]Egg size for *Alloxysta brevis, Asaphes vulgaris* and *D. carpenteri* (Haviland, 1920, 1922, Christiansen-Weniger, 1992; Mackauer and Völkl, 1993), *Syrphophagus inquisitor* (Griswold, 1929).
[2]Data for *Asaphes vulgaris* and *D. carpenteri* (LeRalec, 1995).
[3]Data for *Asaphes vulgaris* (Sullivan and Völkl, 1999).
[4]Demonstrated by Mayhew and Blackburn (1999) for parasitoids.

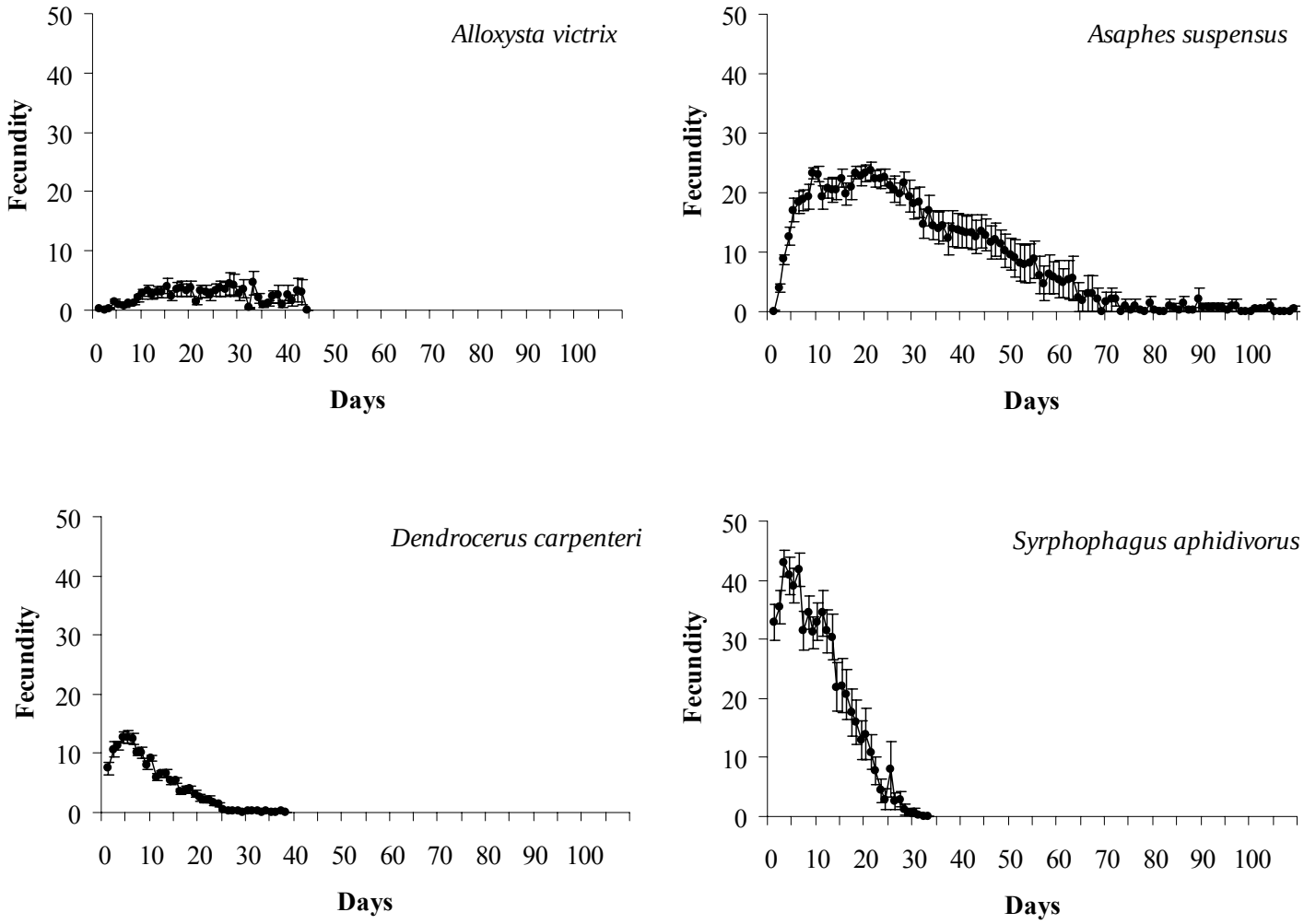


Figure 2-1Realised fecundity (mean ± SE; *Alloxysta victrix* n=9; *Asaphes suspensus* n=7; *Dendrocerus carpenteri* n=13; *Syrphophagus aphidivorus* n=11) as affected by female age of four aphid hyperparasitoids reared on the *Aphidius nigripes*, *Macrosiphum euphorbiae*, potato system

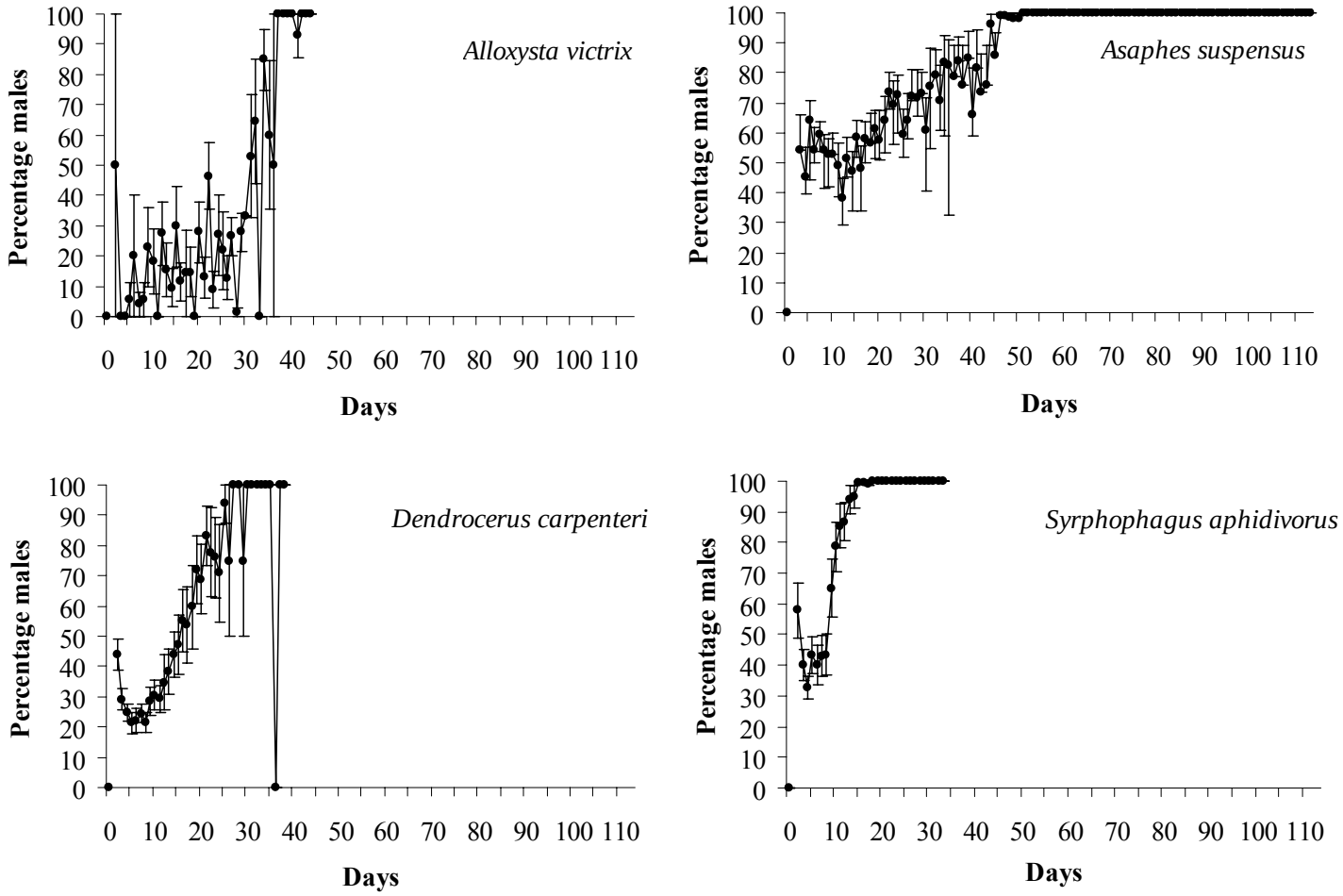
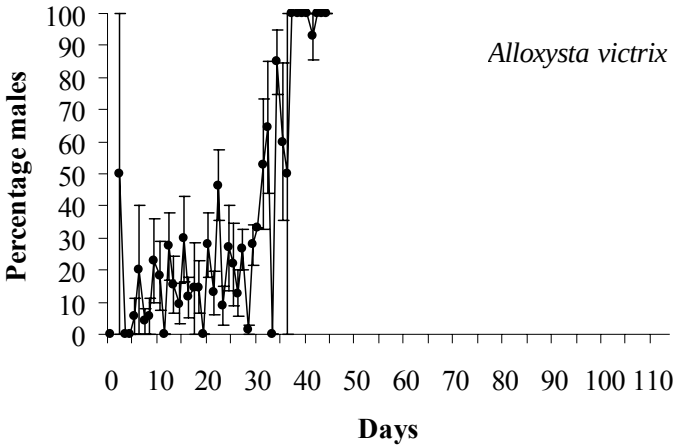
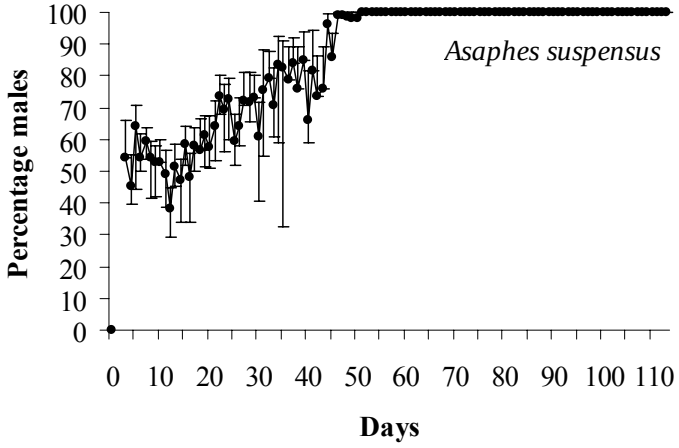
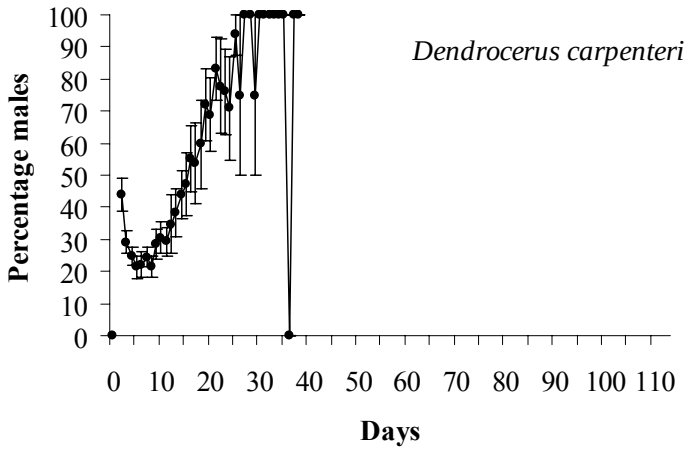
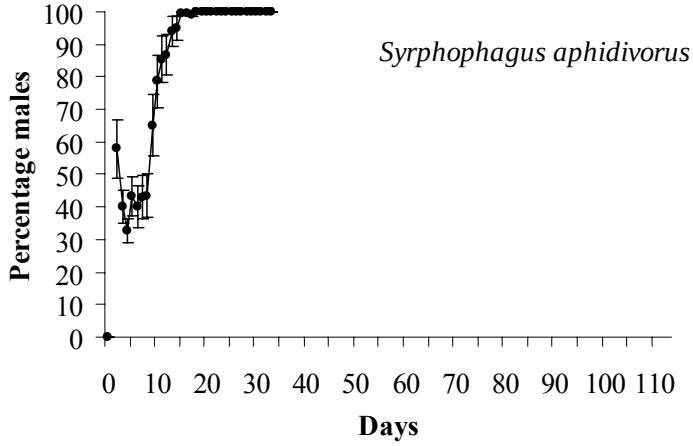


Figure 2-2 Sex ratio of progeny (mean ± SE; *Alloxysta victrix* n=11; *Asaphes suspensus* n=7; *Dendrocerus carpenteri* n=13; *Syrphophagus aphidivorus* n=11) as affected by female age of four aphid hyperparasitoids reared on the *Aphidius nigripes*, *Macrosiphum euphorbiae*, potato system.


Alloxysta victrix
Percentage males
Days


Asaphes suspensus
Percentage males
Days


Dendrocerus carpenteri
Percentage males
Days


Syrphophagus aphidivorus
Percentage males
Days

Chapter 3.

Preference and performance of the hyperparasitoid *Syrphophagus aphidivorus* (Hymenoptera: Encyrtidae):

Fitness consequences of selecting hosts in live aphid vs. aphid mummy

3.1. Abstract

Theoretical models predict that ovipositional decisions of parasitoid females should lead to the selection of the most profitable host for parasitoid development. In the laboratory, we investigated the correlation between host suitability and host preference of the aphid hyperparasitoid *Syrphophagous aphidivorus* (Mayr) (Hymenoptera: Encyrtidae) on the host *Aphidius nigripes* Ashmead parasitising the aphid *Macrosiphum euphorbiae* (Thomas). Female *S. aphidivorus* display atypical oviposition behaviour by attacking either primary parasitoid larvae in live aphids, or parasitoid (pre-)pupae in dead, mummified aphids. The relative suitability of the two host stages was determined by measuring hyperparasitoid fitness parameters (survival, development time, fecundity, sex ratio and adult size of progeny), and calculating the intrinsic rate of population increase (r_m). We further examined host preference by *S. aphidivorus* females and the influence of aphid defence behaviour on host selection. Hyperparasitoid offspring performance was highest when developing from hosts in aphid mummies and females consistently preferred this host to hosts in parasitised aphids. Although aphid defensive behaviour may influence host selection, it was not a determining factor. Ecological and evolutionary processes that might have led to dual oviposition behaviour in *S. aphidivorus* are discussed.

Keywords: Host suitability, oviposition preference, offspring fitness, parasitoid life history

3.2. Résumé

Les modèles théoriques prédisent que les décisions d'oviposition des femelles parasitoïdes devraient mener à la sélection de l'hôte le plus profitable pour le développement du parasitoïde. Dans le laboratoire, nous avons investigué la corrélation entre la convenance de l'hôte et la préférence de l'hôte dans l'hyperparasitoïde de puceron *S. aphidivorus* (Hymenoptera: Encyrtidae). Les femelles *S. aphidivorus* montrent un comportement d'oviposition atypique en attaquant soit la larve de parasitoïde dans le puceron vivant, soit la pupe de parasitoïde dans la momie de puceron. Ces hôtes sont des stades différents de la même espèce d'hôte, et peuvent différer en profitabilité, disponibilité et mortalité. La convenance relative des deux stades d'hôte a été déterminée par des mesures de paramètres de fitness, comme la survie, le développement, la fécondité, le sex ratio et la taille de la progéniture des hyperparasitoïdes, et par le calcul du taux intrinsique de la croissance de la population (r_m). Ensuite nous avons fait des observations de comportement afin d'examiner la préférence d'hôte des femelles *S. aphidivorus* et l'influence du comportement de défense de puceron sur la sélection d'hôte. La performance de la progéniture de l'hyperparasitoïde était le plus élevée quand la progéniture se développait sur des prepupes dans les momies de puceron. Conformément, les femelles préféraient cet hôte. Bien que le comportement de défense du puceron puisse influencer la sélection de l'hôte, ce n'était pas un facteur déterminant. Les procès écologiques et évolutionnaires qui ont pu mener au comportement d'oviposition double de *S. aphidivorus* sont discutés.

3.3. Introduction

For the majority of hymenopteran parasitoids, the relationship between host selection and host profitability is determined by both the physiological capacities of immatures to exploit the host and the behavioural ability of females to locate and use the resource (Godfray 1994). Hosts vary in suitability, availability and detectability (Slansky 1986, Vet and Dicke 1992, Godfray 1994), and it has been demonstrated in several parasitoid species that females show behavioural plasticity towards host acceptance and oviposit preferentially in the most profitable host (reviewed by Godfray 1994). In contrast, imperfect concordance between host selection and offspring performance has also been observed (e.g. Brodeur and Vet 1995, Grasswitz and Reese 1998, Rivero 2000). Recent theories on host-parasitoid relationships have placed more emphasis on determinants related to the physiological and informational state of the foraging female such as her egg load, previous experience, perception of the environment and life expectancy that might influence the dynamic expression of host selection by parasitoids (Roitberg *et al*. 1993, Visser 1995, Rivero 2000).

The great majority of parasitoid species can only parasitize a single host stage and have evolved specific adaptations to exploit either egg, egg-larva, larva, pupa, larva-pupa or adult hosts (Quicke 1997). The most common exceptions are found in parasitoids of hemimetabolous insects which have the capacity to attack nymph and adult hosts from the same species. For example, aphid parasitoids may parasitize all developmental stages of their host (Stary 1970), including the embryo (Mackauer and Kambhampati 1988). Among koinobiont parasitoids of holometabolous insects, the host selection behaviour of the aphid hyperparasitoid *Syrphophagus aphidivorus* (Mayr) (Hymenoptera: Encyrtidae) is atypical. Foraging females display a dual oviposition behaviour as they have the ability to attack either the primary-parasitoid larva when the aphid is alive or the primary-parasitoid prepupa or pupa after the parasitoid has killed and mummified the aphid (Kanuck and Sullivan 1992, and references therein). In both cases, the female lays a single egg inside the primary parasitoid, where the larva first develops as an endophagous parasite, but feeds ectophagously in later larval stages (Kanuck and Sullivan 1992).

The aphid-parasitoid-*S. aphidivorus* system has several favorable attributes for the study of host selection behaviour and offspring fitness from a functional perspective. The two host stages of *S. aphidivorus* may differ in abundance, susceptibility to parasitisation, developmental suitability, and vulnerability to natural enemies. Parasitised aphids and aphid mummies have different morphological characteristics (form, colour, texture) and olfactory profiles (Christiansen-Weniger 1994, Grasswitz and Reese 1998). Aphids may modulate *S. aphidivorus* host choice as they rely on a variety of individual and group defenses to avoid predation and parasitism (Roitberg and Myers 1979, Kouamé and Mackauer 1991, Lucas and Brodeur 2001). On the other hand, aphid mummies are attached to the substrate and confined pupal parasitoids cannot benefit from aphid defensive behaviour. Hyperparasitoids developing in the two host stages may suffer different levels of intra- and interspecific competition (Sullivan 1987, Sullivan and Völkl 1999) and predation as the guilds of competitors and natural enemies associated to parasitised aphids and aphid mummies differ. Finally, the quantity and nutritional quality of the resource available to *S. aphidivorus* are easier to judge for a foraging female because they are relatively fixed in the aphid mummy while they are constantly changing for a growing parasitoid larva within the living aphid.

