

OVIPOSITION DECISIONS AND LARVAL COMPETITION

BETWEEN THE APHID PARASITOIDS

APHIDIUS ERVI AND APHIDIUS SMITHI

(HYMENOPTERA: APHIDIIDAE)

by

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(HYMENOPTERA: APHIDIIDAE)

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Oviposition decisions and larval competition between the

aphid parasitoids Aphidius ervi and Aphidius smithi

(Hymenoptera: Aphidiidae)

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ABSTRACT

Competitive interactions between Aphidius ervi Haliday and A. smithi Sharma and Subba Rao (Hymenoptera: Aphidiidae), solitary endoparasitoids of the pea aphid, were examined in the laboratory. In solitary species, oviposition decisions are expected to be influenced by the relative increase in fitness that results from choosing a particular host. This hypothesis was tested by offering female wasps of each species different host classes of pea aphid: unparasitized, parasitized by a conspecific female, parasitized by a female of the other species, and parasitized by herself.

The potential for competitive interactions between the two parasitoid species was high since both showed the same host instar preference. When offered all nymphal instars simultaneously, A. ervi and A. smithi parasitized more second instar aphids. Studies on aphid responses to parasitoid attack demonstrated that preference was not an absolute value but influenced by experimental design.

Larval competition studies between A. smithi and A. ervi showed that under most conditions, A. ervi was the superior larval competitor. When offered unparasitized pea aphids and those parasitized by a female of the other species, wasps oviposited more often in unparasitized hosts. As the inferior larval competitor, A. smithi was expected to avoid competition with A. ervi.

When given a choice between aphids parasitized by conspecifics and those parasitized by a female of the other species, both species showed a preference for aphids previously attacked by A. smithi. This was predicted because A. ervi is superior to A. smithi in larval competition. When offered conspecific- and self-parasitized hosts, A. smithi females attacked more of the former. This was also predicted, because under the experimental conditions, an A. smithi female cannot increase her fitness by laying two eggs in the same aphid.

Reasons for avoidance of multiparasitism by A. ervi were less apparent. Although larval growth rates were reduced in multiparasitized hosts, variations in host quality were not reflected in parasitoid sex ratios.

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I. GENERAL INTRODUCTION

When selecting hosts for oviposition, hymenopterous parasitoids encounter hosts of varying quality, even when parasitizing only one species. Host quality refers to the status of the host as a food source for the parasitoid's offspring (Waage & Godfray, 1985), and for any given host class, is not an absolute quantity but is measured relative to the other classes available (Waage, 1986). Although quality is influenced by many host attributes, it is often measured using one character as an index, such as host size, age, nutritional status, or whether the host has been previously parasitized. These can affect the quantity of food available to the developing larva and whether it is readily obtained during critical growth phases (Strand, 1986), which in turn may alter parasitoid characteristics such as adult size, developmental time, fecundity, longevity, mating success, or host-finding abilities (Sandlan, 1979; Charnov et al., 1981; Jones, 1982; Lui, 1985; Takagi, 1985; Hurlbutt King, 1987). Lower host quality can reduce larval survival, particularly if the immature parasitoid competes with another during growth. A parasitoid developing in a higher quality host is predicted to have a better chance of survival and/or increased reproductive output compared to one developing in a lower quality host (Stand, 1986; Waage, 1986). Sex ratio shifts are expected when eggs are laid in lower quality hosts if the fitness of one sex (usually female) is more adversely affected by a decrease in host quality than the other (Charnov et al., 1981).

A female parasitoid should assess the quality of each potential host, and

should prefer to select those of higher quality for oviposition (Charnov et al., 1981; Charnov and Skinner, 1985; Waage & Godfray, 1985; Waage, 1986). This assumes that host types are similar with respect to other attributes such as handling time and travel time between individuals. By showing a preference for higher quality hosts, parasitoids are expected to increase their fitness relative to those females which select lower quality hosts or make no choices between host types.

Preference is rarely absolute, but varies with available host classes and environmental conditions, among other factors. The experimental design (Mackauer, 1983) as well as the previous experience of a wasp will also influence the measured preference in any given experiment (van Alphen & Vet, 1986). In the present study, preference is defined to be a relative response, and is not an all-or-none phenomenon. Depending on the situation under discussion, the preferred host class is more often attacked or oviposited in by a searching parasitoid, once any differences in relative frequency of occurrence have been accounted for.

The main objective of the present work is to examine how parasitization by one solitary wasp affects a subsequently attacking wasp's oviposition decisions, and the survival of her offspring. Such oviposition decisions are important for solitary wasps because normally only one parasitoid completes development in a host. Theory predicts that when a wasp encounters a host, her decision to oviposit or not will be influenced by the probability that her larva will survive. Therefore, she should prefer unparasitized hosts to parasitized ones, although there are situations in

which laying an egg in a parasitized host is considered to be adaptive (van Alphen & Nell, 1982; Charnov & Skinner, 1984, 1985; Iwasa et al., 1984; Waage & Godfray, 1985; van Alphen & Vet, 1986; van Alphen & Visser, 1990). The fitness consequences of oviposition into previously-parasitized hosts are expected to vary depending on whether the previously-attacking wasp was herself, a conspecific, or a female of a different species (Waage, 1986; Strand, 1986). The identity of the first-attacking wasp will determine how the quality of an unparasitized host has been altered for the second wasp. When given a choice between hosts parasitized by conspecifics and those parasitized by females of another species, a wasp's oviposition decisions are predicted to depend on the survival probabilities of her offspring in each host type. This in turn varies with the mechanisms of larval competition used by immature parasitoids of each species, since these mechanisms determine larval survival (Mackauer, 1990). Solitary wasps are expected to avoid self-superparasitism (i. e. laying two eggs in the same host) because the offspring which completes development must first kill its sibling (Waage, 1986; Hubbard et al., 1987; Völkl and Mackauer, 1990). Avoidance may not be beneficial when the presence of two eggs in a host increases the chance that one of them will survive (Cloutier, 1984; van Alphen & Visser, 1990; Visser et al., 1990).

Development in a parasitized host may result in a lower growth rate or smaller adult size for the wasp that kills its competitor and eventually emerges. In this situation, the surviving parasitoid will be at a disadvantage when competing for mates or hosts with wasps developing in

singly-parasitized hosts. Parasitoid size has been positively correlated with fecundity or longevity, or both (Sandlan, 1979; Charnov et al., 1981; Jones, 1982; Lui, 1985; Takagi, 1985; Hurlbutt King, 1987) and is assumed to enhance host-finding abilities (Charnov et al., 1981). Sex ratio changes have been observed when parasitoids chose between large and small hosts, with more female wasps emerging from the former (Charnov et al., 1981; Simbolotti et al., 1987; Hurlbutt King, 1987, 1988; Griffiths & Godfray, 1988; Werren & Simbolotti, 1989). Attributes associated with large size are predicted to have a greater effect on female than male fitness (Charnov et al., 1981). A few studies have shown that wasps treat parasitized hosts similarly to small ones, in that more male eggs are allocated to this host type (Wylie 1966, 1973; 1976; Holmes, 1972; van Alphen & Thunnissen, 1983).

Avoidance of parasitized hosts is possible only if parasitoids can distinguish them from unparasitized ones. Discrimination between unparasitized hosts and those parasitized by conspecifics has been demonstrated in many species of hymenopterous parasitoids (van Lenteren, 1981). In contrast, the ability to discriminate between unparasitized hosts and those parasitized by a female of another species has been demonstrated less often (Wylie, 1970, 1971; Chow & Mackauer, 1984; Vet et al., 1984; Bai & Mackauer, unpublished) and is thought to be rare (Bakker et al., 1985; Turlings et al., 1985; van Alphen & Visser, 1990), or to exist only in very closely-related species (Vet et al., 1984). Oviposition in self-parasitized hosts in preference to those parasitized by conspecifics has been shown in some species (Hubbard et al., 1987;

Völkl & Mackauer, 1990) but not in others (van Alphen & Nell, 1982; van Dijken & Waage, 1987; Bai & Mackauer, 1990).