The costs and benefits of attacking either the primary-parasitoid larva within the live aphid (parasitised aphid host hereafter) or the primary-parasitoid prepupa or pupa in the mummified aphid (mummy host hereafter) has yet to be quantified for *S. aphidivorus.* Host stage preference of ovipositing hyperparasitoid females also remains to be determined. While Matteson (1977, cited in Kanuck and Sullivan 1992) observed that host stage do not affect host selection of *S. aphidivorus*, Kanuck and Sullivan (1992) showed that hyperparasitoid females have a preference for the mummy host over the parasitised aphid host.

In this study, we investigated the correlation between host stage preference and host suitability in *S. aphidivorus*. In the laboratory we determined the relative suitability of parasitised aphid vs. mummy hosts for *S. aphidivorus* by measuring several parameters of hyperparasitoid fitness (survival, developmental time, size, longevity, fecundity and sex

ratio) and by calculating the intrinsic rate of natural population increase (r_m). We also measured host preference using paired-choice tests and examined the influence of aphid defensive behaviour on host selection by *S. aphidivorus*.

3.4. Materials and Methods

3.4.1. Insect Colonies

Insects used in the experiments came from laboratory cultures, established for more than five generations from field-collected individuals, and were reared at room temperature (20-22°C) under a 16L:8D photoperiod. The potato aphid, *Macrosiphum euphorbiae* (Thomas) and the primary parasitoid, *Aphidius nigripes* Ashmead originated from commercial potato fields near Québec City, whereas the *S. aphidivorus* colony was established from individuals provided by Dr. W. Völkl, Bayreuth, Germany. The parasitoid was reared on potato aphid colonies feeding on potato seedlings, 'Norland'. The hyperparasitoid was maintained by exposing potato plants, infested with aphid mummies, to *S. aphidivorus* females.

3.4.2. Host Stages Used in the Experiments

The two host stages offered to *S. aphidivorus* consisted of third instar *A. nigripes* larvae in living aphids (parasitised aphid host) or *A. nigripes* pupae in aphid mummies (mummy host). These two developmental stages have been shown to be the most suitable for hyperparasitoids attacking either parasitised aphid or mummy hosts (Kanuck and Sullivan 1992, R.B., unpublished data). To obtain *A. nigripes* cohorts of a specific age class, third-instar aphid nymphs were parasitised by 3-5-d-old mated *A. nigripes* females for a 24-hr period. Parasitised aphids were then reared at 20 ± 1ºC, 75 ± 10% RH, and a 16L:8D photoperiod. Based on embryonic and larval developmental times of *A. nigripes* at 20ºC (Paré *et al*. 1979), third instars in living aphids and pupae in mummified aphids were obtained five and eight days following parasitization, respectively.

3.4.3. Host Suitability

The suitability of both hosts for *S. aphidivorus* was determined by measuring hyperparasitoid survival, development time, longevity, fecundity, sex ratio and size, and by

calculating the intrinsic rate of natural increase (r_m). All experiments were carried out in a growth chamber at 20 ± 1ºC, 75 ± 10% RH, and a photoperiod of 16L:8D.

Development Time. We measured the effect of host stage on total development time (egg-to-adult) of *S. aphidivorus*. Twenty 1-7 day old, mated *S. aphidivorus* females were introduced for 4 h in cages containing potato plants infested with either ca. 400 parasitised aphids or 400 mummies. Following parasitisation, mummies were gently removed from the foliage and individually reared in gelatine capsules whereas aphids were left on the plants until mummification after which they were put in capsules. Hyperparasitoid emergence was monitored every eight hours and the sex of each adult was determined. Temperature in cages and gelatine capsules were monitored regularly using thermocouples (OMEGA HH23). Mummies from which a hyperparasitoid had not emerged 10 days after peak emergence were not included in the analysis.

Longevity. We compared the effect of both host stages on the longevity of adult males and females at a constant temperature of 20°C. Newly emerged *S. aphidivorus* from the development time experiment (n > 60 per host stage and hyperparasitoid sex), were kept individually in small ventilated cylindrical cages (5 cm in diameter and 10 cm in height) with a supply of 40% sugar water, replaced every 3-4 days. Hyperparasitoids did not have access to hosts and were checked daily until death.

Fecundity, Immature Mortality and Sex Ratio. We measured total and age-specific fecundity, immature mortality, as well as the sex ratio of the progeny of *S. aphidivorus* females reared from parasitised aphid or aphid mummy hosts. These females were given mummies to parasitise in this experiment, since previous tests indicated that *S. aphidivorus* females reared on the same host stage were equally fecund when provided with either parasitised aphids or aphid mummies (ANOVA, $F = 2.24$, $df = 1, 28$; $P = 0.1456$; R.B., unpublished data). Females and males used in the experiment were obtained as previously described (see Development time). One newly emerged female and two males were isolated in small ventilated cylindrical cages (5 cm in diameter and 10 cm in height) with a supply of 40% sugar water, and the males removed five days later. From day of emergence to hyperparasitoid death, each female was provided 70 newly formed mummies, glued

(Lepage Bondfast®) on a potato leaf held by the petiole in a glass vial filled with wet cotton wool and placed into the cage. Preliminary tests showed that S. aphidivorus reproduction measured in this way is maximal when offered 70 mummies per day. Less than 5% of the mummies were superparasitised in this set up (R.B., unpublished data). After each 24-h period, mummies were put individually in gelatine capsules, held in a growth chamber, and monitored daily for insect emergence. The secondary sex ratio (proportion of males at emergence) was determined. Mummies from which nothing had emerged were dissected (400X magnification) 10 days after hyperparasitoid peak emergence to determine if they contained a dead primary parasitoid or a dead hyperparasitoid. Fecundity of *S. aphidivorus* was calculated by summing the number of adults emerging and dead hyperparasitoid within the mummies. *Syrphophagus aphidivorus* immature mortality was expressed as the proportion of hyperparasitised mummies containing dead larva, pupa or adult hyperparasitoids. Fifteen females were tested per treatment.

Body size. We compared the effect of host stage on the body size of *S. aphidivorus* adult males and females. Cohorts of parasitised aphid and mummy hosts were produced and parasitised as described above (see Hosts used in the experiments). In this instance, mummies of similar weight (range 0.9-1.1 μg) were selected, so that observed differences in hyperparasitoid adult size would not be affected by mummy size. Following emergence, (maximum delay 24 h) hyperparasitoids were killed at -20ºC, dried for four days at 60ºC and individually weighed on a Mettler Toledo UMT microbalance. The head width and forewing length from humerus to apex were also used as size index and were measured to the nearest 0.01 mm using a stereomicroscope (400X magnification) equipped with an ocular micrometer.

Intrinsic rate of population increase (r_m). The r_m is a demographic parameter used to estimate the population growth potential of an organism under given ecological conditions (Southwood and Henderson 2000). The r_m was estimated for each host stage by repeated iteration of the Birch formula (Birch 1948):

$$\sum e^{-r_m x} l_x m_x = 1$$

x

where x is female age, l_x is the fraction of females surviving to age x and m_x is the age-specific fertility that records the number of living females born per female of age x, calculated from the daily sex ratio data measured in the fecundity experiment.

3.4.4. Host preference

We measured oviposition preference of *S. aphidivorus* females using paired-choice tests. All females were 2–7 day old, mated and had had a 24 h foraging and parasitising experience with both hosts the day prior to the test. Twenty females were individually assigned to a patch of 10 parasitised aphids and 10 mummies on a potato leaf. The patches were created 24 h prior to the test by gluing the mummies in a grid on the upper side of the leaf and introducing the parasitised aphids with a paintbrush. In this experiment, to ensure that parasitised aphids effectively contained a parasitoid larva, aphids were individually exposed to *A. nigripes* females in a gelatine capsule and attack was observed under a stereomicroscope. More than 94% of the aphids are parasitised using this technique (J.B., unpublished data). The patches were enclosed in a 3.5 cm diameter clip-cage to prevent escape of parasitised aphids and favour their settlement within the patch that was formed by the clip cage. Tests were started by introducing a *S. aphidivorus* female on the host patch and recording her behaviour.

Female behaviour was recorded with The Observer® (Version 3, Noldus Information Technology). Each test lasted one hour, or ended when the hyperparasitoid left the patch for more than a few seconds. The duration of the following behaviours was recorded: walking, grooming, host examination and oviposition (drilling and probing of the host). Host acceptance was defined as close examination followed by apparent oviposition and was therefore calculated by dividing the number of ovipositions by the number of examinations and converted into percentages. Multiple oviposition attempts in the same host during a bout were considered as one oviposition, because in many cases the female has to change position on the host to find the ideal angle to lay her egg. However, if a female left a host and returned later, this was counted as a new oviposition (superparasitism). Despite the fact that parasitised aphids were free to move in the patch, it was possible to follow each of

them individually and determine the occurrence of superparasitism. Consequently, for each female superparasitism was calculated by dividing the number of presumably hyperparasitised hosts that was accepted for oviposition, by the total number of hosts that were accepted. Based on the duration of the different recorded behaviours, a time budget was constructed. Twenty replicates were done.

To determine the effect of behavioural defenses of the aphid on the oviposition success of *S. aphidivorus*, we repeated the experiment using motionless aphids. In this instance, just prior to the experiment, the aphid abdomen was glued on the leaf surface, thereby preventing the aphid from walking away, kicking or dropping from the feeding site. Ten replicates were done of this experiment.

3.4.5. Statistical analysis

Differences in longevity, dry weight and wing length between hosts and sexes were tested using two-way ANOVA's. Only head width data were rank-transformed prior to be analysed with conventional two-way ANOVA's as the equivalent of a non-parametric test (Schreirer *et al*. 1976). Development time was analysed using GENMOD (SAS 1999) following a Poisson distribution. Differences among means were tested with Fisher's protected LSD with Bonferroni correction of the significance level. Following arcsine transformation, fecundity and immature mortality data were analysed with Student's t-tests. Lifetime sex ratios, expressed as the proportion of males among each female's progeny, were analysed with a Chi-square test. The percentages of hosts examined and accepted were compared using Fisher's exact ($n < 5$) or Chi-square ($n > 5$) tests. Mean durations of the observed behaviours in host preference tests were analysed using Student's t-tests. All data were handled using SAS (SAS 1999) and significance level was $\propto = 0.05$ in all tests.

3.5. Results

3.5.1. Host suitability

Life history parameters for male and female *S. aphidivorus* developing in both hosts are compared in Table 3-1.

The development time from oviposition to adult emergence was shorter by ca. two days for individuals that developed from mummies than for those that developed from parasitised aphids There was no difference between development times of males and females. (GENMOD; host: $\chi^2 = 110.55$; df = 1; $P < 0.0001$; sex: $\chi^2 = 0.17$, df = 1; $P = 0.6818$; interaction; $\chi^2 = 3.79$, df = 1; $P = 0.0515$).

The individuals reared from parasitised aphid hosts lived much longer than those from mummy hosts, and females lived longer than males irrespective of host (Two-way ANOVA; host: $F = 16.07$; df = 1, 189; $P < 0.0001$; sex: $F = 58.44$, df = 1, 189; $P < 0.0001$; interaction: $F = 0.35$; df = 1, 189; $P = 0.5571$).

Over their life, females from mummy hosts produced twice as much offspring as females from parasitised aphid hosts (*t*-test, $t = 3.90$; df = 16; $P = 0.0013$). Figure 3-1 illustrates the mean daily fecundity of females. Patterns are similar for the two host treatments, but females from mummy hosts produced more progeny per day than females from parasitised aphid hosts. Reproduction started the first day after emergence and peaked from day 3 to 5 in both cases. Females from mummy and parasitised aphid hosts had a maximum daily fecundity of 43 and 25 progenies, respectively.

Immature hyperparasitoid mortality was slightly higher in parasitised aphid hosts, although this was only marginally significant (Table 1; *t*-test, $t = 2.05$; df = 16; $P = 0.0567$). The percentages of *S. aphidivorus* that died during larval and pupal stages ranged from 1.5% to 4.3% in mummy hosts and from 2.7% to 7.5% in parasitised aphid hosts. These values are too small to be meaningfully analysed per developmental stages.

Lifetime sex ratio differed between treatments (Table 3-1; $\chi^2 = 39.65$; df = 1; $P < 0.0001$); it was male-biased for hyperparasitoids developing in mummy hosts (0.7) and unbiased

(0.5) for those developing in parasitised aphid hosts. However, early in reproductive life the pattern was similar for both hosts with sex ratios of 0.51 and 0.52 during the first 10 days for mummy and parasitised aphid hosts, respectively ($\chi^2 = 0.3299$; df = 1; $P = 0.5657$). The overall difference resulted from an increase in the proportion of males produced late in the life of females originating from the mummy host (Fig. 3-2), when reproduction was minimal in the parasitised aphid treatment.

The r_m of *S. aphidivorus* was higher by ca. 24% on mummy host (0.2494 d^{-1}) than on parasitised aphid host (0.1895 d^{-1}; Table 1).

There were significant differences between the hosts in dry weight and wing length of emerging hyperparasitoids, but not in head width. In addition there were significant differences between male and female size for all measurements. (Two-way ANOVA, dry weight: host: $F = 13.94$; df = 1, 205; $P = 0.0002$; sex: $F = 11.10$; df = 1, 205; $P = 0.0010$; interaction: $F = 10.10$; df = 1, 205; $P = 0.0017$; wing length: host: $F = 6.76$; df = 1, 203; $P = 0.0100$; sex: $F = 103.82$; df = 1, 203; $P < 0.0001$; interaction: $F = 2.03$; df = 1, 203; $P = 0.1554$; head width: host: $F = 0.00$; df = 1, 204; $P = 0.9568$; sex: $F = 97.49$; df = 1, 204; $P < 0.0001$; interaction: $F = 0.73$; df = 1, 204; $P = 0.3930$) (Table 3-2). The difference in size between hosts was only observed in females. Male size was the same for both hosts. Furthermore, females were always larger than males, except for dry weight on parasitised aphid hosts

3.5.2. Host preference

In the experiment with free aphids, hyperparasitoid females had a preference for mummy hosts (Fig. 3-3). Mummies were examined much more often than parasitised aphids (χ^2 test, $\chi^2 = 67.21$; df = 1; $P < 0.0001$). Overall, 99.2% of the examined mummies were accepted, whereas only 28.6% of the examined parasitised aphids were accepted (Fisher exact test, $P < 0.0001$). Gluing parasitised aphids to the leaf effectively reduced the mean number of host defensive behaviour (aphid kicking) per observation from 11.4 ± 7.8 to 0.1 ± 0.3. Nevertheless, a similar pattern of host preference was observed (Fig. 3-3), as mummies were examined significantly more often than parasitised aphids (χ^2 test, $\chi^2 = 7.37$; df = 1; $P < 0.0066$). Similarly, the acceptance rate was greater for mummies (98.3%) than for

parasitised aphids (79.2%) (Fisher's exact test, $P = 0.0074$). However, the proportion of parasitised hosts accepted for oviposition was significantly higher for glued aphids than for free-moving aphids (Fisher's exact test, $P < 0.0099$).

Hosts were frequently examined a second time and superparasitism was common in our experimental set-up. With free aphids, 25.4% of all the hyperparasitised mummies were superparasitised (on average 2.2 mummies per patch). Only once a parasitised aphid was examined twice but host defence prevented an attack. With glued aphids, 33.3% of all the hyperparasitised mummies and 13.6% of all the hyperparasitised aphids were superparasitised (2.4 mummies and 0.3 parasitised aphids per patch).

When compared to the situation with free parasitised aphids, immobilising the aphids significantly increased the time spent examining (3 *vs.* 36 seconds) and ovipositing into (70 *vs.* 284 seconds) parasitised aphid hosts. (*t*-tests: examination, $t = 3.05$; df = 28; P = 0.0133; oviposition $t = 2.20$; df = 28; $P = 0.0365$). The duration of the other behaviours were not different (*t*-tests: examination mummy, $t = 0.98$; df = 28; $P = 0.3350$; oviposition mummy, $t = 0.21$; df = 28; $P = 0.8366$; walking, $t = 0.53$; df = 28; $P = 0.6033$; grooming $t = 0.63$; df = 28; $P = 0.5312$) (Fig. 3-4). In both experiments (free and glued parasitised aphid hosts), a hundredfold more time was spent examining and parasitising mummy hosts than parasitised aphid hosts, which confirms the preference for the mummy hosts.

3.6. Discussion

Host selection by insect parasitoids is complex and results from interactions at physiological, ecological, and behavioural levels. Females are expected to prefer hosts that maximize progeny performance and their lifetime reproductive success. Accordingly, they have evolved a variety of behavioural mechanisms enabling them to opt for the superior hosts (Vinson 1984, Bell 1990). Our study indicates that female ovipositional decisions match up with host suitability for *S. aphidivorus*. Females prefer aphid mummies to parasitised aphids, the latter being the least profitable for parasitoid development.

3.6.1. Host Suitability

Syrphophagus aphidivorus is capable of developing in both hosts, but life history parameters indicated that hosts in mummies are more suitable than those in parasitised aphids. No major differences were observed in immature mortality, male size, and sex ratio. Although secondary lifetime sex ratio was biased towards males in the mummy host treatment, the pattern was similar during the first 10 days following emergence when females realized 60-65% of their lifetime fecundity. In our experiment, females had only access to males briefly in their early reproductive life. Apparently, the amount or viability of sperm received during this period was insufficient to fertilize eggs produced late by old females from the mummy host treatment. Such was not the case for less fecund females from the parasitised aphid host treatment.

Increased longevity is the only parameter that may provide a fitness gain to hyperparasitoids from the parasitised aphid host, over those from the mummy host. However, this may hold true only for males, as the reproductive period is similar for females developing in both hosts (Table 3-1); the extended, and unexplained, post-reproductive period of females from the parasitised host treatment apparently does not contribute to parasitoid fitness.

Hyperparasitoids developing in mummy hosts took two days less to reach the adult stage and females were larger and more fecund than those developing in parasitised aphid hosts. These differences led to a higher r_m for *S. aphidivorus* on mummy hosts (0.25 d^{-1}) than on

parasitised aphid hosts (0.19 d^{-1}), the r_m being strongly correlated to developmental rate and early fecundity in arthropods (Roy *et al.* 2003). These r_m values clearly indicate that mummy hosts are more suitable for *S. aphidivorus* than parasitised aphid hosts. Although parasitoid ecologists do not commonly use variations of the intrinsic rate of increase, they represent adequate measures of fitness differences for species with overlapping generations such as *S. aphidivorus* (Stearns 1992, Roitberg *et al*. 2001).

The observed difference in development time between hosts should be interpreted with caution. When a parasitised aphid is attacked by *S. aphidivorus*, hatching of the hyperparasitoid egg is delayed until aphid mummification (Kanuck and Sullivan 1992). The prolonged development of ca. two days in the parasitised aphid host corresponds to the time from oviposition to aphid mummification by the primary parasitoid, when the hyperparasitoid egg remains dormant in the parasitoid larva. We therefore suspect that actual development time from egg to adult is similar in both hosts, once embryogenesis has been initiated. Nevertheless, there are potential benefits associated with a shorter egg phase (excluding dormancy) for individuals developing in mummy hosts. First, it would reduce the time exposed to the immune system of the host, thereby lowering the potential risk of egg encapsulation. Second, it would also reduce the risk of mortality from competitors and natural enemies, as predicted by the slow-growth-high-mortality hypothesis (Clancy and Price 1987, Benrey and Denno 1997).

In theory, because hyperparasitoid egg development is arrested until aphid mummification, *S. aphidivorus* larvae should have access to the same resources for development regardless of the host stage in which the egg was laid. Under experimental conditions, after oviposition either in parasitised aphids of mummies, immature *S. aphidivorus* exploited the same primary parasitoid stage. Therefore, why are mummy hosts more suitable than parasitised aphid hosts? Differences in hyperparasitoid fitness likely originate from factors associated with the pre-mummification period. For instance, parasitism might affect growth of the primary parasitoid larva, thereby the overall quality of the subsequent pupa. At oviposition parasitoid females typically inject virus-like particles and venom into the host, which are important in disarming host defences and disrupting host physiology (Piek 1986,

Stoltz 1993). We do not know if this is the case in *S. aphidivorus*. Preliminary data indicate that parasitism of hosts within live aphids by *S. aphidivorus* does not affect the pre-pupal weight of the primary parasitoid within the mummified aphid (R.B., unpublished data). More information is needed to assess the quality of parasitoid hosts originating from parasitised aphids *vs.* aphid mummies. There might also be a cost associated with arrested development for eggs laid in host larvae in live aphids. *Syrphophagus* species produce relatively large, nutrient rich eggs (Griswold 1929) that could be partially depleted during the resting period, thereby lowering the fitness of the resulting offspring.

3.6.2. Host Preference

Results from the paired choice experiment are straightforward: *S. aphidivorus* females clearly prefer aphid mummies to parasitised aphids for oviposition. Similarly, Kanuck and Sullivan (1992) found a 82% preference for mummy in petri dish choice tests for this species. The host defensive behaviour contributed to host preference in *S. aphidivorus*, as shown for a number of primary parasitoid species (Harvey and Thompson 1995, Brodeur *et al*. 1996, Lauzière *et al*. 2001), including aphid parasitoids (Gerling *et al*. 1990). When parasitised aphids were immobilised, although acceptance rose from 29% to 79%, preference for the mummy host was still predominant. More detailed observations indicated that aphid behaviours (moving away, kicking) interfere mostly during examination. The hyperparasitoid oviposition sequence is rarely interrupted once a female has successfully mounted the aphid.

3.6.3. Origin and Benefits of a Dual Oviposition Behaviour

The ability of *S. aphidivorus* to parasitise two different hosts stages and to develop either as a larval-pupal or a pupal hyperparasitoid is unique among aphid hyperparasitoids. Females have the ability to find and recognize both parasitised aphids and mummies (R.B., unpublished data). They possess an ovipositor that can either drill a hole in a mummy or stab the live aphid and locate the parasitoid larva within the aphid abdomen. Also of interest, the larva first develops as an endophagous parasite, but feeds ectophagously in later larval stages (Kanuck and Sullivan 1992). All other aphid hyperparasitoid species are either koinobiont endophagous larval-pupal parasitoids that attack host larvae in aphids

before mummification, or idiobiont ectophagous pupal parasitoids that attack the host pupae after the mummy is formed (Sullivan, 1987). This atypical dual pattern of oviposition and development might indicate a transitional state from ectoparasitism of hosts within aphid mummies to endoparasitism of hosts within parasitised aphids. Although classified as a koinobiont endoparasitoid, *S. aphidivorus* shows several attributes of many idiobiont ectoparasitoids (Quicke 1997), e.g. attacking sessile hosts, broad host range (Hoffer and Stary 1970, Völkl and Barczak 1990), large eggs (Griswold 1929), and host feeding (Kanuck and Sullivan 1992). Of significance, large eggs in endoparasitoids may reflect how recent the shift from ecto- to endoparasitism has occurred in a given taxon, large eggs being generally associated with ectoparasitism (Strand 2000). On the other hand, *S. aphidivorus* does not exhibit typical behavioural adaptations of hyperparasitoids that attack parasitised aphid hosts. For example, once a potential host has been located, *Alloxysta victrix* (Westwood) (Hymenoptera: Charipidae) appease the aphid by antennal stroking and the secretion of a chemical before mounting it to hyperparasitise (Petersen 2000). Furthermore, unless there is a large advantage of host availability, the poor performance of *S. aphidivorus* on parasitised aphids is unlikely to lead to a host switch. A better knowledge of the phylogeny and natural history of *S. aphidivorus* is a prerequisite to further test the hypothesis of an evolutionary transition from idiobiont ectophagous pupal parasitism to koinobiont endophagous larval-pupal parasitism.

Besides the transition hypothesis, the dual oviposition behaviour of *S. aphidivorus* might also be an evolved strategy to host distribution. Aphid mummies and parasitised aphids can either be found within or near the aphid colony (Brodeu and McNeil 1989, Müller *et al*. 1997) and both hosts could be simultaneously encountered on plants. The ability to attack parasitised aphid and mummy hosts may therefore provide a larger range of potential hosts to foraging *S. aphidivorus* females. As with all other parasitoids, host selection would be determined by the physiological state of the *S. aphidivorus* female and the ecological differences that exist between host stages: suitability, nutritional value, vulnerability to natural enemies, and abundance (Vinson 1984, Bell 1990).

Finally, the dual oviposition behaviour may contribute to reduce competition for hosts between *S. aphidivorus* and other aphid hyperparasitoids, mainly those that attack mummified aphids. Several studies examined aspects of interspecific competition between endo- and ectohyperparasitoid species (Sullivan 1972, Matejko and Sullivan 1984) and between two ectohyperparasitoids (Carew and Sullivan 1993). Competition may occur between wasps during the host selection process or through interactions between immature parasitoids. Ovipositing females can reduce the chance that other females later attack the host by marking it (Roitberg and Mangel 1988), while a parasitoid larva can eliminate a competitor by way of physical attack, chemical suppression or resource competition (Mackauer 1990). Whatever the mechanisms, the outcome frequently depends on the sequence of events: the first female to oviposit, or the first larva to emerge usually wins the competition. For example, Sullivan (1972) and Matejko and Sullivan (1984) examined interspecific larval competition between different associations of two *Alloxysta* species, which attack parasitised aphids, and two mummy attacking hyperparasitoids, *Asaphes californicus* and *Dendrocerus carpenteri*. They concluded that the *Alloxysta* species usually win competition with hyperparasitoids that attack the aphid mummywhen the latter oviposit in older mummies, containing an *Alloxysta* (pre-) pupa. Similarly, ovipositing in parasitised aphids would partially secure the host and could therefore provide a competitive advantage to *S. aphidivorus* over species attacking aphid mummies.

It is unclear whether the atypical dual oviposition behaviour of *S. aphidivorus*, as well as its fitness consequences, as observed here can be extrapolated to predict patterns of host use and interactions with competitors and natural enemies under field conditions. *Syrphophagous aphidivorus* is ubiquitous in many agricultural and natural systems (Sullivan and van den Bosch 1971, Mertins 1985, Völkl and Barczak 1990). This ubiquity might partly be due to its capacity to attack both parasitised aphid and mummy hosts.

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Table 3-1 Life history parameters and intrinsic rate of increase of male and female *Syrphophagus aphidivorus* developing in mummies or parasitised aphids containing *Aphidius nigripes*. Values between parentheses are standard errors. P values refer to statistical differences between hosts.

		***M. euphorbiae* host**				
	Sex	**N**	**Mummy**	**N**	**Parasitised aphid**	***P* value**
Development time (days)[1]	♀	172	17.7 (0.1)	88	19.6 (0.1)	$P < 0.0001$ [2]
	♂	96	17.4 (0.1)	45	19.8 (0.2)	$P < 0.0001$ [2]
Longevity (days)	♀	50	37.4 (3.2)	33	47.3 (1.5)	$P < 0.0001$ [2]
	♂	65	22.2 (1.8)	45	29.6 (0.9)	$P = 0.0281$ [2]
Lifetime fecundity (dead + live offspring)		11 [3]	623 (55)	7 [3]	307 (53)	$P = 0.0013$ [4]
Immature mortality(%)		11 [3]	7.8 (0.9)	7 [3]	15.0 (3.9)	$P = 0.0567$ [4]
Sex ratio (% male)		11 [3]	70.2	7 [3]	49.4	$P < 0.0001$ [5]
Intrinsic rate of increase (r_m)(d^{-1})			0.2494		0.1895	

[1] Development from oviposition to adult emergence.
[2] Two-way ANOVA followed by the Least Significant Difference procedure.
[3] N represents the number of females. Per female, the total of all offspring was used to calculate the lifetime fecundity, immature mortality and sex ratio.
[4] Student's *t*-test
5 χ^2 test

Table 3-2 Size of adult male and female *Syrphophagus aphidivorus* developing on *Aphidius nigripes* host available as aphid mummies or parasitised aphids. Values between parentheses are standard errors. P values refer to statistical differences between hosts.

		***M. euphorbiae* host**			
	Sex	**N**	**Mummy**	**N**	**Parasitised Aphid**	**P value [1]**
Dry weight (mg)	♀	85	0.06 (0.01)	84	0.04 (0.01)	P < 0.0001
	♂	19	0.05 (0.01)	22	0.04 (0.01)	P = 0.6511
Head width (mm)	♀	84	0.42 (0.01)	84	0.43 (0.02)	P = 0.3612 [2]
	♂	19	0.39 (0.02)	21	0.34 (0.03)	P = 0.6132 [2]
Wing length (mm)	♀	85	1.15 (0.03)	83	1.07 (0.03)	P = 0.0001
	♂	19	1.03 (0.03)	21	1.03 (0.03)	P = 0.4569

[1] Two way ANOVA followed by the Least Significant Difference procedure.
[2] Data were rank transformed prior to the analysis.

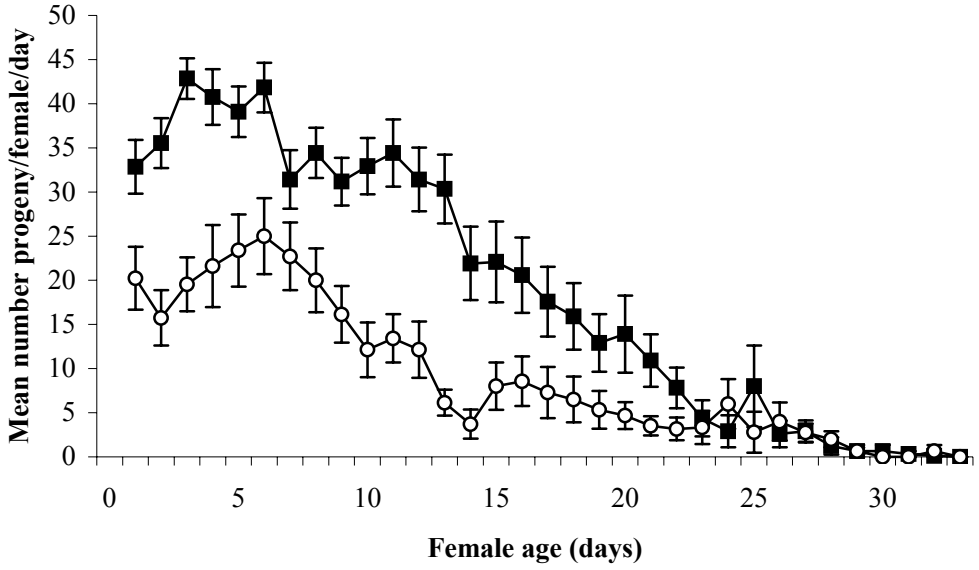

Figure 3-1 Daily fecundity of *Syrphophagus aphidivorus* females that developed on *Aphidius nigripes* available as mummies (■) or parasitised aphids (○) (means ± SE). Throughout their life, females were provided with the mummy host.

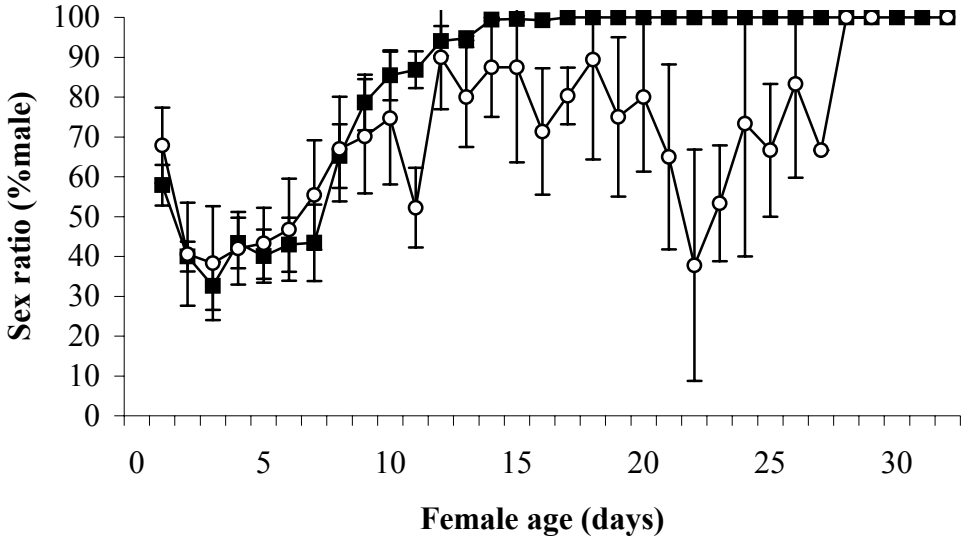

Figure 3-2 Secondary sex ratio (% males) of progeny of *Syrphophagus aphidivorus* females that developed on *Aphidius nigripes* available as mummies (■) or parasitised aphids (○) (means ± SE). Throughout their life, females were provided with the mummy host.

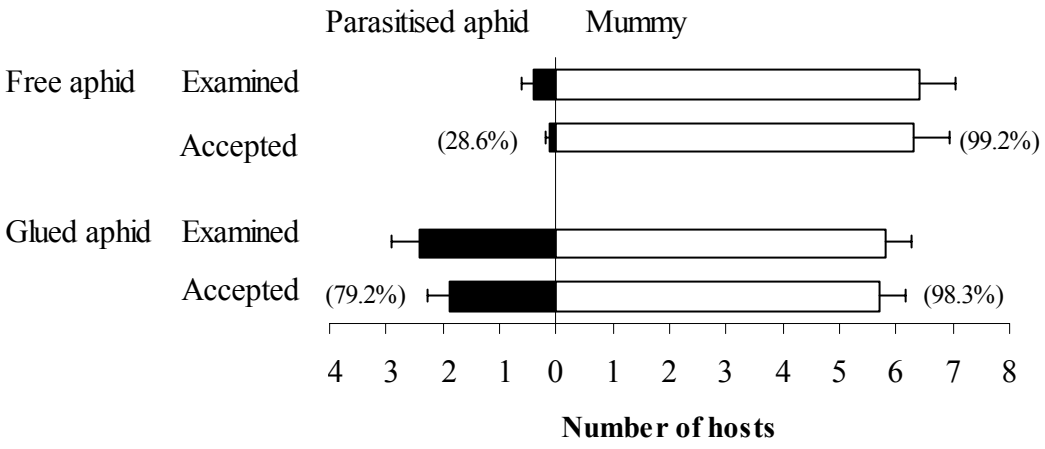

Figure 3-3 Mean number of hosts examined and accepted by a female *Syrphophagus aphidivorus*, searching in a patch of *Aphidius nigripes* hosts available as 10 mummies plus 10 parasitised aphids. Subsequent encounters with the same host are excluded. Percent examined hosts accepted for oviposition indicated in parentheses. I-bars refer to standard errors. Free aphids, n = 20 females; glued aphids, n = 10 females.

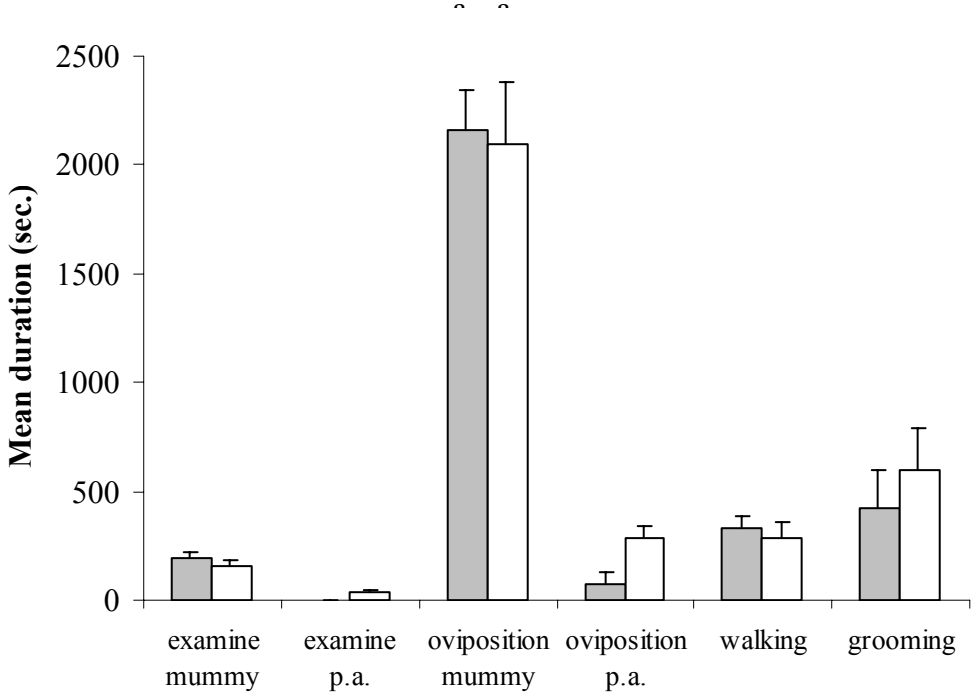

Figure 3-4 Total time (seconds, mean + standard error) allocated to different behaviours by *Syrphophagus aphidivorus* females on a patch of host *Aphidius nigripes*, available as 10 mummies plus 10 parasitised aphids. ■: experiment with free parasitised aphids; □: experiment with immobilised (glued) parasitised aphids. Means (± SE) of a given behaviour followed by different letters are significantly different between experimental conditions (t-tests). (p.a. = parasitised aphid).