Either external or internal cues, or both, are used to recognize parasitized hosts. External cues can be marking pheromones (Roitberg & Mangel, 1988; Mackauer, 1990), physical marks left on the host (Takasu & Hirose, 1988), or on the host patch (Sugimoto et al., 1986). Since marking pheromones are generally assumed to be species-specific (Bakker et al., 1985; Turlings et al., 1985; van Alphen & Visser, 1990), wasps are not expected to use them for recognition of hosts parasitized by another species. Internal cues, however, are not likely to have this kind of specificity, since they are often a result of changes in host quality associated with the developing parasitoid embryo (Fisher, 1971; Beckage & Templeton, 1986; Strand, 1986).

The objective of this study is to examine competitive interactions between Aphidius smithi Sharma and Subba Rao and Aphidius ervi Haliday (Hymenoptera: Aphidiidae), solitary endoparasitoids of the pea aphid Acyrtosiphon pisum Harris (Homoptera: Aphididae). Oviposition decisions made by these parasitoids when offered hosts of varying quality (*i. e.* unparasitized, conspecific-, heterospecific-, and self-parasitized hosts) were examined in the laboratory. Widely distributed in the Palaearctic, A. ervi is sympatric with A. smithi in the Oriental region. Both species were introduced into the United States and Canada for biological control of the pea aphid (Angalet & Fuester, 1977; Gonzalez et al., 1978; Unruh et al., 1986; Kambhampati & Mackauer, 1989). The pea

aphid was introduced into North America from Europe and appeared as a pest of peas, alfalfa, vetch, and clover in Canada and the United States during the late 1890's and early 1900's (Harper et al., 1978).

The objectives of the my thesis are as follows:

1. To determine whether A. ervi and A. smithi have a similar preference for one or more instars of the pea aphid. The potential for interaction between these parasitoid species is greatest if this is the case. Both A. ervi and A. smithi attack and complete development in all instars and the adult stage of the pea aphid, but females may oviposit more often in a particular instar.
2. To examine the outcome and mechanisms of larval competition between A. ervi and A. smithi in multiparasitized pea aphids (i. e. hosts containing immature stages of two or more parasitoid species (Smith, 1916)) by varying the stages of immature parasitoid competing inside a multiparasitized aphid. Estimates of survival probabilities of A. smithi immatures in hosts parasitized by A. ervi and vice versa are necessary to determine the relative fitness consequences of multiparasitism for each species.
3. To determine if A. ervi and A. smithi females are capable of heterospecific host discrimination (i. e. if they can distinguish between unparasitized hosts and those parasitized by the other species).
4. To determine if A. ervi and A. smithi can distinguish between hosts parasitized by conspecifics and those parasitized by the other species. Host choices will be related to predictions based on the survival probabilities of offspring belonging to the second-attacking female in

each type of parasitized host.

5. To determine if A. smithi females can distinguish between hosts parasitized by conspecifics and those previously parasitized by themselves.

6. To test the prediction that wasps should adjust the sex ratio of their offspring according to whether or not the host has been previously parasitized. Since unparasitized hosts are considered to be of higher quality than parasitized hosts, wasps are predicted to lay more female (i. e. fertilized) eggs in the former and more male (i. e. unfertilized) eggs in the latter.

II. INSECT COLONIES AND GENERAL PROCEDURES

1. Parasitoid colonies

The life cycles of A. ervi and A. smithi are similar. The female parasitoid inserts her ovipositor into a host and lays a single egg which hatches two and a half to three and a half days later at 21°C (see Chapter IV). The immatures go through at least three (O'Donnell, 1987) or perhaps four (Chorney & Mackauer, 1979) larval instars and grow by feeding on the host's internal contents. The first larval instar has sickle-shaped mandibles, the intermediate stages are amandibulate, while the mandibles of the last stage are used for cleaning out the aphid's internal contents prior to parasitoid pupation. The immature parasitoid pupates inside the hardened skin of the dead host (called a mummy), and later the adult parasitoid emerges through a hole it has cut through the mummy's skin. Normally only one parasitoid can complete development inside one host (*i. e.* the parasitoids are solitary). At 21°C, developmental time from egg to adult is approximately 2 weeks. The egg and larval stages of A. ervi and A. smithi cannot be separated according to species under the dissecting scope, while the adults are easily distinguished based on colour differences (Mackauer & Finlayson, 1967).

Laboratory colonies of A. ervi and A. smithi were established from mummified pea aphids, A. pisum, collected on alfalfa near Kamloops, British Columbia in 1986. The A. ervi-colony used for experiments described in Chapter VII was collected in 1989 in the same location. Both

parasitoid species were reared on pea aphids feeding on broad bean, Vicia faba L. cv. 'Broad Windsor'. For maintenance of parasitoid stock colonies, third instar pea aphids were parasitized. All stock colonies and experimental material were reared at 21° C, 55 to 70% RH, and continuous light.

After they emerged from the mummies, adult parasitoids were provided with diluted honey. In all experiments, two- to four-day old wasps were used. Unless otherwise stated, female parasitoids were not mated prior to experiments and therefore laid only male (*i. e.* unfertilized) eggs. This eliminated any variation in developmental time or larval competitive abilities caused by a difference in sex. Also, parasitoids were allowed to attack third-instar pea aphids for three to four hours one day before each experiment to gain experience in handling hosts.

2. Pea aphid colony

In British Columbia, the pea aphid A. pisum overwinters as eggs. During the spring, eggs hatch into aphid stem mothers (the fundatrix generation) which give birth to viviparous females. Throughout the rest of the spring and summer, many generations of viviparous females are produced and these aphids may be apterous (wingless) or alate (winged). The latter form permit aerial dispersal, and are often produced if colony conditions become crowded and/or food quality deteriorates. In the fall, male and female sexual forms are produced which mate; the female morph then lays eggs.

Each pea aphid goes through four nymphal instars and an adult form. At 21°C, a viviparous female begins reproducing about 10 days after birth. This short generation time and the fact that embryo development begins inside a viviparous aphid before she is born contribute to the high aphid populations that often occur during summer months.

Pea aphids occur on many Papilionaceae, and are considered to be pests of peas, alfalfa, clovers, and vetch (Harper et al., 1978). In British Columbia, pea aphids are commonly found on alfalfa. Laboratory colonies of pea aphids were reared on broad bean, Vicia faba L. cv. 'Broad Windsor', because these plants are much easier to grow under greenhouse conditions than are most other host plants of the pea aphid.

In my study, the stock colony of aphids consisted almost entirely of apterous (wingless) viviparous females. Under the rearing conditions, (21°C, 55 to 70% RH, continuous light) alate (winged) females were rarely present and sexual forms were not produced.

3. Aphid dissections

When an Aphidius female strikes an aphid with her ovipositor, she does not necessarily lay an egg. Since oviposition lasts only a fraction of a second, the presence of an egg inside a host must be confirmed by rearing attacked aphids and later dissecting them to check for the presence of parasitoid eggs or larvae. Aphids were usually dissected when parasitoids

were in the advanced embryonic or newly-hatched stage to ensure detection of all immature parasitoids.

To check only for the presence or absence of parasitoid eggs or larvae, aphids were killed and dissected in 70% ethanol. However, to observe living parasitoids freshly removed from a host, aphids were dissected in 0.8% saline solution. The latter technique is necessary in larval competition studies to determine which immature parasitoid is alive and which is dead at the time of dissection.

III. HOST INSTAR PREFERENCE

1. Introduction

Although A. ervi and A. smithi attack and are able to complete development in all four nymphal instars as well as the adult of the pea aphid, females of each species may oviposit more often in one instar than another. If A. smithi and A. ervi share a similar preference for the same nymphal instar of the pea aphid, the potential for interaction between these parasitoid species will be greater. If one species oviposits more often in older instars while the other prefers younger instars, interaction will be minimal and will occur if a female of the former species encounters a host containing a fairly old immature of the latter. The preferred instar is defined as the one which has the highest percentage of parasitism, once any differences in relative frequency of each instar have been accounted for. Although the observed host instar preference will be strongly influenced by experimental design, a comparison of A. smithi and A. ervi behaviour under similar conditions will demonstrate whether differences in oviposition patterns exist between these species.