Chapter 4.

Foraging behaviour on the fourth trophic level:

a comparative study of host location in aphid hyperparasitoids.

4.1. Abstract

In studies of foraging behaviour in a multitrophic context, the fourth trophic level has generally been ignored. We used four aphid hyperparasitoid species, *Dendrocerus carpenteri* (Curtis) (Hymenoptera: Megaspilidae), *Asaphes suspensus* Walker (Hymenoptera: Pteromalidae), *Alloxysta victrix* (Westwood) (Hymenoptera: Alloxystidae) and *Syrphophagus aphidivorus* (Mayr) (Hymenoptera: Encyrtidae), to correlate their response to different cues with their ecological attributes such as host range and host stage. In addition, we compared our results with studies of primary parasitoids on the same plant-herbivore system. First, the olfactory response of females was tested in a Y-tube olfactometer (single choice: plant, aphid, honeydew, parasitised aphid, aphid mummy and virgin female parasitoid; dual choice: clean plant, plant with aphids or plant-host complex). Second, their foraging behaviour and pattern was described on plants with different stimuli (honeydew, aphids, parasitised aphids and aphid mummies). The results indicated that olfactory cues are not essential cues for hyperparasitoid females. In foraging behaviour on the plant, all species prolonged total visit time and search time as compared to the control treatment (clean plant). Only *A. victrix* did not react to honeydew. Ovipositions in mummies prolonged total visit time because of their long handling time, but the effect of this behaviour on search time could not be determined. No clear correlation between foraging behaviour and host stage or host range was found. In contrast to specialised primary aphid parasitoids that have strong fixed responses to specific kairomones and herbivore-induced synomones, more generalist aphid hyperparasitoids seem to depend less on volatile olfactory stimuli, but show similarities with primary parasitoids in their use of contact cues while searching on a plant.

4.2. Résumé

Dans les études du comportement de recherche en contexte multitrophique, le quatrième niveau trophique a généralement été ignoré. Nous avons utilisé quatre espèces d'hyperparasitoïdes de pucerons, *Dendrocerus carpenteri* (Curtis) (Hymenoptera: Megaspilidae), *Asaphes suspensus* Walker (Hymenoptera: Pteromalidae), *Alloxysta victrix* (Westwood) (Hymenoptera: Alloxystidae) et *Syrphophagus aphidivorus* (Mayr) (Hymenoptera: Encyrtidae), pour correler leur réponse à différents stimuli avec des facteurs écologiques incluant la spécificité parasitaire et le stade d'hôte. En plus, nous avons comparé nos résultats à ceux d'études des parasitoïdes primaires du même système plante-herbivore-parasitoïde. Premièrement, la réponse olfactive des femelles a été testée dans un olfactomètre en Y (choix simple: plante, puceron, miellat, puceron parasité, puceron momifié et femelles parasitoïdes; choix double: plante non-contaminée, plante avec pucerons ou complexe plante-hôte). Deuxièmement, leur comportement et patron de recherche ont été observés sur des plantes avec différents stimuli (miellat, puceron, puceron parasité et puceron momifié). Les résultats indiquent que les femelles hyperparasitoïdes n'étaient pas attirées par les stimuli olfactifs. Sur la plante, les femelles de toutes les espèces ont prolongé leur temps de visite total et leur temps de recherche, comparé au traitement témoin (plante non-contaminée), excepté *A. victrix* qui n'a pas réagi au miellat. L'oviposition dans les momies a prolongé le temps de résidence total, à cause du temps accru de manipulation, mais l'effet de ce comportement sur le temps de recherche n'a pas été déterminé. Nous n'avons pas trouvé de corrélation du comportement avec le stade d'hôte ou la spécificité parasitaire de ces hyperparasitoïdes. Contrairement aux parasitoïdes primaires, les hyperparasitoïdes de pucerons sont plus généralistes et semblent moins dépendants des stimuli olfactifs volatiles. Par contre, ils font un usage similaire de stimuli de contact dans leur recherche sur une plante.

4.3. Introduction

In the last two decades, much interest has been given to the foraging behaviour of natural enemies in a multitrophic context. Insect parasitoids are known to be influenced by cues from different trophic levels to find their herbivore hosts. Among these cues are plant volatiles, herbivore induced volatiles, and direct and indirect cues from the hosts (Vet *et al.*, 1995; Vinson, 1998). Their strategy is to zoom in on long distance cues, thereby slowly confining their search area, shifting from long range cues to short range cues. Within this gradual transition we usually observe a shift from indirect, often unreliable cues, such as plant cues, to more direct and reliable cues, such as contact chemicals directly derived from the host itself, thereby increasing the chance of locating the host (Vet *et al.*, 2002).

Parasitoids attacking herbivores are not necessarily the highest trophic level of vertical foodwebs. In many systems there are one or more higher trophic levels, exploiting the parasitoids, for example hymenopterous hyperparasitoids. Although the degree of similarity between primary and secondary (or hyper-) parasitoids is obvious because of their common evolutionary origins and life-history strategies, hyperparasitoids are likely to possess specific biological attributes enabling them to exploit resources from the third trophic level (Brodeur, 2000). To find their host, hyperparasitoids can potentially make use of many cues from all trophic levels. However, as yet we have very little insight concerning cues that are actually used by hyperparasitoids. Another intriguing and unanswered question is whether hyperparasitoids use the same host search strategies as primary parasitoids.

The present study aims at shedding light on the unknown searching behaviour of hyperparasitoids and make comparisons with the behaviour of primary parasitoids and between different hyperparasitoid species. Aphid hyperparasitoids are an ideal model as their host relations are relatively well known and they include a great diversity of species with different life histories and host ranges (Sullivan, 1987; Sullivan and Völkl, 1999). Using a comparative approach, we studied the host search behaviour of four obligate hyperparasitoid species from four different families. *Dendrocerus carpenteri* (Curtis) (Megaspilidae) and *Asaphes suspensus* Walker (Pteromalidae) are generalist ectophagous idiobiont hyperparasitoids that attack the prepupae or the pupa of the primary parasitoid

after it has killed and mummified the aphid (mummy host). In contrast, *Alloxysta victrix* (Westwood) (Alloxystidae) is an endophagous koinobiont hyperparasitoid that lays an egg in the parasitoid larva in the still-living aphid (parasitised aphid host), where it remains to hatch only after mummification of the aphid. The host range of hyperparasitoids of this family is more restricted than that of idiobiont hyperparasitoids (van den Bosch, 1981; Sullivan and Völkl, 1999; Brodeur, 2000). Finally, *Syrphophagus aphidivorus* (Mayr) (Encyrtidae) is also an endophagous koinobiont, but it has a dual oviposition behaviour. It attacks both parasitoid larvae in live aphids and parasitoid prepupae or pupae in mummified aphids. The latter are preferred as they are more suitable hosts for development (Kanuck and Sullivan, 1992; Buitenhuis *et al.*, submitted). Furthermore, the encyrtid hyperparasitoids have been reported to attack many different parasitoids of aphids (Aphididae) and even psyllae (Psyllidae) (Hoffer and Stary, 1970).

We tested the prediction that the relatively host specific alloxystid hyperparasitoid uses general cues associated with aphids (aphids and honeydew), and specific cues from primary parasitoid females and/or host plant volatiles from the specific plant – aphid – host system (Sullivan and Völkl, 1999). By contrast, ecto-hyperparasitoids with a broad host range are predicted to depend less on specific cues, and to use general cues associated with aphids (aphids and honeydew) and aphid mummies on different plant – aphid – host systems (Sullivan and Völkl, 1999). The species with the dual oviposition behaviour, *S. aphidivorus*, is predicted to resemble the ecto-hyperparasitoids because of its broad host range and its preference for mummies.

We focussed on two components of foraging behaviour, attraction by olfactory stimuli and behavioural modification by contact stimuli on a plant. The use of olfaction by aphid hyperparasitoids was studied by testing different potentially attractive odours in a Y-tube olfactometer. Odours from all trophic levels were included, such as plant, aphid, female parasitoid, parasitised aphid and mummified aphid odours, as well as the aphid fecal waste product, honeydew. Furthermore, plant odours possibly induced by aphids and the attraction of the whole plant-aphid-host complex were tested. A second experiment tested the influence of different short distance cues such as honeydew, aphids, parasitised aphids

and mummified aphids, on the search behaviour of hyperparasitoids. The behaviour of females was observed while they were searching on a plant that was treated with one or more of these cues.

4.4. Materials and Methods

4.4.1. Insect material

Colonies of the four hyperparasitoids were established on the primary parasitoid *Aphidius nigripes* Ashmead. This parasitoid was reared on the potato aphid *Macrosiphum euphorbiae* (Thomas) on potato seedlings, *Solanum tuberosum* L. cv. Norland according to techniques of Brodeur and McNeil (1994). All four hyperparasitoids have been reported in the field on this experimental system (Shands, 1965; Brodeur and McNeil, 1994). The hyperparasitoid *A. victrix* originated from a laboratory strain in Newport, England, *A. suspensus* from a field population in Quebec, Canada, *D. carpenteri* from a laboratory strain in Burnaby, Canada. and *S. aphidivorus* from a laboratory strain in Bayreuth, Germany. All insects had been held in the laboratory for more than ten generations before being used in the experiments.

Hyperparasitoid colonies were maintained by exposing potato plants, infested with mummified aphids (for *A. suspensus*, *D. carpenteri* and *S. aphidivorus*) or live parasitised aphids (for *A. victrix*) to the hyperparasitoid females. Colonies were held in the laboratory at room temperature under a 16L:8D photoperiod.

For both experiments, hyperparasitised mummies were individually collected in the rearing colonies, and kept as groups of 100 mummies in a cage with a vial of sugar water as a food source at 20±1ºC, 75±10% RH, under a 16L:8D photoperiod. Males were added to ensure that at emergence females had access to potential mates. From these cages 1-6 day old females were taken for use in the bioassays. As these hyperparasitoids live more than 1 month under these experimental conditions (Christiansen-Weniger, 1992; Chow and Mackauer, 1996; R. Buitenhuis, unpublished data), females were not time-limited.

To obtain parasitised aphids and mummies for biossays, third-instar aphid nymphs were exposed to parasitism by 3-5 days old mated *A. nigripes* females for a 24-hr period. Presumably parasitised aphids were then reared at 20±1ºC, 75±10% RH, under a 16L:8D photoperiod. Based on embryonic and larval developmental times of *A. nigripes* at 20ºC (Paré *et al.* 1979), third instar larvae in living aphids and prepupae in mummified aphids,

were obtained five and eight days following parasitisation, respectively. In the text these hosts will be referred to as parasitised aphids and mummies.

4.4.2. Olfaction

Experimental set-up. Tests were carried out at room temperature (20-22ºC) in a Y-tube olfactometer (3.6 cm diameter, length of the arms 30 cm, distance until junction of the arms 17.5 cm). For each arm, air was pumped through activated charcoal, humidified, adjusted to 4 cm/s (0.53 l/min) with an air flow meter (Omega© FL-1405), and led through a chamber containing the odour source. The air speed was chosen based on similar studies of primary and hyperparasitoids of aphids (Bouchard and Cloutier, 1985; Singh and Srivastava, 1987b). All parts of the apparatus were connected using Tygon© tubing. The Y-tube was placed in a black box and its Y-end was oriented towards the one semi-transparent side, behind which a light source was placed (circular Philips 22W cool white fluorescent tube).

To ensure the functionality of the olfactometer, two types of pre-tests were done. When both arms carried clean air only, hyperparasitoids (*A. victrix*, *A. suspensus* and *S. aphidivorus*) chose each of them at the same frequency (χ^2 test, for all species, $p>0.05$, $n>20$). In the second pre-test, males of the primary parasitoid *A. nigripes* chose significantly more often the arm of the olfactometer with conspecific virgin females, against clean air in the other arm ($\chi^2=7.6190$, $p=0.0058$, $n=26$ males).

Treatments. Treatments were chosen according to the quantities and concentrations that were shown to be attractive to primary parasitoids and hyperparasitoids (Read *et al*., 1970; Bouchard and Cloutier, 1985; Siri, 1993).

(1) Single cues originating from all trophic levels.

From the first trophic level, we tested a clean potato seedling (Norland variety). A 15 cm high plant was washed, air dried, cut and immersed in water sealed with Parafilm© to exclude possible interference of volatiles from the cut edges. From the second trophic level, we tested potato aphids. One hundred aphids of all stages were collected in a gauze-covered container. In addition we tested honeydew that was collected as described by Bouchard and Cloutier (1984) (40 mg dried honeydew dissolved in 150 µl distilled water). Finally, from

the third trophic level, we tested parasitised aphids, mummies or female *A. nigripes*. For these treatments either 100 4-5 days parasitised aphids, 100 newly (0-24 h) mummified aphids, or six 1-5 days old virgin *A. nigripes* females were collected in a gauze covered container. Odours were tested in single choice tests against air (pumped through activated charcoal and humidified). A dual-choice test was performed for *S. aphidivorus* to determine preference for mummies vs. parasitised aphids.

(2) Complex cues.

Aphid and possible aphid-induced plant volatiles were tested with a potato seedling infested with 50 potato aphids two days before the test. The attraction of the whole plant-host complex was tested with a potato seedling infested for two days with 25 healthy aphids, 25 parasitised aphids and 25 mummies, obtained as previously described. Mummies were glued on the leaves with non-toxic Lepage© white glue before the experiment. To exclude the possibility that hyperparasitoids were attracted to uninfested plant odours, the plant-host complex was tested in a dual choice test against a clean plant (washed and air dried potato seedling).

Bioassay. Mated 1-6 days old hyperparasitoid females were given an oviposition experience of 24 hours the day before the test with ten mummies and five live parasitised aphids on a potato leaf to standardise their searching and parasitising experience before the test. The females were individually released in the Y-tube, and used only once. After five minutes the position of the female was recorded. This duration was shown to be sufficient for the majority of the females to make a choice. If a female was found more than 15 cm into one of the arms of the olfactometer, this was recorded as a choice. Females recovered before this point, and at or before the intersection of the olfactometer arms were not considered to have made a choice. Effectively, in the experiment, females were either found at the end of the tube, or at the intersection. The Y-tube and the containers for the odour sources were washed with hot water and acetone and air-dried between each treatment. For each experiment (single and dual choice), all treatments were tested in a random order in a two-day period. In each treatment, five females per hyperparasitoid species were tested in a

random order. This was repeated eight times for a total of 40 females per species per treatment.

4.4.3. Foraging behaviour

Experimental set-up. Observations of the influence of several potential cues on the foraging behaviour of hyperparasitoid females were made on 'Norland' potato plants under fluorescent lightning. All plants were selected to have ten leaves (numbered from the base to the top), the same height (20-25 cm) and roughly the same shape and leaf surface area. A protocol similar to that of Cloutier and Bauduin (1990) was designed in order to compare the behaviour of primary parasitoids and hyperparasitoids on the same plant-aphid system.

Treatments. Each plant was randomly allocated to one of the following treatments: control (uncontaminated plant), aphids (plant infested with 100 aphids for two days), honeydew (plant infested with 100 aphids for two days after which aphids and exuviae were removed with a paintbrush before the experiment), aphids + parasitised aphids (PA) (plant infested for two days with 50 aphids and 50 parasitised aphids) and plant-host complex (PHC) (aphids + parasitised aphids, and two mummies glued on the underside of leaves 4, 6 and 8). Parasitised aphids were marked on the abdomen with a non-toxic marker (Sharpie©) to distinguish them from unparasitised aphids during observations. This did not seem to disturb the aphids or to change their behaviour.

Bioassay. Mated 1-6 days old females were given an oviposition experience of 24 hours the day before the test, individually in cages with a potato leaf with hosts (for *A. suspensus* and *D. carpenteri* two mummies; *S. aphidivorus* two mummies and two parasitised aphids; *A. victrix* two parasitised aphids), before being used in the experiments.

At the beginning of a test, one female was released from a gelatine capsule on the upper side of leaf number 4. Her behaviour was observed with the Observer© (Noldus, 1997, version 3 for Macintosh) for one hour, or until the female left the plant for more than 5 seconds. One plant was used for one female of each hyperparasitoid species. Hosts that were parasitised by a hyperparasitoid female were replaced after each observation.

The duration of the following behaviours was recorded: walking, resting, grooming, feeding, flying, examining (aphid, parasitised aphid or mummy) and ovipositing (aphid, parasitised aphid or mummy). Furthermore the position of the female was recorded continuously by noting the leaf number and plant part (upper- or under side of the leaf, petiole or stem).

The order in which the hyperparasitoid species were tested was randomised within treatments. Ten females of each species were tested per treatment. Because the treatment with the parasitised aphids and the plant-host complex were the same for *A. victrix*, this species was not tested on the plant with parasitised aphids but only on the plant-host complex.

From the timetable that was created by the Observer© the following parameters were calculated: The total visit time was defined as the time spent on the plant from release to departure. The search time was defined as the time spent walking. The search time was subdivided between time spent on the upper and lower surface of the leaves. Aphid hyperparasitoids have long handling times of several minutes per host (Sullivan, 1987). The handling time was defined as the total time that a female spent examining and parasitising hosts during the visit. Finally, the number of different leaves that were visited was calculated.

Only females that had come in contact with the offered stimuli were used in the analysis. Also, observations where females immediately left the plant after aphid defence were discarded from the analysis because these did not represent a comparable visit (max. 2 cases out of 10 for *S. aphidivorus* where the aphid kicked and caused the female to fly up).

4.4.4. Statistical analysis

The results of the olfactometer experiment were analysed using a χ^2 test, as the number of females that chose a certain odour was never lower than five.

Because of the presence of censored data in the foraging behaviour, the visit time and search time, these data were analysed per species using the LIFEREG procedure, using a log-normal error function. The number of leaves visited was analysed with General Linear

Models (GENMOD) using a Poisson error function. The time spent on the upper- and under side of the leaves was compared with a paired t-test.

For all analyses, the level of significance was α=0.05 and all data were analysed using SAS (SAS, 1999).

4.5. Results

4.5.1. Olfaction

Overall, 82% (min. 60%, max. 97% in any comparison) of the females made a choice for either of the two olfactometer-arms. However, statistical tests showed that there was no significant preference of the four hyperparasitoid species for any odour source (Fig. 4-1a-c), except for *S. aphidivorus* that showed a preference for the odour of parasitised aphids over that of mummies in the dual choice test (χ^2=4.5151 , p=0.0336) (Fig. 4-1b).

4.5.2. Foraging behaviour

The total visit time of females of all hyperparasitoid species was affected by the different plant treatments (LIFEREG *A. victrix*: χ^2=12.9934, df=3, p=0.0047; *A. suspensus*: χ^2=11.9707, df=4, p=0.0176; *D. carpenteri*: χ^2=42.3305, df=4, p<0.0001; *S. aphidivorus*: χ^2=47.0480, df=4, p<0.0001) (Fig. 2a). For all species, females tended to prolong total visit time with increasing complexity of the stimuli.