Host instar preference has been shown in other species of aphid parasitoids (Stary, 1970; Lui et al., 1984; Sequeira & Mackauer, 1987). This was first investigated for A. smithi by Wiakowski (1962), who found that second and third instars were parasitized more often than first or fourth instars or adults. Unfortunately, Wiakowski's (1962) experimental design was not carefully controlled, with five A. smithi females exposed

to a mixture of aphids for 24 hours. Fox et al. (1967) suggested that A. smithi preferred early first instar aphids when each instar was offered one at a time, and exhibited no clear choice when more than one instar was offered simultaneously. However, their results were based on small sample sizes and should be verified. When Mackauer (1973) offered individual A. smithi females a choice between two host classes (48-h-old "standard" aphids and "test" aphids of various ages), wasps parasitized first instars with less frequency than older nymphs and pre-reproductive (viviparous) adults. Reproductive aphid adults were parasitized less often than second or third instars. However, Mackauer (1973) did not find good evidence that parasitoids distinguished between second, third, and fourth instars. No formal studies have examined host instar preference by A. ervi, although observations by Stary (1962) suggest that second- and third-instar pea aphids are preferred.

As long as all aphid stages are suitable for development of parasitoid offspring, the observed oviposition patterns are influenced by at least two factors. From among the available hosts, a parasitoid may choose the ones of highest quality for her offspring. Also, some aphid instars are better able to escape parasitization than others, which will influence whether a parasitoid can successfully oviposit.

Host size is an important aspect of quality, since it directly influences the amount of food available for the growth of parasitoid offspring. Large hosts are expected to be of higher quality than small ones because they contain more resources and will produce larger offspring (Charnov et

al., 1981). In some studies, host size at oviposition has been positively correlated with adult parasitoid size. This, in turn, has been positively correlated with parasitoid fecundity and longevity, although the relationship between parasitoid size and developmental time is not consistent (e.g. Sandlan, 1979; Charnov et al., 1981; Bellows, 1985; Lui, 1985; Opp & Luck, 1986; Hurlbutt King, 1987; Mackauer & Kambampati, 1988). For males, large size may also improve the ability to obtain effective matings and may increase sperm production (Charnov et al., 1981; Hurlbutt King, 1987). Any advantage associated with being a small parasitoid is probably involved with shorter developmental time (Hurlbutt King, 1987). If female offspring gain more than males in terms of increased reproductive capacity by being large, wasps would then be expected to lay more female (*i. e.* fertilized) eggs in larger hosts (Charnov et al., 1981).

Aphid behaviour influences the frequency at which a particular instar is parasitized. When attacked by a parasitoid, aphids often respond by knocking away the parasitoid, shaking or jerking the body, or walking or falling from the plant. Such defensive mechanisms are often stronger in older instars and adult aphids (Calvert, 1973; Lui et al., 1984; Gardner et al., 1984; Hofsvang and Hagvar, 1986; Sequeira & Mackauer, 1987; Gerling et al., 1990). However, younger instars are more likely to escape parasitism because they are hidden on a plant where wasps cannot find them.

The first objective of this chapter is to determine if A. smithi and A.

ervi females show the same pattern of host instar preference. Parasitoid females will be presented simultaneously with equal numbers of all four instars of the pea aphid. Preference will be determined by comparing the number of each aphid instar parasitized. This experimental design, where a parasitoid is exposed to all instars at the same time, more closely approximates a field situation than paired comparisons where a wasp chooses between only two host classes (Sequeira & Mackauer, 1987).

The second objective is to examine the effect of aphid instar at the time of oviposition on the dry weight of adult parasitoid offspring. This is a preliminary study on the effects of host instar selection on parasitoid fitness, with parasitoid dry weight used as an index of host quality. However, the study will not be carried any further than estimating this simple index of parasitoid fitness.

The third objective is to examine the influence of aphid behaviour on frequency of parasitization. Preliminary observations showed that aphids often drop from a plant when disturbed by a parasitoid, which could be a defensive reaction to avoid parasitism. This behaviour cannot be used to escape parasitism in the experiments described above, since aphids and parasitoids are caged on a bean stalk until the test is complete. I will investigate (1) how dropping behaviour affects the relative proportions of each instar parasitized and (2) whether dropping off the stalk appears to be a defensive behaviour used by the aphids to avoid parasitism. The effect of aphid density will be tested by using a high (20 aphids per stalk) and low (5 aphids per stalk) density.

2. Materials and Methods

a. Hosts and parasitoids

Pea aphid nymphs were produced by placing reproductive viviparous adults on bean stalks for eight hours and then removing the nymphs and placing them on fresh plants. Nymphs were then reared at 20°C for 24, 48, 96, or 144 h to obtain first-, second-, third-, and fourth-instar aphids respectively. Fourth and second instars were marked by clipping the distal end of one antenna (Mackauer 1972). Parasitoids were two- to four-days old, had no previous experience with aphids, and were allowed to mate.

b. Host instar preference of A. smithi and A. ervi

Fifteen individuals of each aphid instar were placed simultaneously on a bean stalk enclosed in a plastic cage with a screened lid (16.0 cm diameter). The base of the stalk was immersed in a vial of water. After the aphids had been allowed to settle on the stalk for two to three hours, one female parasitoid was introduced into each cage for 4 hours and then removed. Twenty wasps of each parasitoid species, one wasp per cage, were tested. Aphids were left on the bean stalks in their cages and reared for three to four days. At this time, ten individuals of each instar were randomly removed from each cage and dissected; the remaining aphids were discarded. The number of immature parasitoids per aphid was recorded.

c. Influence of host instar at oviposition on parasitoid weight

The procedure was the same as described above, except that aphids were reared until parasitoid pupation. If a parasitoid female did not produce any female offspring, her progeny were excluded from the analysis because she may not have mated and would therefore not be capable of producing daughters. Adult parasitoid offspring were dried at 100°C for 72 h, weighed immediately, and their sex recorded. I tested only A. ervi since similar data for A. smithi have already been reported (Henkleman, 1974).

d. Aphid response to searching parasitoids

Aphids of one nymphal instar only were placed on a bean stalk with a height of 10 cm and allowed to settle overnight. All instars were tested, but only one instar was present on a bean stalk during a trial. Each bean stalk had been placed in a vial of water, had two fully-opened leaves, two newly-opened leaves near the tip, and leaves at the tip which had not yet unfurled. The following day, stalks were placed one at a time in a plexiglass cage (45 cm X 34 cm X 31 cm) and a female A. ervi was released at the base of each stalk. Each parasitoid was observed continuously as it searched for aphids. As soon as an aphid left the stalk, it was removed from the cage so the parasite could not encounter that aphid again. A trial ended when the parasite flew away, walked off the stalk, or one hour had elapsed. All aphids were reared and later dissected, and the number of immature parasitoids per aphid recorded.

Preliminary observations had shown that under the experimental conditions, aphids responded to the presence of a searching parasitoid mainly by dropping off the stalk. For each trial, records were kept of the time a parasitoid spent actively searching for aphids, the time a parasitoid spent on other activities (*i. e.* standing still or preening), and the reason that an aphid left the stalk. Causes of an aphid leaving the stalk were divided into four categories:

1. the parasitoid attacked an aphid (*i. e.* struck it with her ovipositor);
2. the parasitoid touched the aphid with her antennae or another part of her body, but did not attempt to oviposit;
3. aphids fell in a group, (*i. e.* two or more aphids fell simultaneously after disturbance);
4. the reason was unknown since the parasite was not near the aphid when it left the stalk.