The total visit time (Fig. 4-2a) was divided in three categories of behaviours (Fig. 4-2b-d): search time, time spent with hosts (examining and ovipositing into parasitised aphids or mummies), and other behaviours (resting, grooming, flying, feeding and examining healthy aphids).

The search time was influenced by different stimuli for all hyperparasitoid species (LIFEREG *A. victrix*: χ^2=16.0711, df=3, p=0.0011; *A. suspensus*: χ^2=16.0553, df=4, p=0.0029; *D. carpenteri*: χ^2=38.9377, df=4, p<0.0001; *S. aphidivorus*: χ^2=36.8214, df=4, p<0.0001) (Fig. 4-2b). Female *A. victrix* searched longer on plants with aphids and on the plant-host complex than on the other plant treatments. The other three species searched longer on all treatments as compared to the control. Female *D. carpenteri* searched the longest time on plants with honeydew and the plant–host complex. Search time of *S. aphidivorus* females was significantly longer on plants with parasitised aphids as compared to the other treatments.

The long total visit times of females of the three mummy-attacking hyperparasitoids on plants with their hosts were actually caused by the time spent with mummies (Fig. 4-2c). *Asaphes suspensus* spent 60 ± 4% (mean ± SE) of the total visit time examining and parasitising mummies, *D. carpenteri* 31 ± 21%, and *S. aphidivorus* 42 ± 18%. The time required to parasitise a mummy was very long (*A. suspensus* 1888 ± 204 s, *D. carpenteri* 462 ± 312 s and *S. aphidivorus* 390 ± 138 s). In contrast, time spent with parasitised aphids did not take such a substantial proportion of total visit time (Fig. 4-2c). *Alloxysta victrix* spent time parasitising its hosts (larvae within live aphids) for only 7 ± 9% of the total visit time, and *S. aphidivorus,* 10 ± 15% (PA) and 2 ± 2% (PHC). The time required to parasitise a host within a parasitised aphid was only 96 ± 18 s for *A. victrix,* and 102 ± 48 s for *S. aphidivorus*.

When hosts were present, a significant proportion of females stayed on the plant for the whole duration of the experiment (1 hour; *A. suspensus* 100%, *A. victrix* 20%, *D. carpenteri* 70%, *S. aphidivorus* (PA) 71%, *S. aphidivorus* (PHC) 43%). It is likely that in these cases visit and search time would have been longer if the experiment would have been permitted to last longer.

The number of leaves that was visited was generally small as compared to the number of leaves available (10). There were differences in the number of leaves visited between species, and between some treatments (2-way GENMOD, treatment $\chi^2 = 11.43$, df = 4, p=0.0221, species, $\chi^2 = 41.48$, df = 3, p<0.0001, treatment*species $\chi^2 = 30.87$, df = 11, p=0.0012) (Table 4-1). In general, *D. carpenteri* visited more leaves than the other three species. For each species, the differences in number of leaves visited between the treatments are similar to the results for search time. *Asaphes suspensus* visited an equal number of leaves in each treatment, *A. victrix* visited more leaves on the plant with aphids, *D. carpenteri* visited more leaves on the honeydew, parasitised aphid and plant-host complex treatments and *S. aphidivorus* visited more leaves on the parasitised aphid and plant-host complex treatments as compared to the control.

After release, female hyperparasitoids explored the plant mainly by walking. Only occasionally were females observed to use short flights to move between the leaves (1.1 ±

0.2 SE flights·female^{-1}·observation^{-1}). Females searched both sides of leaves, often alternating rapidly between the upper and under sides. The time allocated to searching on the upper and lower surfaces of leaves did not differ significantly for any species or treatment, except for *A. victrix* (Table 4-1), which searched longer on the upper than the lower surface of leaves on honeydew-contaminated plants (paired t-test t_8=4.59, p=0.0018). When visiting different leaves, *A. suspensus* moved slightly upward on the plant in all treatments. *Alloxysta victrix* always moved to the highest leaves before taking off. *Dendrocerus carpenteri* and *S. aphidivorus* moved up and down on the plant without a clear pattern.

4.6. Discussion

Our results indicate that airborne olfactory cues are not essential cues in host search by the four aphid hyperparasitoids studied here, while cues that are encountered on a plant do provide information that induces searching in most species.

4.6.1. Olfaction

Even though the hyperparasitoid females had been given an oviposition experience before the test, the odours of the potato - *M. euphorbiae* - *A. nigipes* system that we offered in the olfactometer apparently were not attractive to females.

Although we cannot exclude the doubt that our olfactometer set-up was not functional for aphid hyperparasitoids, several arguments imply that our results are valid. First, a similar set-up has been used successfully for aphid hyperparasitoids before (Read *et al*., 1970; Singh and Shrivastava, 1987a/b; Siri, 1993). Second, the pre-tests showed that the set-up was functional for primary parasitoids. Male *A. nigripes* was attracted to the odour of conspecific virgin females. Third, we obtained one positive response of *S. aphidivorus*, that was attracted to the odour of live parasitised aphids vs. aphid mummies. However, we cannot explain why *S. aphidivorus* preferred the odour of live parasitised aphids to that of aphid mummies in the dual choice test, while in the single choice test it was neither attracted nor repelled by any of these odour sources. Finally, attempts to test the hyperparasitoid species in a windtunnel with the same odour sources did not succeed, because females would not fly, even at low windspeeds.

Other studies, with similar set-ups, report varying results. *Alloxysta fuscicornis* (=*Charips brassicae*) was attracted to female primary parasitoids, but not to plant or aphid odours (Read *et al*., 1970). On the other hand, *Alloxysta pleuralis* is attracted to volatiles from various plants (Singh and Shrivastava, 1987a/b). Furthermore, *A. victrix* was attracted to herbivore-induced volatiles and a synthetic aphid alarm pheromone, and *D. carpenteri* was attracted to herbivore-induced volatiles, conspecific females and mummies, but neither species reacted to aphids, plants or primary parasitoid females (Siri, 1993).

The differences between these studies and our results might be explained by differences in the hyperparasitoid species that were tested, or may be due to differences in plant – aphid – primary parasitoid systems (oat – *Sitobion avenae* - *Aphidius uzbekistanicus*) (Siri, 1993). Also, in the above-mentioned studies, a confounding effect of attraction between females that were tested simultaneously in groups of 10-30 in the olfactometer cannot be excluded. While volatiles might play a role in host searching in some hyperparasitoid species, our results suggest that they are not strong cues in aphid hyperparasitoids.

4.6.2. Foraging behaviour

Once arrived on a plant, aphid hyperparasitoid females are arrested by aphid and host-derived stimuli. Honeydew acted as a search stimulant for *A. suspensus*, *D. carpenteri* and *S. aphidivorus*, but not for *A. victrix*. These results confirm that honeydew is a source of kairomones used in host finding by some hyperparasitoids (Budenberg, 1990; Buitenhuis *et al*., submitted), which was never previously demonstrated on a whole plant. When honeydew is offered on a filter paper disk or a glass slide, *A. victrix* is reported to be arrested (Budenberg, 1990; Grasswitz, 1998; Buitenhuis *et al*., submitted), which is in contrast with our findings here on a plant. The observed indifference of *A. victrix* towards honeydew might be caused by the relatively young age of the *A. victrix* females that were tested (mostly two days old). More recent experiments showed that this species has a pre-oviposition period of 2.1 days (Buitenhuis *et al*., unpublished). Consequently, older females of this species might be more stimulated to search and might show different behaviour.

As could be expected, *S. aphidivorus* females spent more time searching on plants with parasitised aphids, than on plants with unparasitised aphids. However, this was not observed on plants with mummies (plant-host complex). This is curious because, of the two hosts, mummies are reported to be the preferred and most suitable one (Kanuck and Sullivan, 1992; Buitenhuis *et al*., submitted). Perhaps the different proportions of parasitised aphids and mummies in the plant treatments had an influence on the females' perception of the patch. Further study will have to point out how oviposition in one of the two hosts influences searching time in this species.

The presence of hosts did prolong visit time in most cases, an effect that would probably be even stronger if females would be permitted to stay longer than 60 minutes on the plant. This increase in visit time was due to the long handling times of mummies (6 ± 2 to 32 ± 3 minutes) for *A. suspensus* and *D. carpenteri*. Longer search following successful oviposition (success-motivated search) could not be demonstrated in this experiment, but might be if females would be observed until they left the plant.

4.6.3. Influence of host stage and host range

There were no differences between species that correspond with the host (mummy vs. parasitised aphid). We found that airborne direct cues from the host were not detectable by olfaction. Therefore, to find hosts from a distance these hyperparasitoids would have to rely on indirect cues, which are the same for both hosts. In contrast, direct contact cues from the host probably play a greater role in the host acceptance phase where potential hosts are probably recognised by contact chemicals or by ovipositor probing (Christiansen-Weniger, 1992, 1994; Siri, 1993; Grasswitz, 1998; Grasswitz and Reese, 1998).

Another potential determinant of searching behaviour is the host range. Generally, the use of cues can be transposed on a specialist-generalist continuum: from intense and specific through weak and non-specific, to the absence of cue use (Vet and Dicke, 1992). Similarly, the four tested hyperparasitoids ranged from one relatively host specific species (*A. victrix*, attacking parasitoids of only one genus) to three species with a very large host range (*D. carpenteri*, *A. suspensus* and *S. aphidivorus*, attacking a wide variety of genera) (van den Bosch, 1981; Höller *et al*., 1993; Brodeur, 2000). However, in this study, no differences between hyperparasitoid species could be observed.

4.6.4. Differences between trophic levels

Do primary parasitoids and hyperparasitoids use the same host searching strategy? For several aphid primary parasitoids, attraction to olfactory cues from plants, plant-aphid complexes, aphids (Powell and Zhang, 1983; Bouchard and Cloutier, 1985; Wickremasinghe and van Emden, 1992; Reed *et al*., 1995; Du *et al*., 1996; Vaughn *et al*., 1996; Du *et al*., 1997; Völkl, 2000; Storeck *et al*., 2000), honeydew (Bouchard and Cloutier, 1985) and aphid sex pheromone (Powell *et al*., 1998; Glinwood *et al*., 1999) is

reported. On a plant, honeydew, aphids, aphid sex pheromone, and honeydew collecting ants arrest primary parasitoid females and induce them to search (Ayal, 1987; Cloutier and Bauduin, 1990; Powell *et al*., 1998; Völkl, 2000).

We designed this study for realistic comparison between the behaviour of aphid hyperparasitoids and the host search behaviour of the primary parasitoid *A. nigripes* (Bouchard and Cloutier, 1985; Cloutier and Bauduin, 1990) on the same potato – *Macrosiphum euphorbiae* system. Contrary to the behaviour of *A. nigripes* which was attracted to the odours of several aphid species and to aphid honeydew (Bouchard and Cloutier, 1985), none of the four hyperparasitoids was attracted to olfactory cues. On the other hand, there were similarities in the search behaviour on a plant of the primary parasitoid and hyperparasitoids. *Aphidius nigripes* showed longer residence and searching times, visited more leaves and spent more time per leaf in response to honeydew and aphids (Cloutier and Bauduin, 1990). This arrestment and search stimulation was also found in the hyperparasitoid species. Not all hyperparasitoids were arrested by honeydew, in contrast to what was found for *A. nigripes*. Both upper and lower leaf surfaces were searched equally by most hyperparasitoids, contrary to *A. nigripes* that searched more on the lower leaf surface, where it is more likely to find *M. euphorbiae* aphids.

In summary, our study suggests that aphid hyperparasitoids may not resemble primary parasitoids in attraction to olfactory stimuli, but it demonstrates that their behaviour on a plant shows several similarities, although this depends on the hyperparasitoid species. There are two non-exclusive explanations for differences between primary parasitoids and hyperparasitoids. First, many of the cues that are direct and reliable for primary parasitoids, are indirect cues for hyperparasitoids and therefore less reliable. First, the presence of aphids on a plant, a reliable cue for primary parasitoids, does not guarantee the presence of suitable parasitised aphids to hyperparasitoids. Secondly, compared to primary parasitoids, hyperparasitoids generally have a broader host range (Gordh, 1981; Sullivan, 1987; Sullivan and Völkl, 1999; but see van den Bosch, 1981 and Brodeur, 2000). Vet and Dicke (1992) hypothesised that contrary to specialists, the use of kairomones by generalists should be weak and non-specific, or could even be impossible because the great diversity of

potentially useful chemical information would generate a physiological constraint on sensory processing, and common chemical components would be very limited. The hyperparasitoids tested here have been reported on many different plants and aphids (e.g. Gutierrez and van den Bosch, 1970; Sullivan and van den Bosch, 1971; Johnson *et al*., 1979; Thiboldeaux *et al*., 1987; Mertins, 1985; Höller *et al*., 1993, Müller *et al*., 1999). In the absence of common, detectable cues it is therefore likely that aphid hyperparasitoids search mainly in the habitat where they are born, or select a habitat at random and that search is induced by contact stimuli on the plant.

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4.8. References

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Table 4-1 Number of leaves (mean ± SE) visited and total time spent on the upper and under leaf sides by four aphid hyperparasitoids searching on differently treated potato plants. Treatments are control (clean plant), honeydew (aphids removed from plant previously infested with *Macrosiphum euphorbiae*), aphids (plant infested with *M. euphorbiae*), parasitised aphids (plant infested with *M. euphorbiae* aphids, both unparasitised and parasitised by *Aphidius nigripes*), PHC (plant-host complex, as parasitised aphids plus *A. nigripes* mummies).

Species	**Treatment**	**N** [1]	**Nb. leaves visited**		**Total time on upper surface**	**Total time on under surface**
Alloxysta victrix	Control	10	1.9 ± 0.4	a[2]	188 ± 68	122 ± 66
	Honeydew	9	1.7 ± 0.4	a	206 ± 72	117 ± 50 *** [3]
	Aphid	7	3.3 ± 0.9	b	463 ± 93	495 ± 136
	PHC	5	2.0 ± 0.8	ab	204 ± 71	538 ± 195
Asaphes suspensus	Control	10	2.2 ± 0.9	a	187 ± 100	156 ± 99
	Honeydew	9	1.6 ± 0.2	a	342 ± 114	174 ± 37
	Aphid	4	3.5 ± 1.6	a	505 ± 187	464 ± 219
	Parasitised aphid	4	2.5 ± 1.0	a	599 ± 216	414 ± 170
	PHC	2	1.5 ± 0.5	a	320 ± 28	416 ± 92
Dendrocerus carpenteri	Control	10	3.0 ± 0.5	a	168 ± 37	109 ± 29
	Honeydew	10	6.5 ± 0.9	b	668 ± 111	576 ± 69
	Aphid	8	2.7 ± 1.0	a	485 ± 175	330 ± 163
	Parasitised aphid	10	5.2 ± 0.9	b	707 ± 146	429 ± 109
	PHC	10	6.0 ± 1.0	b	723 ± 107	599 ± 54
Syrphophagus aphidivorus	Control	10	1.4 ± 0.3	a	233 ± 100	100 ± 100
	Honeydew	10	2.5 ± 0.5	ab	604 ± 179	403 ± 69
	Aphid	6	3.2 ± 1.1	ab	567 ± 405	600 ± 197
	Parasitised aphid	7	4.4 ± 0.7	b	1073 ± 235	894 ± 160
	PHC	7	3.6 ± 0.9	b	405 ± 123	630 ± 165

[1] Number of females observed.
[2] Data were analysed with a GLM using a Poisson error function. Within species in the same column, means with the same letter do not differ significantly ($p>0.05$).
[3] Significantdifference between time spend on upper and under side of the leaf, paired t-test.

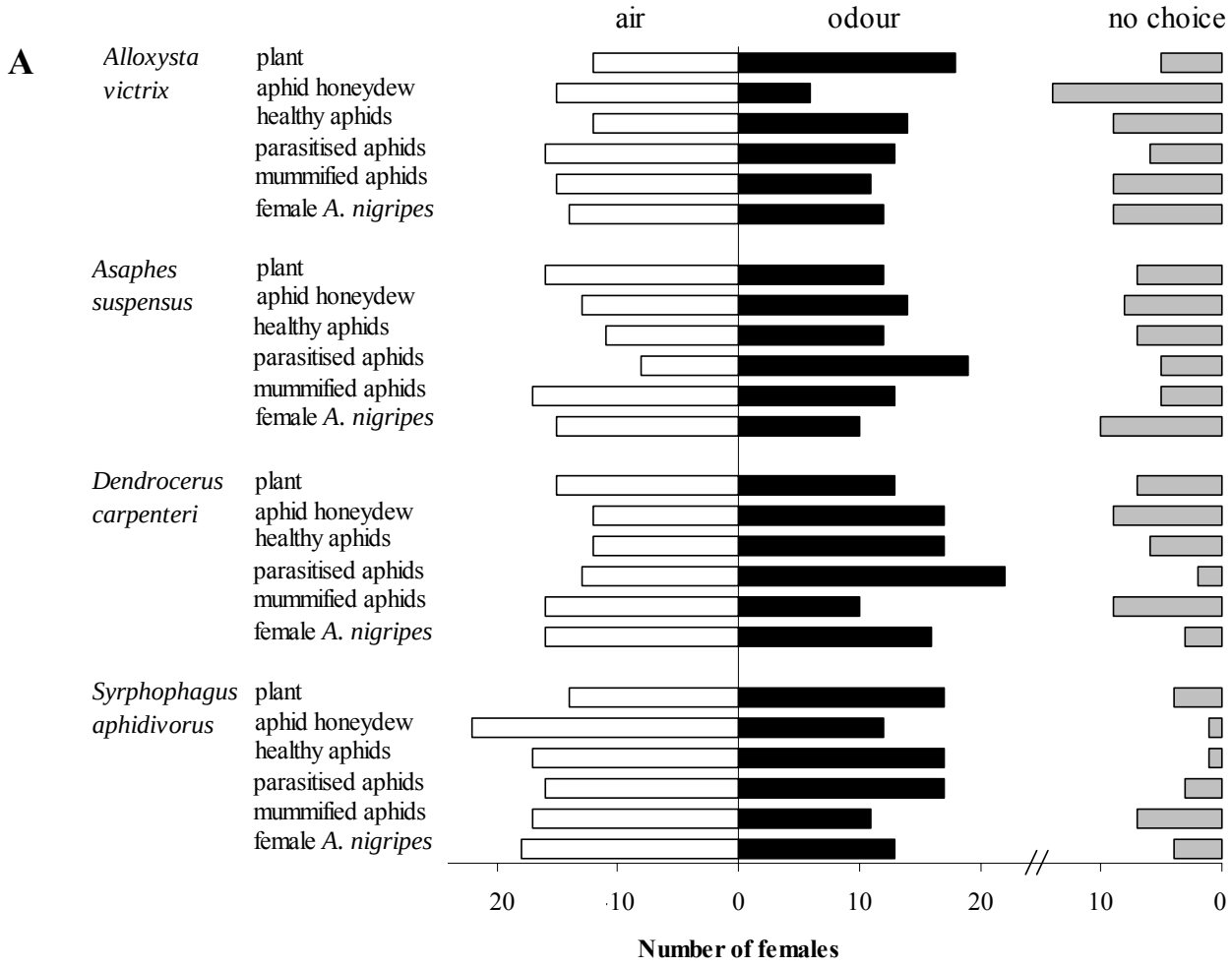
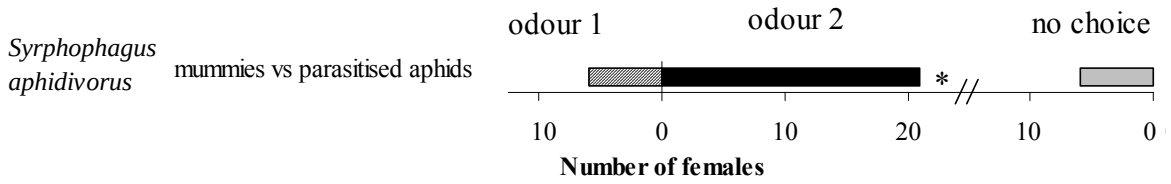
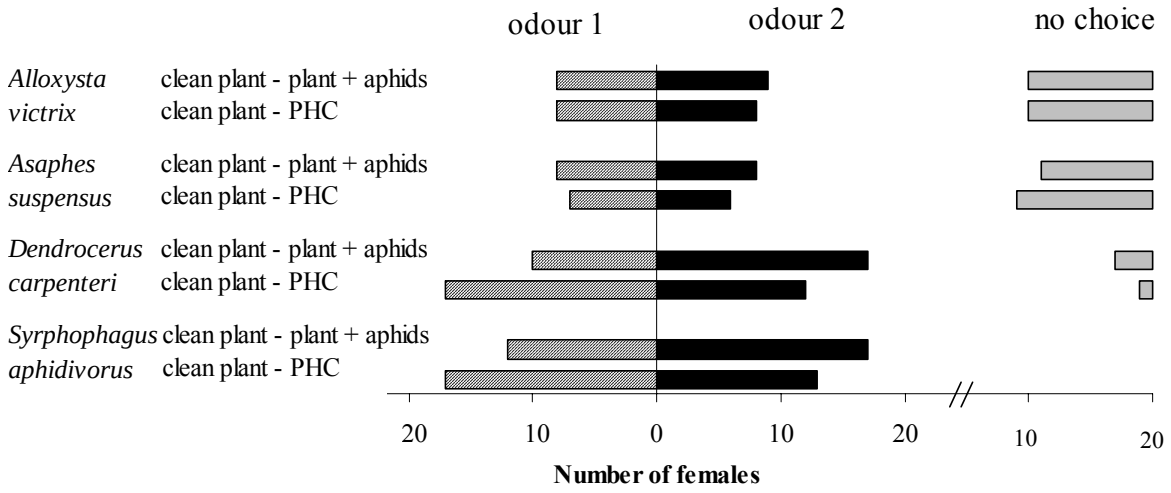