Two aphid densities were tested: 20 and 5 of each aphid instar per stalk. At each density, data for 15 *A. ervi* females were pooled for each aphid instar. For each density, I compared percent parasitism among instars and percentage of aphids dropping off the stalk among instars by fitting a log-linear model to the pooled data, using the SAS statistical software package (SAS, 1985). In this experiment, host instar "preference", as measured by percent parasitism, was assessed by giving wasps only one instar to chose from at any given time.

3. Results

a. Instar preference of A. smithi and A. ervi

Females of A. ervi and A. smithi parasitized more second-instar pea aphids and also laid more eggs in these hosts (Tables 1 and 2) (ANOVA; for number of hosts parasitized, A. ervi : $F=5.87$, $df = 3,76$, $P=0.001$; A. smithi : $F=8.93$, $df=3,76$, $P<0.001$; for numbers of eggs laid, A. ervi : $F=5.41$, $df=3,76$, $P=0.002$; A. smithi : $F=13.50$, $df=3,76$, $P<0.001$; a Newman-Kuels multiple range test was done after each ANOVA). For both species, there was no significant difference in number of hosts parasitized when first, third, and fourth nymphal instars were compared. Similarly, number of eggs laid per parasitoid did not differ when these three aphid instars were compared. According to these oviposition patterns, host instar preference shown by both parasitoid species was:

instar II > (I = III = IV).

Frequencies of superparasitism were low, as shown by the numbers of eggs laid per parasitized host (Tables 1 and 2). A value of one indicates no superparasitism, and except for A. smithi ovipositing in second-instar aphids, experimental values were less than 1.26. For A. ervi there was no significant difference between mean number of eggs laid per parasitized host when all instars were compared (Table 1) (ANOVA, $F=0.50$, $df=3,75$, $P=0.69$). This shows that the higher number of eggs laid by A. ervi females in second-instar aphids was a result of each female parasitizing more aphids of this instar. For A. smithi, the mean number of eggs laid

Table 1. Host instar preference by Aphidius ervi when all four instars of the pea aphid were presented simultaneously.

Aphid instar	No. of hosts parasitized	No. of eggs laid	No. of eggs laid/ parasitized host
1st	4.00±0.44 a	4.90±0.64 a	1.17±0.07 a
2nd	6.10±0.38 b	7.75±0.60 b	1.25±0.07 a
3rd	3.90±0.57 a	4.60±0.66 a	1.17±0.06 a
4th	4.10±0.54 a	4.90±0.54 a	1.16±0.04 a

Data show means (n = 20 parasitoids) ± standard errors. In each column, means joined by the same letter are not significantly different (P > 0.05) (ANOVA, followed by a Newman-Keuls multiple range test if necessary).

Table 2. Host instar preference by Aphidius smithi when all four instars of the pea aphid were presented simultaneously.

Aphid instar	No. of hosts parasitized	No. of eggs laid	No. of eggs laid/ parasitized host
1st	4.80±0.43 a	5.65±0.56 a	1.17±0.05 a
2nd	7.65±0.41 b	11.20±0.87 b	1.44±0.07 b
3rd	4.80±0.48 a	5.95±0.65 a	1.21±0.05 a
4th	5.65±0.47 a	7.05±0.67 a	1.25±0.05 a

Data show means (n = 20 parasitoids) ± standard errors. In each column, means joined by the same letter are not significantly different (P > 0.05) (ANOVA, followed by a Newman-Keuls multiple range test if necessary).

per parasitized host was significantly greater for second-instar aphids (Table 2) (ANOVA, $F=5.28$, $df=3,76$, $P<0.005$, followed by a Newman-Keuls multiple range test). Therefore, the higher number of eggs laid in this instar was a result of females ovipositing in more second-instar aphids and laying more eggs in each parasitized host.

b. Influence of host instar at oviposition on parasitoid weight

Aphid instar at the time of oviposition and the dry weights of adult A. ervi emerging from these aphids are shown in Table 3. Within each aphid instar, female parasitoids were heavier than males (see Table 3). Within each sex, aphid instar at the time of oviposition significantly affected parasitoid dry weight in the following way:

(weight of parasitoid from Instar 1 = weight from Instar 2) <

(weight from Instar 3 = weight from Instar 4)

(ANOVA; for females, $F=62.52$, $df=3,131$, $P<0.001$; for males, $F=146.88$, $df=3,231$, $P<0.001$; followed by a Student Newman-Kuels multiple range test for each sex).

c. Aphid response to a searching parasitoid

The percentage of each aphid instar parasitized by A. ervi is shown in Fig. 1. For each aphid density, there was a significant difference in percent parasitism among instars (for density = 20, $X^2=9.65$, $df=3$, $P=0.022$; for density = 5, $X^2=13.55$, $df=3$, $P=0.004$). Next, I tested the null hypothesis that percent parasitism was highest for second-instar

Table 3. Comparison between dry weights of adult male and female Aphidius ervi emerging from each host instar.

Aphid instar ¹	Males		Females		t	df	P
	n	weight (mg)	n	weight (mg)			
1	42	0.1498±0.0025	21	0.1779±0.0038	6.31	61	**
2	61	0.1515±0.0024	30	0.1776±0.0027	6.56	89	**
3	61	0.2111±0.0035	46	0.2414±0.0050	5.14	105	**
4	71	0.2219±0.0034	38	0.2377±0.0039	2.89	107	*

¹aphid instar at time of oviposition

Data show means ± standard errors.

Within each instar, means were compared using a two-sample t-test.

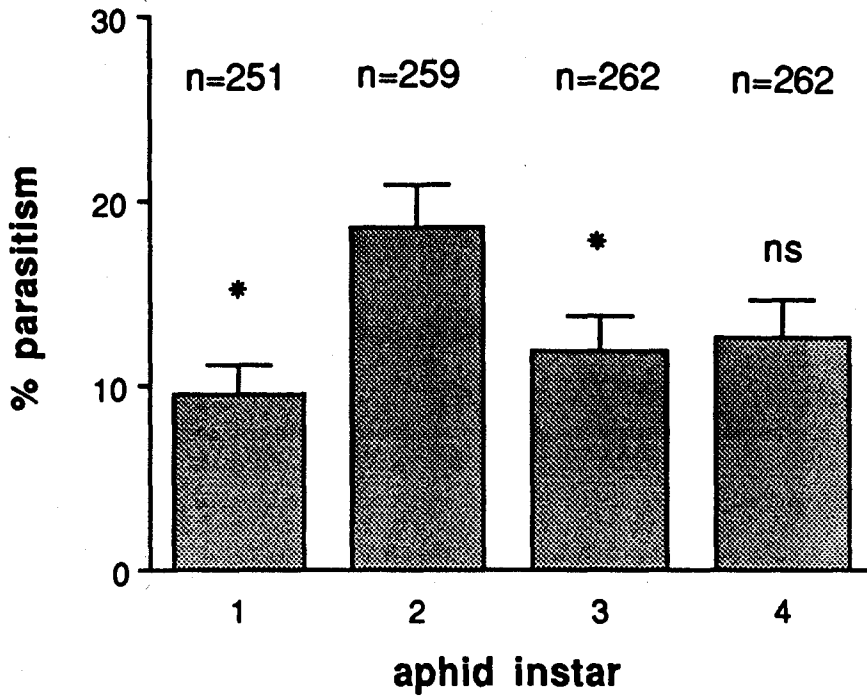
** P<0.001 * P<0.005

Fig. 1. Percent parasitism of each aphid instar
at two aphid densities. Data show mean and 1 SEM.
Parasitism of each instar was compared to that of
second instars.

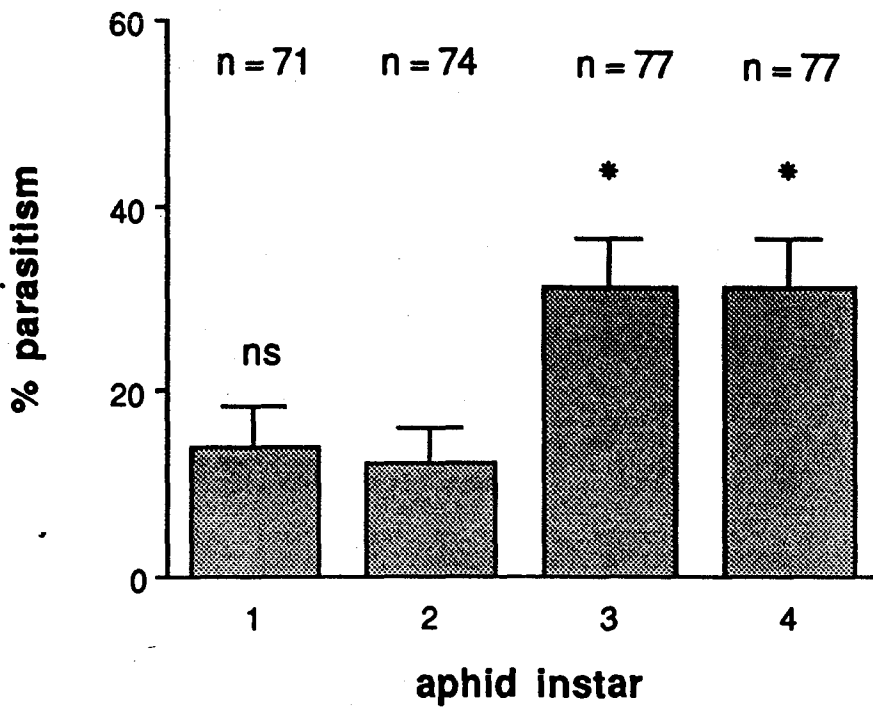
* $P < 0.05$

ns $P > 0.05$

20 APHIDS PER STALK



5 APHIDS PER STALK



aphids at each density, since this was the preferred host instar in a previous experiment (see section III.3.a). At a density of 20 aphids/stalk, percent parasitism of second instars was significantly greater than that for first ($X^2=8.19$, $df=1$, $P=0.004$) or third ($X^2=4.48$, $df=1$, $P=0.034$) instars. However, it was not significantly different from fourth instars ($X^2=3.46$, $df=1$, $P=0.063$), although percent parasitism of second instars tended to be higher and would likely be so given a larger sample size. At a density of 5 aphids/stalk, percent parasitism of second-instar aphids was the same as for first instars ($X^2=0.12$, $df=1$, $P=0.732$) but significantly lower than that for third ($X^2=7.51$, $df=1$, $P=0.006$) and fourth ($X^2=7.51$, $df=1$, $P=0.006$) instars. Therefore, instar "preference" as measured by oviposition patterns depended on whether aphid density was high or low. At the higher density parasitoids tended to "prefer" second-instar aphids, while at the lower density, a higher proportion of larger instars (third and fourth) were parasitized.

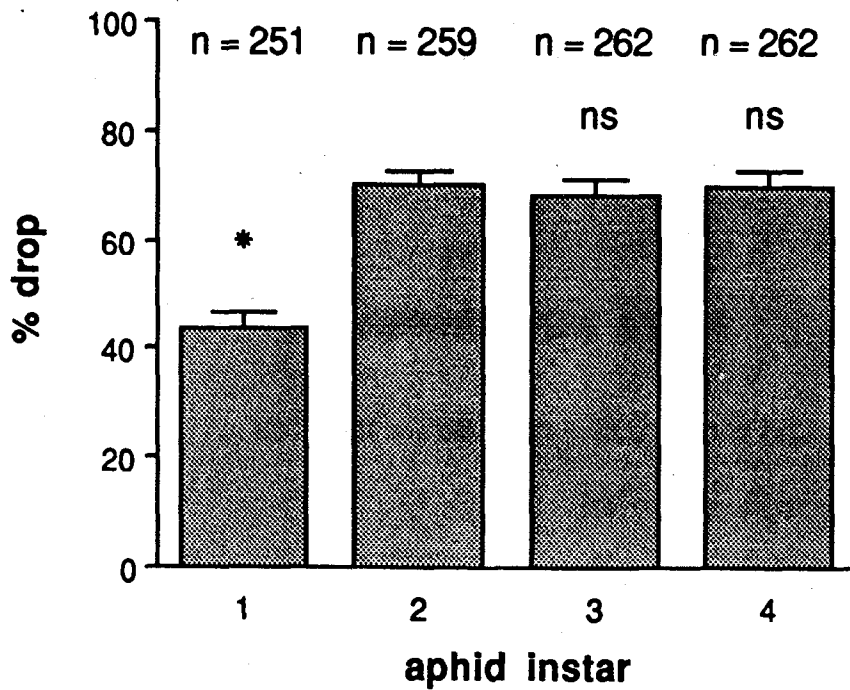
I classified aphid response to the presence of a parasitoid on the stalk as "dropping off the stalk" or "remaining on the stalk" (Fig. 2). For each aphid density, there was a significant difference among instars in the percentage of aphids dropping off the stalk (for density = 20, $X^2=52.16$, $df=3$, $P<0.001$; for density = 5, $X^2=12.24$, $df=3$, $P=0.007$). Once again, I compared the response of second instar aphids to that of the other three instars. At density = 20 aphids/ stalk, 70% of second instar aphids dropped off the stalk, a response similar to that of third ($X^2=0.15$, $df=1$, $P=0.669$) and fourth instars ($X^2=0.01$, $df=1$, $P=0.917$) but significantly greater than that of first instar aphids ($X^2=35.41$, $df=1$,

Fig. 2. Percentage of each aphid instar dropping from the stalk at two aphid densities. Data show mean and 1 SEM. The mean value for each instar was compared to that of the second instar.

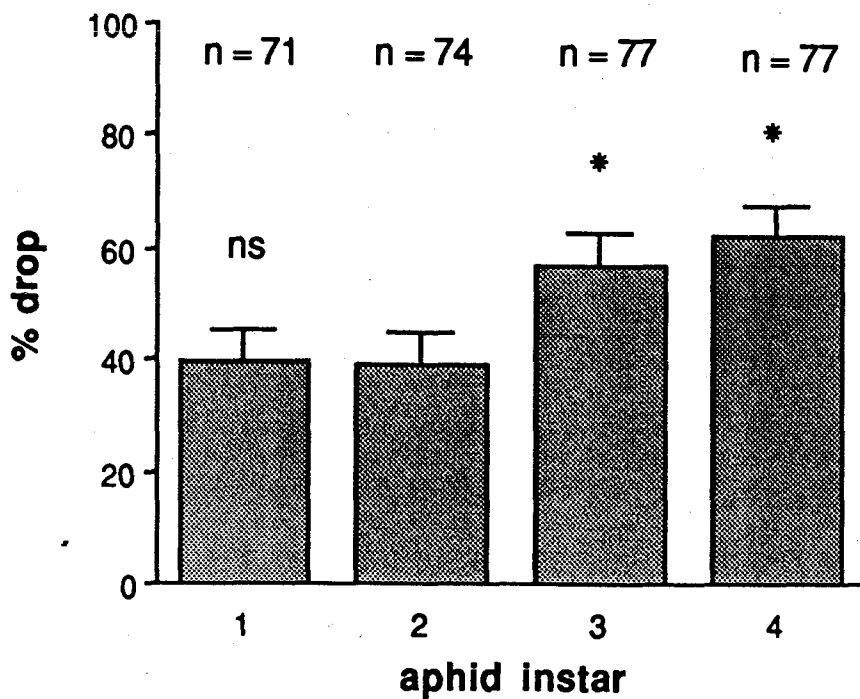
* $P < 0.05$

ns $P > 0.05$

20 APHIDS PER STALK



5 APHIDS PER STALK



$P=0.001$) (43% dropped). At density = 5 aphids/stalk, second-instar aphids behaved like first instars ($X^2=0.00$, $df=1$, $P=0.976$), with only 39% leaving the stalk. In contrast, a significantly higher percentage of third ($X^2=4.82$, $df=1$, $P=0.028$) and fourth instars ($X^2=7.94$, $df=1$, $P=0.005$) left the stalk (over 57%).