Figure 4-1 Preference of four aphid hyperparasitoid species for olfactory stimuli in an Y-tube olfactometer using the potato - *Macrosiphum euphorbiae* - *Aphidius nigripes* system. A. Single choice test (odour vs. air); B. Dual choice test of odours from the two hosts of *Syrphophagus aphidivorus*; C. Dual choice test (odour 1 vs. odour 2). Treatments indicated by an asterisk show significant differences (χ^2 test, $p<0.05$).

B


Syrphophagus aphidivorus
mummies vs parasitised aphids
odour 1
odour 2
no choice
*
10
0
10
20
10
0
Number of females

C


odour 1
odour 2
no choice
Alloxysta victrix
clean plant - plant + aphids
clean plant - PHC
Asaphes suspensus
clean plant - plant + aphids
clean plant - PHC
Dendrocerus carpenteri
clean plant - plant + aphids
clean plant - PHC
Syrphophagus aphidivorus
clean plant - plant + aphids
clean plant - PHC
20
10
0
10
20
10
20
Number of females

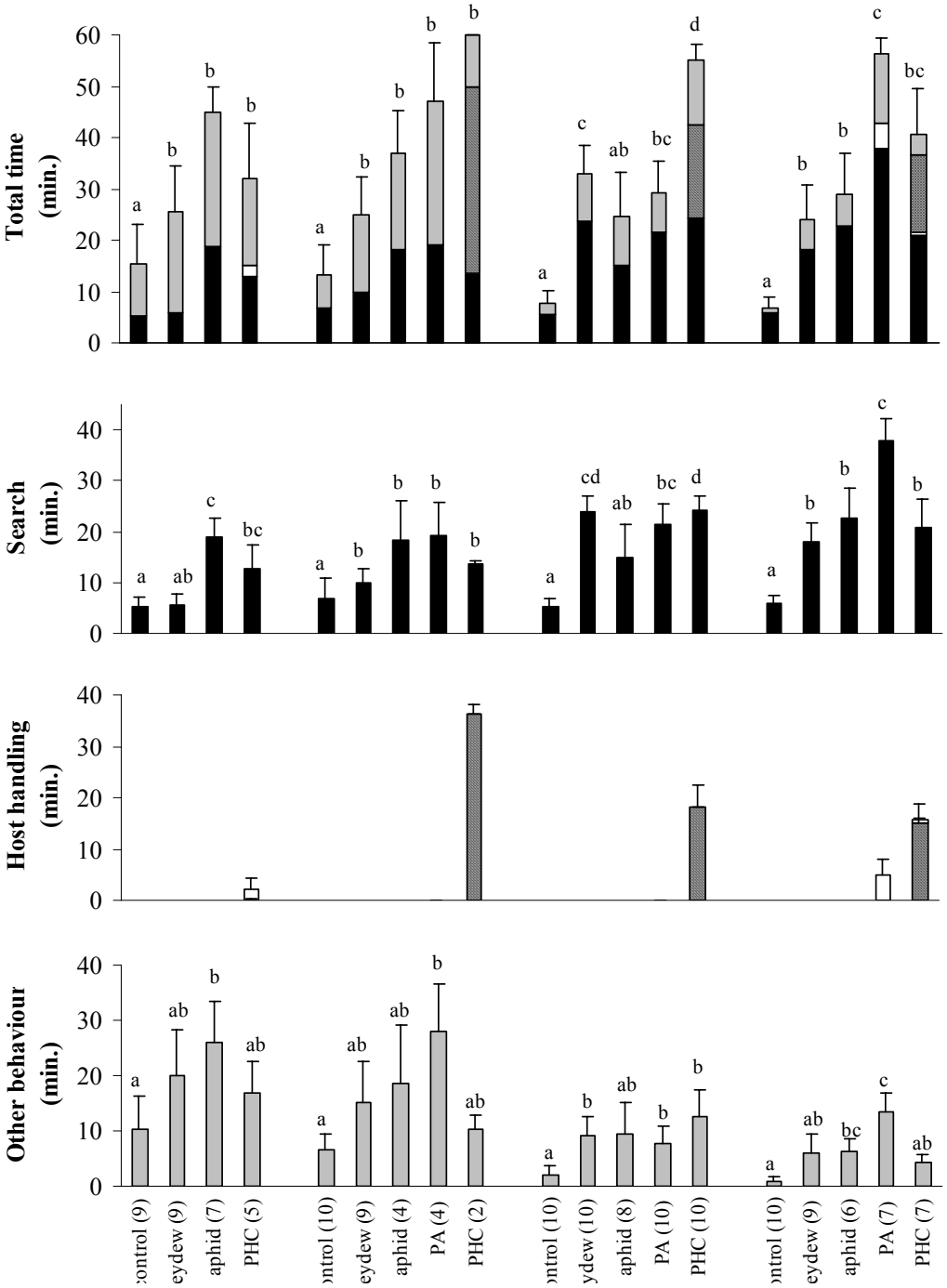


Figure 4-2 Effect of stimuli on host searching behaviour (mean ± SE) of four aphid hyperparasitoid species searching on a potato plant. A. Total visit time; B. Search time; C. Host attack; D. Other behaviours (rest, groom, fly, feed and examining aphids). Maximum observation time 60 minutes. Between parentheses the number of females that were observed is indicated. Treatments are control (clean plant), honeydew (plant with *Macrosiphum euphorbiae* honeydew), aphid (plant infested with *M. euphorbiae*), PA (parasitised aphid, plant infested with *M. euphorbiae*, both healthy and parasitised by *Aphidius nigripes*), PHC (plant-host complex, as parasitised aphid treatment with additional *A. nigripes* mummies). Data were analysed per species with the LIFEREG procedure

Chapter 5.

The role of honeydew in host searching of aphid hyperparasitoids

5.1. Abstract

Foraging in many insect parasitoids is mediated by chemicals associated with their hosts. For example, honeydew, the faeces of feeding aphids induces and/or prolongs the searching behaviour of aphid parasitoids. In the laboratory, we tested if aphid hyperparasitoids, which belong to a higher trophic level, also rely on aphid honeydew to locate their hosts. To do this we used the potato aphid, *Macrosiphum euphorbiae,* the primary parasitoid, *Aphidius nigripes* and four hyperparasitoids, *Asaphes suspensu*s, *Dendrocerus carpenteri, Alloxysta victrix*, and *Syrphophagus aphidivorus* that possess different biological attributes and host ranges. In addition we determined if foraging hyperparasitoid females could discriminate between (i) honeydew from a host and a non-host (the potato aphid and the soft brown scale, *Coccus hesperidum*), and (ii) honeydew from healthy aphids and those parasitised by *A. nigripes*. Females of *A. suspensus* did not react to any of the honeydew treatments. While the presence of non-aphid honeydew did not modify the behaviour of *A. victrix*, *D. carpenteri* and *S. aphidivorus* females, they exhibited a significant increase in searching time and path length, but not walking speed when in the presence of honeydew from aphids. However, there were no changes in host searching behaviours, such as antennation or ovipositor probing, that have been reported for primary aphid parasitoids. There was no significant difference in the response of hyperparasitoid females to honeydew from healthy and parasitised aphids. These results indicate that hyperparasitoids may use aphid honeydew, a conspicuous cue from the second trophic level, as an infochemical to locate their hosts.

Key Words - Honeydew, aphid, aphid parasitoid, hyperparasitoid, host searching behaviour, trophic interactions, infochemical detour.

5.2. Résumé

Chez plusieurs parasitoïdes d'insectes, la recherche de l'hôte est modulée par des infochimiques associés à l'hôte. Par exemple, le miellat, ou l'excrétion fécale des pucerons, induit ou prolonge le comportement de recherche chez les parasitoïdes de pucerons.

Au laboratoire, nous avons determiné si les hyperparasitoïdes de puceron, qui appartiennent à un niveau trophique supérieur, utilisent également le miellat pour localiser leurs hôtes. Nous avons utilisé le puceron de la pomme de terre, *Macrosiphum euphorbiae*, le parasitoïde, *Aphidius nigripes* et quatre hyperparasitoïdes, *Dendrocerus carpenteri*, *Asaphes suspensus*, *Alloxysta victrix* et *Syrphophagus aphidivorus*. Nous avons déterminé si les femelles hyperparasitoïdes avaient la capacité de discriminer entre (1) le miellat excrété par le puceron *M. euphorbiae* et celui de la cochenille, *Coccus hesperidum*, laquelle n'abrite pas d'hôtes potentiels, et (2) le miellat de pucerons sains et celui des pucerons parasités par *A. nigripes*.

Les femelles *A. suspensus* n'ont répondu à aucun des traitements de miellat, alors qu'aucun hyperparasotoïde n'a répondu au miellat de cochenille. Au contraire en présence du miellat de puceron, le temps de recherche et la longueur des tracés, mais pas la vitesse de marche, ont augmenté chez *A. victrix*, *D. carpenteri* et *S. aphidivorus*. Toutefois, leurs femelles n'ont pas réagi au miellat par certains comportements spécifiques observés chez les parasitoïdes primaires de pucerons, tel l'investigation avec les antennes ou l'ovipositeur. De plus, les femelles hyperparasitoïdes n'ont pas discriminé entre le miellat de pucerons parasités et non-parasités.

Ces résultats montrent que les hyperparasitoïdes pourraient utiliser le miellat de puceron, une substance manifeste du deuxième niveau trophique, comme infochimique pour localiser leurs hôtes.

5.3. Introduction

Honeydew, a complex mixture of chemical compounds, of which the most important are sugars and amino acids (Auclair, 1963) is excreted by phloem-feeding Homoptera, such as aphids, whiteflies and scale insects. Differences in chemical composition of aphid honeydew have been studied in detail and may vary depending on the host plant species (Hendrix *et al.*, 1992; Douglas, 1993; Fisher and Shingleton, 2001), the aphid species (Hendrix *et al.*, 1992; Völkl *et al.*, 1999; Fisher and Shingleton, 2001), the aphid age (Fisher *et al.*, 2002), the sugar concentration in the diet (Mittler and Meikle, 1991; Wilkinson *et al.*, 1997), the level of ant tending (Fisher and Shingleton, 2001; Yao and Akimoto), the presence of bacterial intracellular symbionts (Sasaki *et al.*, 1990; Wilkinson and Douglas, 1995; Wilkinson *et al.*, 1997), and parasitism (Cloutier 1986). Honeydew may serve as a source of carbohydrates for many insects, for example ants and parasitoids (Völkl *et al.* 1999; Wäckers and Steppuhn, 2003).

Honeydew is also used as an infochemical by foraging parasitoids (e.g. Bouchard and Cloutier 1984) and predators (e.g. Budenberg and Powell, 1992). Its role in host searching of aphid parasitoid females has been studied extensively. Honeydew attracts foraging parasitoid females (Wickremasinghe and van Emden, 1992; Bouchard and Cloutier, 1985) and/or arrests them on contaminated areas (Bouchard and Cloutier, 1984; Gardner and Dixon, 1985; Ayal, 1987; Budenberg, 1990; Cloutier and Bauduin, 1990; Hågvar and Hofsvang, 1991; Budenberg *et al.*, 1992; Grasswitz and Paine, 1993). Honeydew may also contain substantial specific information for natural enemies, for while *Aphidius rhopalosiphi* females respond to honeydew of both host and non-host aphids, they spend less time in areas contaminated with honeydew from the non-host species (Budenberg, 1990).

Aphid parasitoids can in turn be parasitised by different species of hyperparasitoids. Contrary to primary parasitoids, honeydew from healthy aphids does not appear to attract hyperparasitoids towards contaminated areas (Buitenhuis *et al.*, unpublished). This is not altogether surprising for while honeydew is a direct cue to the presence of aphids for parasitoids, it would only be an indirect cue for hyperparasitoids as it provides females no

reliable information about the presence of their aphid parasitoid hosts. On the other hand, honeydew does act as a contact synomone, inducing hyperparasitoid females to stay and search longer on contaminated surfaces and plants (Budenberg, 1990; Grasswitz, 1998; Buitenhuis *et al.*, unpublished). However, parasitism by braconid wasps may also induce changes in both the quantity and composition of honeydew produced by aphids (Cloutier and Mackauer 1979, Cloutier 1986, Rahbé *et al.*, 2002). Therefore, honeydew could be a direct and reliable cue for hyperparasitoids if females have evolved the capacity to discriminate between honeydew from healthy and parasitised aphids.

In this study, we examined the innate response of aphid hyperparasitoids to different types of honeydew. We predicted that foraging hyperparasitoid females not only have the ability to detect honeydew but also show a preference for honeydew from aphid rather than non-aphid species and, more specifically, for honeydew from parasitised aphids. We tested these predictions in the laboratory by measuring behavioural components of hyperparasitoid females exposed to water extract of honeydew applied to filter paper discs following the study of Bouchard and Cloutier (1984). We used the potato aphid, *Macrosiphum euphorbiae* (Thomas), its primary parasitoid, *Aphidius nigrip*es Ashmead and four hyperparasitoids, *Asaphes suspensu*s Walker (Pteromalidae), *Dendrocerus carpenteri* (Curtis) (Megaspilidae), *Alloxysta victrix* (Westwood) (Alloxystidae), and *Syrphophagus aphidivorus* (Mayr) (Encyrtidae). These species were chosen for while they all naturally exploit *Aphidius* spp. they possess different biological attributes and host ranges. *A. suspensus* and *D. carpenteri* are generalist ectoparasitoids which attack primary parasitoid prepupae or pupae following mummification of the aphid. *A. victrix* is an endoparasitoid that lays its egg in parasitoid larvae prior to aphid mummification and has a more restricted host range. Finally, *S. aphidivorus* is a generalist hyperparasitoid with the capacity to attack either primary parasitoid larvae in live aphids or parasitoid prepupae or pupae following mummification.

5.4. Materials and Methods

5.4.1. Insects.

Colonies of the aphid, parasitoid and four hyperparasitoids were reared on potato seedlings following the techniques of Brodeur and McNeil (1994) and Buitenhuis *et al.* (submitted).

To prevent contact with honeydew stimuli before the test, hyperparasitised aphid mummies were collected, put in individual gelatine capsules and kept at 20±1ºC, 75±10% RH, under a 16L:8D photoperiod until adult emergence. The adults were then sexed and females were put in small individual ventilated cylindrical cages (5 cm in diameter and 10 cm in height) with a supply of 40% sucrose solution and held under the same conditions until used in the experiment.

5.4.2. Honeydew Collection.

Aphid honeydew was collected by placing Parafilm™ sheets for 24 h under potato plants infested with either healthy aphids from all developmental stages or parasitised aphids. Parasitised aphids were obtained by exposing third instar aphids for 24 hr to 3-5 day-old mated females at a parasitoid: host ratio of 1:2 (resulting in 90-95% parasitism). Honeydew was collected 4-7 days later. The response of hyperparasitoid females to honeydew from a non-host was tested using honeydew from scale insects (*Coccus hesperidum* L.: Coccidae) collected on *Ficus benjamina* L. (Moraceae) plants. In this instance, both the herbivore and the plant species were different from the potato-aphid system. This honeydew was collected in the same manner as described above but, due to the lower insect density, the Parafilm sheets were removed after 2 days. Honeydew was allowed to dry for 30 minutes at 40°C, collected by scraping the sheets with a glass microscope slide and then stored at -20°C until use. Before the bioassay, the honeydew was weighed and dissolved in distilled water, filtered through a cloth, and adjusted to a concentration of 0.26 mg/µl (following Bouchard and Cloutier, 1984). Between bioassays the solution was stored at 4°C for a maximum of 8 days.