The reason for an aphid dropping off the stalk was classified into one of four categories; the numbers of aphids in each category are shown in Table 4. At both densities, 80 to 90% of aphids leaving the stalk did so immediately after disturbance by the searching parasitoid. Only at the higher density did aphids fall in a group (*i. e.* two or more aphids fell simultaneously after a nearby aphid was disturbed by a parasitoid). This "group effect" was not present at the lower density because the aphids were less crowded on the stalk and their behaviour was not influenced as much by what happened to their neighbours. Note that in Table 4, the number of each instar dropping off the stalk is higher than the values recorded for Fig. 2. Table 4 is compiled from direct observations while data for Fig. 2 were obtained after the aphids had been reared for four days and then dissected. However, since mortality rates of aphids were low and spread evenly among instars, they did not significantly affect results.

At both aphid densities, percent parasitism of aphids dropping off the plant did not differ among instars (for density=20, $X^2=7.18$, $df=3$, $P=0.066$; for density=5, $X^2=0.33$, $df=3$, $P=0.954$) (Table 5). Within an instar, when the percent parasitism of aphids dropping off the stalk was

Table 4. Reason for aphids dropping off the bean stalk.

Aphid instar	n	Numbers of aphids			
		struck by a wasp	touched by a wasp ¹	fell in a group ²	reason unknown
20 APHIDS/STALK					
1st	121	42	8	45	26
2nd	197	37	46	87	27
3rd	190	52	43	75	20
4th	203	87	34	42	40
5 APHIDS/STALK					
1st	31	24	2	0	5
2nd	31	15	10	0	6
3rd	44	21	17	0	6
4th	51	36	6	0	9

¹Wasp did not attempt to oviposit.

²Two or more aphids fell simultaneously after disturbance.

Table 5. Comparison between the number of parasitized aphids dropping off and remaining on the stalk for each aphid instar.

Aphid instar	Dropping aphids			Remaining aphids			G	P
	n	par	unpar	n	par	unpar		
20 APHIDS/STALK								
1st	109	16	93	142	8	134	5.586	*
2nd	181	38	143	78	10	68	2.490	ns
3rd	179	22	157	83	9	74	0.111	ns
4th	182	22	160	80	11	69	0.133	ns
5 APHIDS/STALK								
1st	28	8	20	43	2	41	7.319	**
2nd	29	9	20	45	0	45	16.972	***
3rd	44	14	30	33	10	23	0.020	ns
4th	48	13	35	29	11	18	0.945	ns

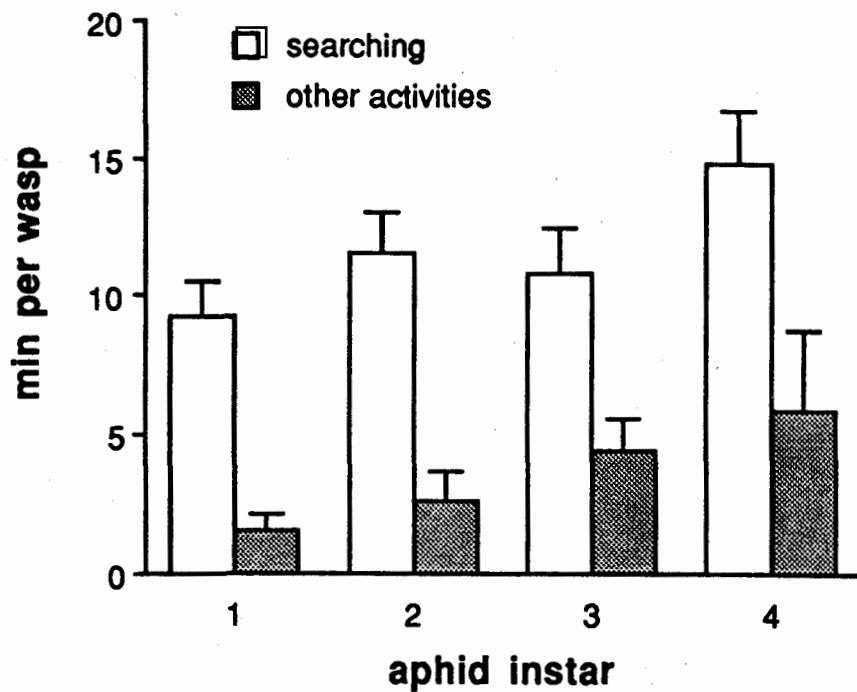
Abbreviations: n = number of aphids dissected, par = parasitized, unpar = unparasitized
 Data were pooled over all Aphidius ervi females (n = 15 for each instar).
 Comparisons were made using a G-test with William's correction factor (df = 1).
 *** P<0.001, ** P<0.01, * P<0.025,
 ns = not significant (P>0.05)

compared to that of aphids remaining, differences were found in some cases. At the higher aphid density, first instar aphids that dropped off the plant were more likely to be parasitized than those that did not (see Table 5). This was also true for both first- and second-instar aphids at the lower density. In fact, at this density, all second-instar aphids that stayed on the plant escaped parasitism. When other instars were tested, there was no relationship between dropping off the stalk and being parasitized.

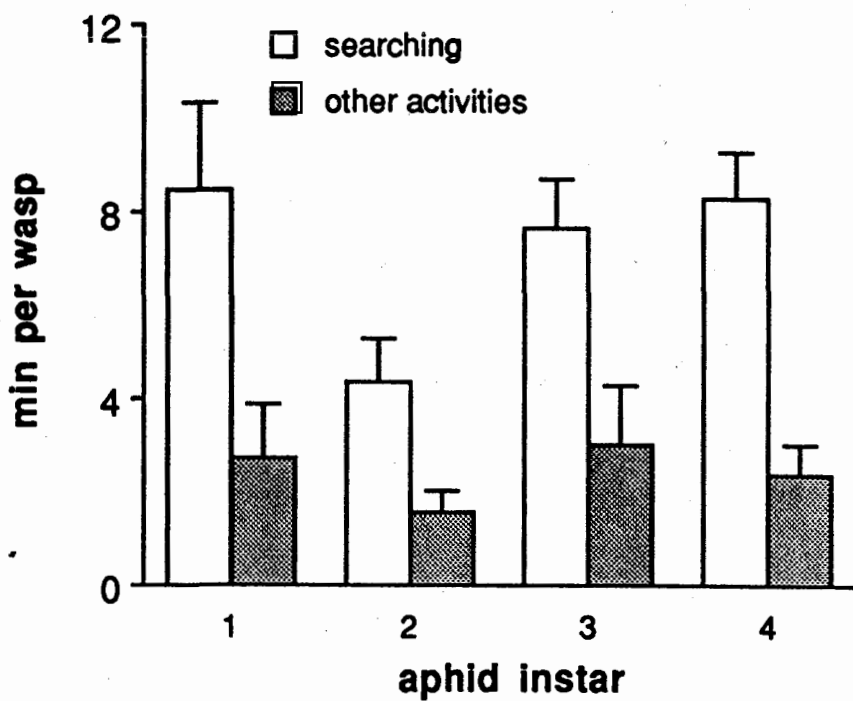
Differences in rates of parasitism among instars cannot be accounted for by differences in parasitoid activity since there was no significant difference among instars in the time parasitoids spent searching for aphids (Fig. 3; ANOVA; for density = 20, $F=2.23$, $df=3,56$, $P=0.095$; for density=5, $F=2.38$, $df=3,56$, $P=0.080$). The same applied to time spent on other activities, such as resting or preening (Fig 3; ANOVA; for density = 20, $F=1.30$, $df=3,56$, $P=0.283$; for density = 5, $F=0.45$, $df=3,56$, $P=0.718$).

Fig. 3. Time spent by female Aphidius ervi (n=15) searching for hosts or on other activities (e.g. resting, preening) at each aphid density. Data show mean and 1 SEM.

20 APHIDS PER STALK



5 APHIDS PER STALK



4. Discussion

When equal numbers of all four instars of the pea aphid were presented simultaneously, females of A. ervi and A. smithi showed the same pattern of instar preference: they parasitized second instars more frequently, while there was no difference among the parasitization rates of the other three instars. This is not in disagreement with the results obtained by Mackauer (1973), who offered A. smithi females a choice between two different instars at one time, and not all four simultaneously as in this study. Preference tests are influenced by the experimental conditions (Mackauer 1983; Lui et al., 1984), as illustrated by results of a study by Sequeira and Mackauer (1987) on host instar preference of the aphid parasitoid Praon pequodorum Viereck. When presented with all four pea aphid instars simultaneously, that parasitoid preferred instar I < (II = III = IV). However, when compared in pairwise combinations, instars were ranked I < (II = IV) < III.

Another important factor in preference tests is the time for which a parasitoid is exposed to hosts, because differences among numbers of each host instar parasitized will decline with the test duration (Mackauer, 1983). This problem can be avoided by observing parasitoids individually and replacing a host immediately with one of the same kind once it is attacked. Disadvantages of this method are the increased effort involved to obtain a sufficient number of replications for analysis and disturbance of hosts and parasitoids caused by host replacement during the experiment. Regardless of whether another experimental design will show a different

pattern of instar preference for A. smithi and A. ervi, results of this study show that females of these species share a similar instar preference. Therefore, the potential for interaction between A. smithi and A. ervi is high, particularly if unparasitized aphids are scarce as sometimes occurs in the field (see Appendix I, Tables 12 and 13).

For both A. ervi and A. smithi, oviposition into younger (and smaller) aphids yielded relatively small offspring (Table 3; Henkleman, 1974). However, there was an upper limit to parasitoid size; wasps originating from fourth instar aphids did not weigh any more than those from third instars. Generally, larger hosts produced larger wasps, and if females preferred hosts which produced larger parasitoid offspring, more third and fourth instar pea aphids should have been parasitized. However, since a pea aphid continues to grow after parasitization, host quality continues to change during the immature parasitoid's course of development (Lui, 1985; Mackauer, 1986), and the relationship between host size and quality is expected to be more complex than a simple relationship between host size at oviposition and final parasitoid weight or size (Waage, 1982; Sequeira & Mackauer, unpublished). Using A. ervi developing in the pea aphid, Sequeira and Mackauer (unpublished data) have shown that there is not a simple linear relationship between host size and quality in this system. The quality of a particular instar, as measured experimentally, depends on which parasitoid attribute is being examined (e.g. fecundity, developmental time, longevity, sex ratio). In terms of parasitoid fitness, the highest quality host is not determined by optimization of one character, but is a complex relationship between a number of traits.

Aphid behaviour also plays a role in the host instar preference expressed by a parasitoid in any given environment (Calvert, 1973; Lui et al., 1984; Sequiera & Mackauer, 1987; Gerling et al., 1990). This was demonstrated in the present study when aphids were permitted to drop off the stalk in response to a searching parasitoid (section III.3.c). Aphids could also do this when they were caged with parasitoids on a bean stalk for four hours (section III.3.a), but the wasp could re-encounter these aphids on the floor or walls of the cage. At a density of 20 aphids/stalk (section III.3.c), percent parasitism tended to be highest for second-instar nymphs, while at 5 aphids/stalk parasitism of larger instars was higher (Fig. 1). Host instar "preference" (as defined by oviposition rates) depended on the density of aphids on the bean stalk.

At both aphid densities, most first instars were concealed in crevices on the bean plant (eg. inside small leaves, between the stem and petiole of a leaf) at the beginning of each trial. Second instars were able to do this only when few aphids were present on the bean stalk. In this situation, smaller aphids were able to escape parasitism because the searching parasitoid could not find them. Evidence for this is seen in Table 5. At 20 aphids/stalk, only first instar aphids that dropped off the stalk were more likely to be parasitized than those remaining on the stalk. This was not true for the other instars. At 5 aphids/ stalk, first and second instars that dropped off the stalk were more likely to be parasitized. In fact, of the 45 second instar aphids remaining on the stalk, none contained a parasitoid egg. Once an aphid had left its place

of concealment and was exposed on the bean stalk where the parasitoid could find it, the probability of that aphid being parasitized greatly increased. The probability of dropping off the plant also increased, since at least 80% of aphids dropping off the stalk did so in response to disturbance by the searching parasitoid (Table 4). In many cases, a wasp was observed to strike an aphid with her ovipositor and immediately afterwards the aphid dropped off the plant.

For second instar aphids, dropping off the stalk in response to parasitoid activity occurred more frequently at the higher aphid density (Fig. 2), where they behaved more like third and fourth instar aphids. At the lower aphid density, second instars behaved more like first instar aphids. This also indicates that second instars did not often escape parasitism by concealment in crevices and inside unfurled leaflets at the higher aphid density, but were able to do so at the lower density as first instars did. Dropping from the stalk is mainly caused by parasitoid disturbance and will not occur if the parasitoid cannot find the aphids. The tendency for second instars to be more heavily parasitized at the higher density can likely be accounted for by the following:

1. Being bigger, they were easier to find than first-instar aphids;
2. They defended themselves less vigorously against parasitoid attack by kicking or running away than older instars did;
3. Dropping off the stalk was frequently a result of parasitoid attack and oviposition often occurred before second instar aphids fell.

At both aphid densities, first instar aphids were more likely to stay on the stalk than drop off (Fig. 2). Other studies have suggested that a tendency for first instar aphids to remain on the plant is adaptive (Fraser & Gilbert, 1976; Roitberg & Myers, 1978, 1979; Roitberg et al., 1979). Roitberg & Myers (1978) found that few first instar aphids dropped off a plant in response to aphid alarm pheromone alone, but almost all responded to a pheromone-vibratory stimulus. First instars may require a higher stimulus to drop than older instars because they are more susceptible than adults to high ground temperatures and are exposed longer on the ground due to difficulties in locating host plants and walking over terrain in the field (Roitberg and Myers, 1979; Roitberg et al., 1979).

Parasitism rates were generally lower when 20 aphids were initially present on the stalk (Fig. 1). Aphids often fell in a group at the higher aphid density, (i. e. two or more aphids fell simultaneously after disturbance by the parasitoid) (Table 4), a behaviour which Stary (1962) observed when A. ervi foraged in dense aphid colonies and caused aphids to "rain to the ground". At the beginning of each trial, aphids were often clustered together in groups. Once one member of the group was disturbed by the parasitoid, its neighbours responded by dropping or moving away from the area. These aphids were likely responding to alarm pheromones given off by their neighbours (Nault et al., 1973) and to movement of adjacent aphids. When several aphids fell at one time, which often occurred at the beginning of each trial, the parasitoid was able to strike only one or two of these aphids before the group left the stalk. This lowered the rate of parasitism, because aphids dropped before the

parasitoid had an opportunity to attempt oviposition. This "group effect" did not occur at the lower aphid density where aphids were spaced further apart on the bean stalk and at least 80% of the aphids fell after direct contact with the searching parasitoid (Table 4). Third and fourth instar aphids were more readily parasitized at a density of 5 aphids per stalk than at the higher density (Fig. 1). The reason for this difference is that aphids interacted more strongly at the higher density (Table 4), as discussed above.

Results presented in sections III.3.a and III.3.c showed that host instar "preference" as indicated by oviposition frequency in the laboratory is influenced by experimental design. In the field, percent parasitism of each aphid instar will be a function of the age structure and density of the aphid population, as well as the spatial distribution of each nymphal instar on the host plants. Which instar is most often parasitized is probably also a combination of wasps choosing the highest quality hosts for offspring development and aphid reaction to parasitoid disturbance.