5.4.3. Bioassay.

100 µl of the distilled water, honeydew from either healthy or parasitised aphids, or from non-host scales was applied in the middle of a filter paper (12.5 cm diameter; Schleicher & Schuell #595), giving a treated circle about 4 cm in diameter. A circle of 12 cm in diameter was drawn inside the perimeter of the disc. The paper was dried under laboratory conditions and used within 5 hours of preparation. For each assay the test paper was placed in a 14 cm diameter glass petri dish, covered by a glass plate, located in a tent lit by a circular 22W fluorescent tube. One virgin, naïve, female hyperparasitoid (2-7 day-old) was released onto the middle of the filter paper and her behaviour recorded on video until she either crossed the 12 cm circle or until she flew to the side or top of the arena. Females that immediately flew off the filter paper or that did not move were excluded from the analysis. One female of each of the four hyperparasitoid species was tested on the same filter paper. The filter papers treated with aphid honeydew were only used once, but in the case of scale honeydew assays they were used twice due to the shortage of scale honeydew solution. Such a procedure had no effect on any of the measured parameters: residence time, path length, walking speed (2-way ANOVAs using hyperparasitoid species and $1^{st}/2^{nd}$ repetition as factors, all p-values >0.05). In all assays the hyperparasitoid species were randomised within the treatments, and 20 replicates per treatment were done within a 8 day period.

The time spent inside and outside the contaminated area was determined from the videotape using the Observer© (Noldus, 1997, version 3 for Macintosh), while the locomotory behaviour was quantified by tracing each female's path on a transparency and then measuring its length. Walking speed (cm/s) was calculated by dividing the total path length by the total time.

5.4.4. Statistical Analysis.

Duration and path length data were log(x+1) transformed, whereas speed data were square root transformed prior to be analysed using a two-way ANOVA. Differences between treatments were determined by contrasts. Given that statistical models had 3 degrees of freedom per factor, only 3 orthogonal contrasts (α=0.05) were allowed. To test the predictions, we selected a priori (i) honeydew, regardless of origin vs water, (ii) aphid vs

non-aphid (scale) honeydew, and (iii) honeydew from healthy and parasitised aphids. One additional contrast analysis, (iv) non-aphid (scale) honeydew vs water, was done using Scheffé's adjustment of the p-value (Steel and Torrie, 1980). Differences between hyperparasitoid species were determined by Fisher's protected LSD (α=0.05). All analyses were done using SAS (SAS Institute, 1999).

5.5. Results

Most females of all hyperparasitoid species walked on the filter paper: *A. victrix* (77%), *A. suspensus* (88%) *D. carpenteri* (94%), and *S. aphidivorus* (100%). In all species, walking was continuous or could be interrupted with short jumps. The trajectories of females that did or did not respond to honeydew were very different. Females in the control treatment, and those not responding to honeydew, usually walked rapidly across the treated area without showing evidence of arrestment (Figure 5-1). In contrast, a positive response was characterised by a klinotactic response, and the resulting tortuous path ensured that the females searched most of the treated area (Figure 5-1).

Overall, there were significant effects of both treatment and species on residence times and path length (Table 5-1, Figures 5-2 and 5-3). However, while there were species specific differences in walking speed, for any given species, this parameter was unaffected by treatment (Table 5-1, Figure 5-4).

Clearly, these treatment effects are due to overall differences in response to aphid honeydew compared with those to water and honeydew from scale insects (Table 5-2, Figures 5-2 and 5-3). However, the contrast analyses underlined specific treatment differences between the four hyperparasitoid species (Table 5-2). One noticeable point is that *A. suspensus* female foraging behaviours remained unchanged in all assays (Table 5-2, Figures 5-2 and 5-3). The apparent increase in time spent in the scale insect treatment on Figure 5-2 was non-significant and resulted from the behaviour of two females, one which spent a long time walking in the treated patch and the other which remained outside the patch for a prolonged period. When the pooled responses to honeydew, regardless of source, and water were contrasted, *D. carpenteri* females showed significant changes in foraging while *A. victrix* and *S. aphidivorus* did not. However, there are no differences between water and scale honeydew for any given species (Table 5-2), while all respond to aphid honeydew.

Contrary to our initial hypothesis, there were no differences between honeydew from healthy and parasitised aphids (Table 5-2, Figures 5-2 and 5-3).

5.6. Discussion

Our results, together with those of Budenberg (1990) on *Alloxysta macrophadna* and *Phaenoglyphis villosa* (Alloxystidae), and Grasswitz (1998) on *A. victrix*, indicate that aphid honeydew may modify female foraging behaviour in species from each of the three superfamilies (Cynipoidea, Ceraphronoidea, Chalcidoidea) where aphid hyperparasitoids are found. The existence of such a common response among evolutionary diverse groups of aphid hyperparasitoids would suggest that aphid honeydew is a reliable cue to host finding and may thus serve as a contact synomone that transcends trophic levels. A parallel study, at a different spatial scale using whole plants, also showed that the foraging behaviour of hyperparasitoid females was significantly modified by the presence of aphid honeydew (Buitenhuis *et al.*, unpublished). This conclusion is supported by the fact that the behavioural changes observed were not in response to all sources of honeydew, but rather to honeydew produced by insects serving as a host for the primary parasitoid. This ability to discriminate between aphid and non-aphid honeydew would result in females making extensive searches in areas where aphid parasitoids are most likely to be found. Honeydew composition is in a large part determined by the elements of phloem sap, and is thus partly plant specific (Hendrix *et al.*, 1992), so we cannot exclude the possibility that the different patterns we observed may be associated with the different host plants, i.e. potato vs *Ficus* plants used by the two herbivore species. However, discrimination between host and non-host honeydew has been reported, as the whitefly parasitoid *Encarsia formosa* responded differently to whitefly and aphid honeydew, when both species are reared on the same host plant (Romeis and Zebitz, 1997).

It is evident that not all hyperparasitoids respond in the same way to honeydew from hosts exploited by primary parasitoids, for while three of the four species modified their behaviour, one, *A. suspensus*, did not. Furthermore, no consistent patterns of response to aphid honeydew are found when considering aphid hyperparasitoids with different life-history strategies (endo- vs ectoparasitoids, koino- vs idiobiont parasitoids) and the stage of primary parasitoid host attacked (parasitoid larva in live aphid or parasitoid pupa in aphid mummy). Similarly, host specificity does not appear to shape aphid hyperparasitoid responses to honeydew. For example, *A. victrix*, a koinobiont hyperparasitoid, has a

narrower host spectrum than most idiobiont hyperparasitoids, including those tested in this study (Brodeur 2000), but showed the same type of response. In contrast, the foraging of *A. suspensus*, a cosmopolitan and polyphagous species (Höller *et al*. 1993), was unaffected by honeydew on the substrate (this study). However, it was arrested by aphids/honeydew on plants (Buitenhuis *et al.*, unpublished). Nevertheless, it is possible that the use of honeydew as a foraging cue can be learned. Clearly more species must be examined in order to explain such marked differences in preference or absence of response.

Despite the potential advantages of recognising honeydew from parasitised aphids, females of the hyperparasitoid species we tested did not discriminate between honeydew from healthy and parasitised aphids. Several non-exclusive explanations may account for this. First, differences between honeydew from healthy and parasitised aphids are mostly reflected in quantitative differences in the concentrations of amino acids being measured (Cloutier, 1986). Furthermore, while the presence of primary parasitoid larvae may modify aphid honeydew, several other factors may result in similar changes. These include aphid and host plant species, which may modify the nature and concentration of amino acids and sugars present (Douglas, 1993; Völkl *et al.*, 1999; Fisher and Shingleton, 2001). In addition, hyperparasitoid females foraging in an aphid colony under natural conditions will encounter a mix of new and decomposing honeydews from both healthy and parasitised aphids, which could mask any subtle quantitative differences associated with the origin of the synomone. One must therefore conclude that differences between honeydew from healthy and parasitised aphids do not provide sufficiently reliable cues to modify foraging behaviour.

Primary parasitoids and hyperparasitoids of aphids both use aphid honeydew in host searching and the response to this cue appears to be innate as naïve females respond to the infochemical (Bouchard and Cloutier, 1984; Grasswitz 1998; Grasswitz and Paine, 1993; this study). There are, however, distinct differences between the two trophic levels. While both use honeydew as an arrestment cue, primary parasitoids use volatiles from honeydew in long distance search (Bouchard and Cloutier, 1985), while hyperparasitoids do not (Buitenhuis *et al*., unpublished). Furthermore, when primary parasitoids contact host

honeydew there are a series of behavioural changes, including increased antennation, abdominal extension/flexing, reduced walking speed and increased turning rate (Bouchard and Cloutier, 1984; Budenberg, 1990; Hågvar and Hofsvang, 1991). Female hyperparasitoids also spend longer times and follow tortuous paths when encountering honeydew patches, yet they maintain a constant walking speed and do not perform the specific behaviours seen in the primary parasitoids. Such differences may arise from differences in the reliability of aphid honeydew as a foraging cue for primary and secondary parasitoids. Honeydew comes from the aphid and represents a reliable, abundant, and direct source of information about the presence of hosts to primary parasitoids (Vet and Dicke, 1992). In contrast, aphid honeydew provides no reliable information about the availability of suitable stages of the primary parasitoid that the hyperparasitoid females exploit. This situation represents an original example of an « infochemical detour », where the cue is only indirectly related to its host/prey (Vet and Dicke, 1992). Aphid hyperparasitoid females could benefit from searching in habitats contaminated by honeydew, as parasitised aphids and aphid mummies can either be found within or near the aphid colony (Brodeur and McNeil, 1989, 1992; Müller *et al*., 1997). Furthermore, by keeping a constant walking speed, females possibly cover a greater area and thus gain the greatest benefit from an indirect cue for host availability.

5.7. Acknowledgements

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Table 5-1 Results of 2-way ANOVAs on different parameters describing the behaviour of four species of aphid hyperparasitoids foraging on a filter paper disc treated with different honeydew extracts.

Parameters	**Factors**		
	Honeydew treatment	**Hyperparasitoid species**	**Interaction**
Total residence time	$F_{3,252}$=11.78, *P*<0.001	$F_{3,252}$=3.43, P=0.018	$F_{9,252}$=1.40, P=0.186
In treated patch	$F_{3,252}$=8.62, *P*<0.001	$F_{3,252}$=4.91, P=0.002	$F_{9,252}$=1.79, P=0.071
Outside treated patch	$F_{3,252}$=9.16, *P*<0.001	$F_{3,252}$=3.63, P=0.014	$F_{9,252}$=0.56, P=0.827
Path length	$F_{3,252}$=16.62, *P*<0.001	$F_{3,252}$=26.78, *P*<0.001	$F_{9,252}$=1.03, P=0.413
Walking speed	$F_{3,252}$=1.53, P=0.207	$F_{3,252}$=54.42, *P*<0.001	$F_{9,252}$=0.51, P=0.868

Table 5-2 P-values of contrast analyses on the effect of different types of honeydew on the behaviour of four species of aphid hyperparasitoids. Contrast treatments were: Control (distilled water); All honeydew (combination of all honeydew treatments); Aphid (honeydew produced by *Macrosiphum euphorbiae*, both healthy and parasitised by *Aphidius nigripes*); Scale insect (honeydew produced by *Coccus hesperidum*); Healthy aphid (honeydew produced by *M. euphorbiae*); Parasitised aphid (honeydew produced by *M. euphorbiae* parasitised by *A. nigripes*). Significant contrasts are indicated by asterisks (P<0.05)

Species	**Contrast**	**Visit time**			**Path length**	**Walking speed**
		Inside honeydew	**Outside honeydew**	**Total**		
Alloxysta.	Control vs all honeydew	0.386	0.133	0.187	0.252	0.879
victrix	Scale insect vs aphid	0.018*	0.080	0.020*	0.009*	0.569
	Healthy aphid vs parasitised aphid	0.844	0.685	0.762	0.844	0.840
	Control vs scale insect [1]	0.410	0.988	0.641	0.437	0.650
Asaphes	Control vs all honeydew	0.127	0.240	0.177	0.130	0.571
suspensus	Scale insect vs aphid	0.178	0.494	0.722	0.418	0.090
	Healthy aphid vs parasitised aphid	0.656	0.943	0.774	0.409	0.544
	Control vs scale insect [1]	0.052	0.639	0.223	0.517	0.554
Dendrocerus	Control vs all honeydew	0.004*	0.308	0.008*	0.002*	0.310
carpenteri	Scale insect vs aphid	0.002*	0.001*	<0.001*	<0.001*	0.547
	Healthy aphid vs parasitised aphid	0.098	0.595	0.386	0.452	0.389
	Control vs scale insect [1]	0.770	0.244	0.796	0.983	0.687
Syrphophagus	Control vs all honeydew	0.092	0.128	0.057	0.003*	0.125
aphidivorus	Scale insect vs aphid	0.038*	0.004*	0.003*	0.004*	0.873
	Healthy aphid vs parasitised aphid	0.736	0.234	0.348	0.628	0.478
	Control vs scale insect [1]	0.948	0.571	0.755	0.558	0.204

[1]Additional contrast with Scheffé adjustment.

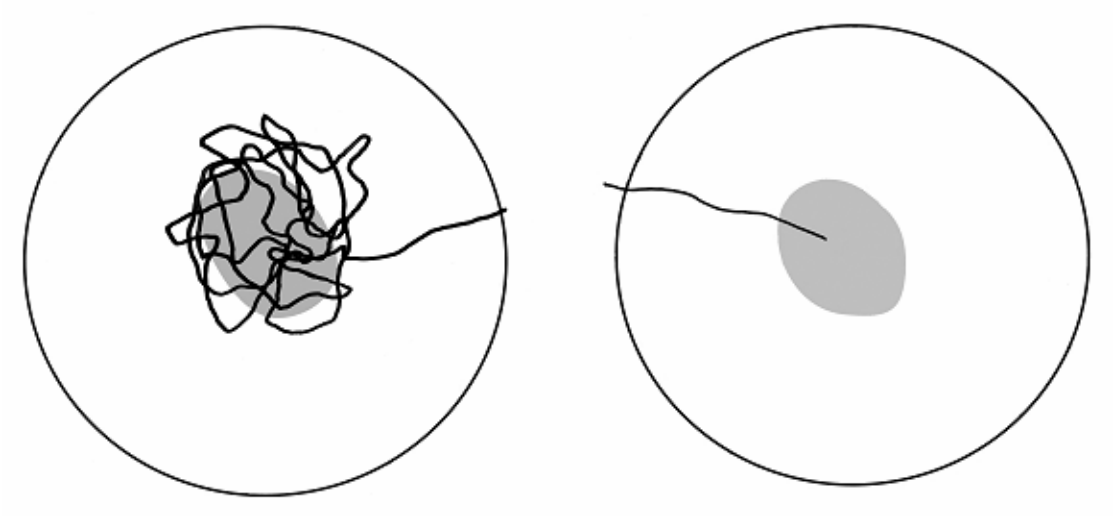

Figure 5-1 Typical path tracings of aphid hyperparasitoid females that responded (left, *Dendrocerus carpenteri* on honeydew from parasitised aphid) or not (right, *D. carpenteri* on honeydew from scale insect) to honeydew.

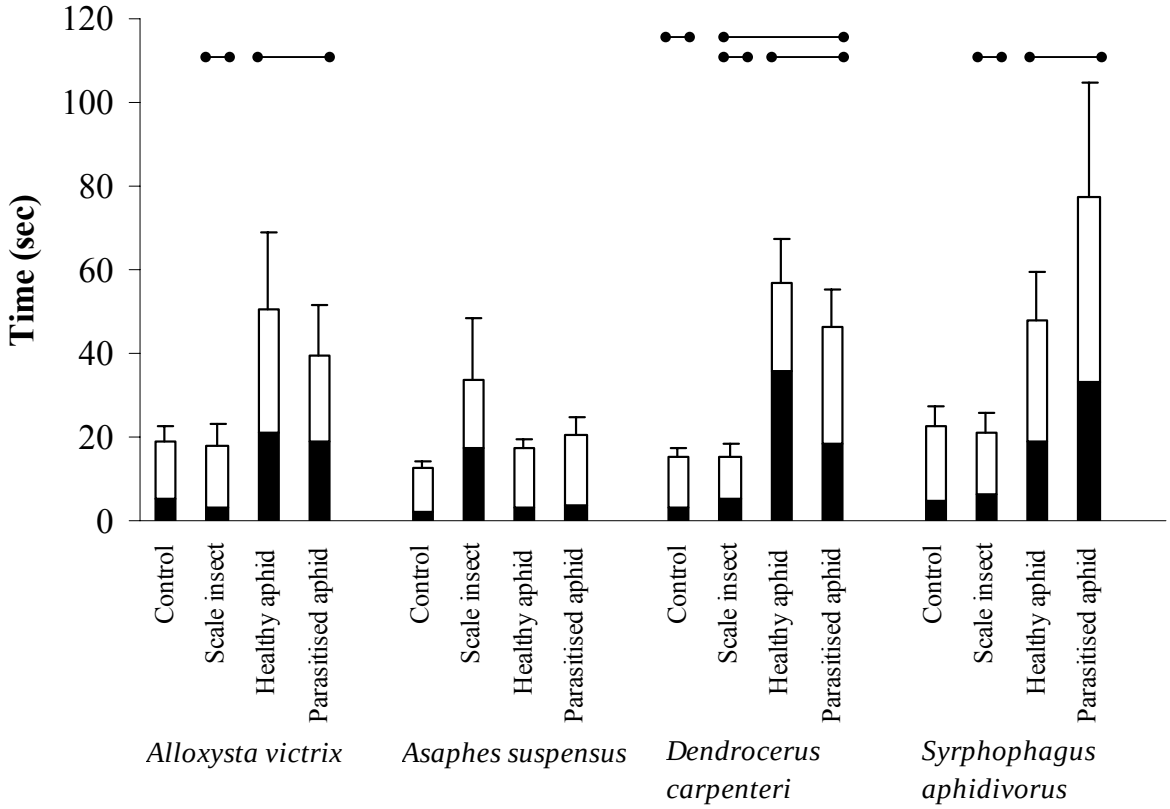

Figure 5-2 Residence time (mean + SE) of female of four species of aphid hyperparasitoids foraging on a filter paper disc (12 cm in diameter) treated with different honeydew extracts. The bars further indicate the time spent within (black) and outside (white) of of the treated area. Per species, significant contrasts are indicated with horizontal bars. For details on all statistical differences, see Table 2.

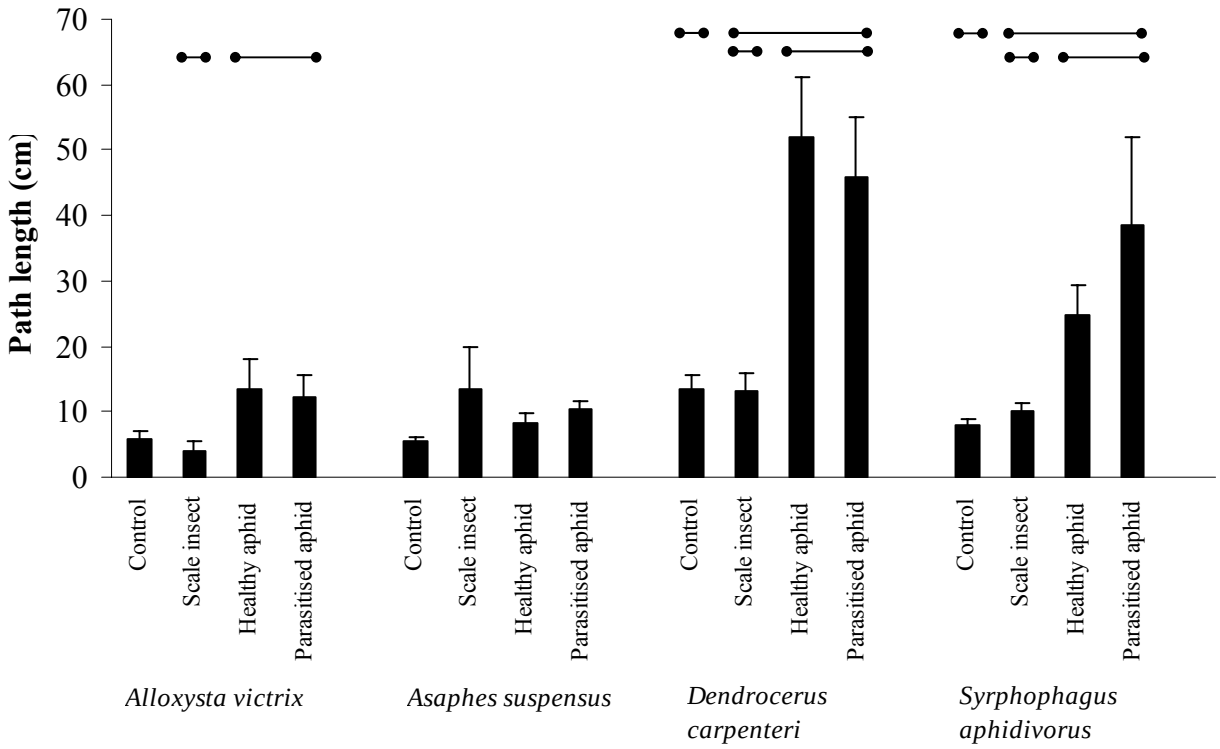

Figure 5-3 Path length (mean + SE) of female of four species of aphid hyperparasitoids foraging on a filter paper disc (12 cm in diameter) treated with different honeydew extracts. Per species, significant contrasts are indicated with horizontal bars. For details on all statistical differences, see Table 2.

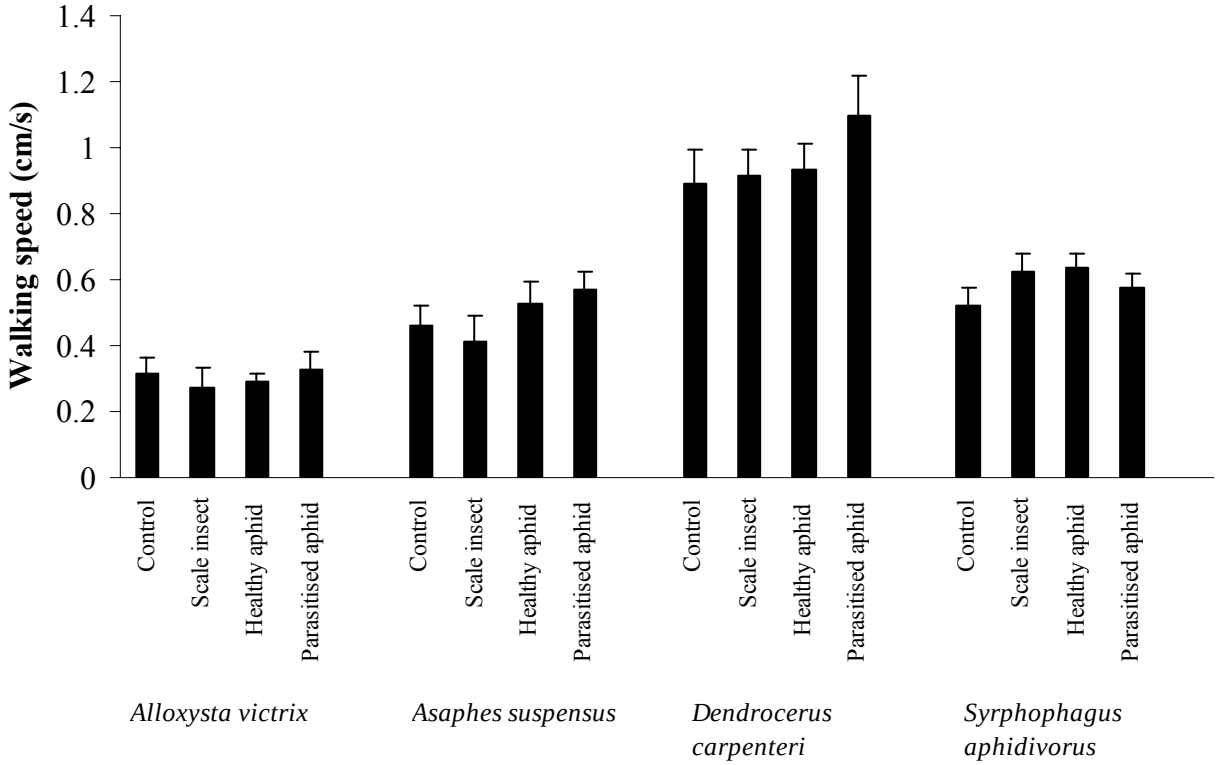

Figure 5-4 Walking speed (mean + SE) of female of four species of aphid hyperparasitoids foraging on a filter paper disc (12 cm in diameter) treated with different honeydew extracts. No species showed significant differences between treatments.

Chapter 6.

Conclusion

6.1. Conclusion

This study is the first to directly compare the life history traits of different species of hyperparasitoids of four families on the same plant-aphid–primary parasitoid system. Without the confounding influence of different systems of previous studies, this should allow a more valid comparison between species, and increase the possibility to find correlations between life history traits or host searching behaviour, and factors such as host range or host stage relationship. The results of this study have demonstrated that aphid hyperparasitoid systems can be very complex. Because of many exceptions, it is difficult to generalise.

6.2. Life history

In the literature, aphid hyperparasitoids are generally classified into two groups according to their host stage for oviposition and the so-called development mode: Koinobiont endo-hyperarasitoids attack parasitoid larvae in live aphids, and idiobiont ecto-hyperparasitoids attack parasitoid pupae in aphid mummies. In primary parasitoids, this dichotomy (koinobiont endoparasitism – idiobiont ectoparasitism) has been hypothesised as a major organiser of parasitoid life histories (Godfray, 1994; Mayhew and Blackburn, 1999). In chapter 2, I found a great variation in life history parameters between four aphid hyperparasitoid species. However, this variation could not be totally explained by the predictions of the dichotomy hypothesis. Although the data for the idiobiont ectohyperparasitoids (*D. carpenteri* and *A. suspensus*) mostly confirmed the dichotomous hypothesis, the results obtained for koinobiont endohyperparasitoids (*A. victrix* and *S. aphidivorus*) did not match its predictions .

I argue that the classification of aphid hyperparasitoids as idiobiont ecto-hyperparasitoids or koinobiont endo-hyperparasitoids is an oversimplification. I found great differences between species that would be classified in the same group. The two representatives of the idiobiont ecto-hyperparasitoids, *D. carpenteri* and *A. suspensus* had profound differences in life history. *Asaphes suspensus* had a significantly longer development time and a higher fecundity than *D. carpenteri*. The two koinobiont endo-hyperparasitoids also had very different traits. For example, *S. aphidivorus* had the

highest intrinsic rate of increase of all four species, while *A. victrix* had the lowest. Furthermore, *A. victrix* only attacks parasitoid larvae in live aphids and is incapable to attack parasitoids in aphid mummies (Grasswitz and Reese, 1998). In contrast, parasitoids in aphid mummies are the most suitable and preferred host for *S. aphidivorus*, although it can parasitise both (chapter 3). It is clear that based on these differences, *S. aphidivorus* and *A. victrix* should belong to different groupings based on similar life history.

6.3. Host location

In chapter 4 the role of airborne and contact cues from all trophic levels in host location was tested. It was found that aphid hyperparasitoid females were not attracted by olfactory cues from distant sources in a Y-tube olfactometer, but reacted to contact cues while searching by walking on a plant. All species prolonged visit time and searching time. Our results suggest that olfactory cues from distant sources are not essential in host search by aphid hyperparasitoids, while close cues that are encountered on a plant do provide information that induces searching in most species.

If hyperparasitoids do not use long distance olfactory stimuli, an interesting question is how do they select a site to search for hosts? Probably all plants are likely sites. The tested species have been reported from many different plant-aphid-primary parasitoid systems in agricultural fields in North America and Europe (e.g. Gutierrez and van den Bosch, 1970; Sullivan and van den Bosch, 1971; Johnson *et al*., 1979; Thiboldeaux *et al*., 1987; Mertins, 1985; Höller *et al*., 1993), and natural habitat with mixed vegetation (Müller *et al*., 1999). Each of these systems is probably associated with different volatiles, depending on plant, aphid and host species composition. In the absence of common, detectable cues it is therefore likely that aphid hyperparasitoids search in the habitat where they are born, or select a different habitat at random and that search is then induced by contact stimuli that are encountered on the plant.

I have observed that direct cues from the host, either aphid mummies or live parasitised aphids, apparently are only perceived at short distance, a few centimetres away from the searching female (personal observation). Therefore, hyperparasitoids may have to rely mainly on indirect cues. Aphids and honeydew are useful such indicators of the presence

of hosts, as parasitised aphids and aphid mummies can either be found within or near the aphid colony (Brodeur and McNeil, 1989, 1992; Müller *et al*., 1997). The reliability of these indirect cues depends on the predictability of aphid parasitism over space and time (Vet and Dicke, 1992). If parasitism is common in aphid colonies, the information from aphid and honeydew cues is reliable for host search of aphid hyperparasitoids.

In chapter 5, I tested the use of honeydew as a host-searching cue in more detail. Honeydew is an abundant, easily detectable, common substance associated with healthy and parasitised aphids. The composition of honeydew potentially contains information on aphid species (Hendrix *et al.*, 1992; Völkl *et al.*, 1999; Fisher and Shingleton, 2001), and parasitism (Cloutier and Mackauer, 1979; Cloutier, 1986; Rahbé *et al.*, 2002). Females of *A. suspensus* did not react to any of the honeydew treatments. The other three species were able to discriminate between honeydew from an aphid and the soft brown scale, *Coccus hesperidum*, but made no difference between honeydew from healthy aphids and those parasitised by *A. nigripes*. The ability to discriminate between aphid and non-aphid honeydew would result in extensive searches in areas where aphid parasitoids are most likely to be found. There were no changes in host searching behaviours induced by honeydew, such as antennation or slower walking speed as reported for the primary aphid parasitoid *A. nigripes* by Bouchard and Cloutier (1984). By keeping a constant walking speed, hyperparasitoid females possibly cover a greater area and thus gain the greatest benefit from an indirect cue for host availability.

6.4. Differences between hyperparasitoid species

It has been difficult to correlate the results in life history or host search behaviour to development mode, host stage or host range. Neither of these factors would singly explain all variation between the four species. So the results provide no support for my prediction that differences in life history and host location behaviour between hyperparasitoid species would be due to only one of these factors. Probably the effect of all factors on hyperparasitoid biology is combined.

Darwin (1859) supposed that a balanced interpretation of an evolutionary pattern requires two components: adaptation and lineage specific effects. The effect of adaptation is that

life history traits are adapted to each other and to local environmental conditions. At the same time, some life history traits are fixed at high taxonomic levels (lineage specific effects).

It is therefore likely that differences between species are also partly determined by their different phylogenetic origins. In this thesis, I chose four species from different families with aphid hyperparasitoids. These different species have probably evolved within phylogenetic constraints specific to their origin to exploit the same resource. Effectively, it is proposed that hyperparasitism might have evolved independently several times in the Hymenoptera (Gordh, 1981). This is likely because the expression of hyperparasitism is spotty (within the parasitoid taxa), different development modes occur among hyperparasitoids and adult female hyperparasitoids have different ovipositional strategies. Further progress to explain the evolution of different forms of hyperparasitism will be slow until good phylogenies are available for parasitoid taxa that include hyperparasitoids (Godfray, 1994).

6.5. Comparison between hyperparasitoids and primary parasitoids.

Arguments for the impact of hyperparasitism on primary parasitoid populations often rest on the notion that those characteristics that define an effective primary parasite are the same ones that make a hyperparasitoid deleterious (Luck *et al*., 1981). Following this reasoning, data obtained from primary parasitoids are extrapolated to the next trophic level. This extrapolation is not necessarily valid. Following Brodeur (2000), I stated that hyperparasitoids should have developed specific biological attributes enabling them to exploit resources from the third trophic level, attributes that are not necessary found in primary parasitoids. Indeed, comparing my results to studies of aphidiine primary parasitoids (Force and Messenger, 1964), and *A. nigripes* in particular (Bouchard and Cloutier, 1984; 1985; Cloutier and Bauduin, 1990), I found differences in life history and host location between the primary parasitoid and secondary aphid hyperparasitoids. In general, aphid hyperparasitoids have a higher longevity, a longer development time and a lower fecundity than primary parasitoids of aphids (meaning that they are more k-selected than the trophic level immediately below them). They do not seem to use

volatiles in long distance host location and although they do use contact stimuli while searching on a plant, they do not show specialised behaviour such as slowing down and antennation in response to contact cues such as honeydew, which to them is only an indirect cue to suitable hosts.

There are two non-exclusive explanations for the above differences between primary parasitoids and hyperparasitoids. Most of these differences could be explained first by the fact that hyperparasitoids generally have a broader host spectrum than primary parasitoids (Gordh, 1981). They attack many different aphid parasitoid species in different aphid species (e.g. Hoffer and Stary, 1970; Andrews, 1978; Fergusson, 1980; Mertins, 1985; Sullivan, 1987; Höller *et al*., 1993). As I argued in section 6.2, each of these hosts is associated with different volatiles, depending on plant, aphid and host species. Vet and Dicke (1992) hypothesised that contrary to specialists, the use of kairomones by generalists should be weak and non-specific, or could even be ineffective because the great diversity of chemical information generates a physiological constraint on sensory processing and chemical components common to several host species will be very limited.

As second explanation, hyperparasitoid increased longevity and lower fecundity compared to parasitoids might reflect the fact that the abundance of their hosts is lower (Quicke, 1997; Heimpel and Rosenheim, 1998; Heimpel *et al*, 1998; Rosenheim *et al*., 2000). For instance, the incidence of parasitism by Aphidiine wasps is mostly below 20% in the field (Shands *et al*., 1965; Sullivan and van den Bosch, 1971; Höller *et al*., 1993). Aphidiine wasps that oviposit in aphid colonies usually lay only a few eggs per colony and show high dispersal (Dettner *et al*., 1997), and most species of aphidiine parasitoids rarely exploit more than a small percentage (1%-10%) of the host resources available in the field (Mackauer and Völkl, 1993). Further research will lend more insight in the above suppositions.

6.6. Future studies

For this thesis I chose species representative of four families that contain aphid hyperparasitoids. However, the life history traits and searching behaviours should be

tested on more species to establish patterns. It will be interesting to study the host location behaviour of other *Alloxysta* species, to see if other species with a small host range show similar results as *A. victrix*. Another intriguing question is how exceptional is the dual oviposition behaviour of *S. aphidivorus*? There are indications that other species in this genus also show this behaviour. For example *Aphidencyrtus* (=*Syrphophagus*) *cassatus* attacks primary endo- and ectoparasitoids of psyllae (McDaniel and Moran, 1972; Tamesse *et al*., 2002). This species is an egg-larval hyperparasitoid when it attacks ectoparasitoids and a larval hyperparasitoid of endoparasitoids (McDaniel and Moran, 1972). In contrast, other *Syrphophagus* species are only reported from parasitoid pupae in aphid mummies (*Syrphophagus mamitus*, Müller and Godfray, 1998; Müller *et al*., 1999). Further investigation of the diverse host relations of this genus might give clues on the evolution of hyperparasitism in this group. Several lines of evidence indicate that aphid hyperparasitoids have evolved from parasitoids of aphid predators (Pschorn-Walcher 1957; Gauld and Bolton, 1988). In some cases it is the cocoon like structures of the host that seem to be the common link between the old and the new host (Brodeur, 2000). This might be a likely mechanism in the evolution of hyperparasitism in *Syrphophagus* because at least one species (*Aphidencyrtus* (=*Syrphophagus*) *staryi*) is suspected to parasitise a group of syrphid flies that are aphid predators (Hoffer and Stary, 1970).

In addition to studies of the biology of separate groups of hyperparasitoids, research should be conducted of their population dynamics. Estimates of field hyperparasitism may be obtained by placing cohorts of primary parasitoid hosts in the field for fixed periods (Kidd and Jervis, 1996). Such data should be collected as part of a life table study covering several generations. Information should be obtained for (a) the average level of hyperparasitism per primary parasitoid host generation, (b) its variability from generation to generation and whether or not hyperparasitism is a key-factor responsible for host population fluctuations, (c) the extent to which hyperparasitism tends to act as a density-dependent factor, either spatially within a generation or between generations, (d) other primary parasitoid host mortalities that combine with hyperparasitism to counter the host's potential rate of increase, and (e) any important mortalities suffered by hyperparasitoids that reduce their effectiveness as natural enemies of primary parasitoids (after Hassel and Waage, 1984).

Furthermore, we should investigate interactions between hyperparasitoids such as the levels of competition between hyperparasitoids species. Species like *Dendrocerus* and *Asaphes* are capable of tertiary parasitism, which means that the larva of a hyperparasitoid is parasitised by another hyperparasitoid. Several laboratory studies have already been conducted to discover which species is the strongest competitor in this situation (Sullivan, 1972; Bennet and Sullivan, 1978; Levine and Sullivan, 1983; Matejko and Sullivan, 1984; Carew and Sullivan, 1993). Studies like these will indicate the level of interference between different hyperparasitoid species, which might influence the impact of these species on populations of primary parasitoids.

These data will contribute to the construction of more realistic models that can be used in simulation studies. Eventually, we should consider multitrophic interactions on a landscape scale, outside an agricultural field. Even though parasitoid mortality due to hyperparasitism is often high in agro-ecosystems, parasitoid survival may rest on the availability of hosts that are feeding on wild plants. The dynamics and persistence of multitrophic interactions will be influenced by the ability of herbivores and their antagonists to exploit natural plant communities that are considerably more heterogeneous than simple monocultures that frequently characterise agro-ecosystems (Harvey *et al*., 2003).

Hyperparasitoids are part of the ecosystem and the effects of those species already present, good or bad, are unlikely to be altered drastically by the intervention of man (Bennett, 1981). However, when we know their role in agricultural ecosystems, it will be easier to evaluate the efficacy of parasitoids to suppress herbivore populations and the choice of natural enemies for biological control purposes.

6.7. References

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