IV. HOST DISCRIMINATION AND LARVAL COMPETITION

1. Introduction

Discrimination between parasitized and unparasitized hosts has evolved in many species of hymenopterous parasitoids to avoid the potential loss of offspring and search time resulting from oviposition in previously parasitized hosts (van Lenteren, 1981; Waage, 1986). In solitary parasitoid species, supernumerary larvae usually are eliminated by direct combat or physiological suppression (Fisher, 1961, 1971; Salt 1961), so that only one larva completes development in a superparasitized host. The outcome of larval competition depends mainly on:

1. the species of parasitoids that compete for host resources,
2. the sequence in which different females attack a host,
3. and the time interval between the first and later ovipositions.

These factors determine the developmental stage of each immature at the time of interaction, the mechanisms involved in competition, and which parasitoid will eventually complete development.

A parasitoid's decision to oviposit in a parasitized host will not depend only on her ability to discriminate, but on other factors as well. These include her information about the availability of unparasitized or other high-quality hosts (Hubbard *et al.*, 1987; van Dijken & Waage, 1987), her knowledge of how many other parasitoids are searching in the same patch (van Alphen, 1988), her age and physiological condition, which determine her supply of mature eggs (Völkl & Mackauer, 1990), and the probability

that her offspring will survive in a previously parasitized host (Chow & Mackauer, 1986; Waage, 1986; Hubbard et al. 1987). As a general rule, a parasitoid should be more likely to oviposit in a parasitized host as the probability that her offspring will survive increases (Waage, 1986).

In general, previous studies on host discrimination and larval competition have focused mainly on interactions between females of the same species, concentrating on conspecific host discrimination and more recently on discrimination between conspecific- and self-parasitized hosts. In comparison, heterospecific (= interspecific) host discrimination, which is the ability of a parasitoid to recognize a host parasitized by another species, has been reported less often (Wylie, 1970; Chow & Mackauer, 1984; Vet et al. 1984). Absence of heterospecific discrimination and a lack of ovipositional restraint results in multiparasitism (= heterospecific superparasitism (Mackauer, 1990)).

Both A. ervi and A. smithi can discriminate between unparasitized pea aphids and those parasitized by conspecifics (Chow & Mackauer, 1984; B. Bai, unpublished). However, no studies have examined heterospecific host discrimination and mechanisms of larval competition in these two species.

The objectives of this chapter are:

1. to determine whether A. smithi and A. ervi are capable of interspecific host discrimination and
2. to examine the mechanisms and outcome of larval competition between A. smithi and A. ervi.

These objectives can be achieved by examining the influence of oviposition

sequence and the time interval between ovipositions on:

1. the acceptance or rejection of parasitized hosts and
2. larval survival.

2. Materials and Methods

a. Early larval developmental time

In general, I followed the method of Chow & Mackauer (1984, 1986). I estimated the rate of early larval development of A. ervi growing in third-instar pea aphids at 21°C. The median developmental time (ET_{50}) was 80.1 h (95% CI, 79.4 h - 80.8 h) from oviposition to the beginning of the first instar and 115.2 h (95% CI, 114.1 h - 116.2 h) to the beginning of the second instar. The corresponding times for A. smithi were given by Chow & Mackauer (1984) as 62.3 h (95% CI, 61.8 h - 62.9 h) and 90.3 h (95% CI, 89.8 h - 90.6 h) respectively. These data were needed to determine which parasitoid instars were competing in multiparasitized aphids.

b. General terms

The terms "species A" and "species B" refer to the first- and second-attacking parasitoid female in a sequence of controlled trials. To obtain aphids parasitized by species A, I placed an aphid of known age in a gelatin capsule (size 00) containing a wasp; the aphid was removed after it had been attacked once. I adjusted host age at the time of the first attack by species A so that aphids were in their third instar by the time of the second attack by species B. This procedure minimized any possible effects of differences in age or size on the aphids' defensive behaviour.

c. Host discrimination

I used two procedures to assess host discrimination by A. smithi and A. ervi. The first test was designed to provide general information about the extent, if any, of heterospecific host discrimination by experienced females (*i. e.* females previously exposed to unparasitized aphids). Wasps of species B, kept individually in gelatin capsules, were provided one at a time with hosts previously attacked by species A. I varied the sequence of attacks (A = A. ervi, B = A. smithi, and vice versa) and the intervals (t_0 , in h) between the first and second attack (see Tables 6 and 7). All attacked aphids were dissected in 0.8% saline, and the number of immature parasitoid offspring present in each host was recorded. However, I was able to distinguish between offspring of A. smithi and A. ervi only if they were at different developmental stages. In all cases, two parasitoid eggs or larvae in a dissected aphid showed a lack of oviposition restraint by species B. Because aphids containing fewer than two parasitoid eggs or larvae could have been rejected by either the first or second female or by both females, they could not be classified.

In the second test, a female of species B was placed in a screened waxed-paper cup (12 cm diameter, 6 cm height) that contained 10 unparasitized aphids and 10 aphids parasitized by species A. Unparasitized aphids were marked by amputation of the distal third of one antenna (Mackauer 1972). Aphids were permitted to disperse freely in the arena. I removed any struck aphid immediately and replaced it by one of the same type. All aphids were reared and later dissected (see above).

A trial was completed when a wasp had attacked about 50 aphids or after 30 min, whichever came first. Females that attacked fewer than 15 aphids during the 30 min observation period were not included in the analysis. There were not many of this type of parasitoid, but their behaviour indicated that at the time they were not ready to search for hosts and were also unlikely to lay eggs. From each group of aphids presumably parasitized by species A, I set aside a subsample (= control); these aphids were dissected four to five days later to estimate the proportion of successfully parasitized aphids (p_1).

For each trial, I tested the hypothesis of no oviposition restraint by comparing the observed proportion of multiparasitized aphids with the proportion expected ($p_1 \times p_2$), where p_1 is the proportion of control aphids parasitized by female A and p_2 is the proportion of aphids expected to be parasitized by female B if she treated all aphids in the arena as unparasitized (*i. e.* she did not discriminate). I obtained p_2 by counting, in the choice tests, the number of initially unparasitized aphids that were parasitized by species B. In each series (A = A. ervi, B = A. smithi, and vice versa), I tested the behaviour of female B ($n = 10$) at each of three intervals ($t_0 = 0, 2, 24$ h (± 20 min)), where t_0 is the interval between the first oviposition by female A and the introduction of female B into the arena. I did not run a separate test for $t_0 = 24$ h and A = A. ervi and B = A. smithi because other experiments had shown that A. smithi almost always rejected pea aphids containing a 30-h-old egg of A. ervi.

d. Larval competition

I determined the outcome of direct competition between the immature stages of A. smithi and A. ervi in multiparasitized pea aphids in the following way. For the first series, (A = A. ervi and B = A. smithi), I set t_0 to 18 h (± 12 min), *i. e.* eggs of both species were expected to hatch at the same time. For the second series (A = A. smithi, B = A. ervi), I set $t_0 = 0$ (± 3 min), 7, 24, and 48 h (± 12 min). In this second series, A. smithi eggs hatched earlier than those of A. ervi. To obtain multiparasitized hosts, I placed individual aphids that had been attacked by species A in gelatin capsules containing a female of species B, as described above.

I considered two hypotheses regarding larval competition. First, I tested the hypothesis that the development and survival of species A did not differ between multiparasitized aphids and those parasitized only by species A (= control group). I compared the number of A adults that emerged from mummies in the control group (mean number of aphids struck per 10 females \pm s.d. = 112 ± 10 ; n = 5 groups) with the number of A adults emerging from mummified aphids that had been attacked by both parasitoid species (mean \pm s.d. = 114 ± 5 ; n = 5). Second, I tested the alternative hypothesis that species A died (and species B survived) in multiparasitized hosts. I compared the number of A adults emerging from aphids that had been struck by both parasitoid species with the number expected if all A larvae died and all B larvae survived, using the sample of dissected aphids that contained two parasitoid larvae as the reference. Note that A adults could emerge from two kinds of aphids struck twice:

from aphids that had been parasitized only by A females (and not by B) and from aphids that had been multiparasitized and in which A larvae had survived. Similarly, B adults could emerge from two kinds of aphids: from aphids that had been parasitized only by B females (and not by A) and from multiparasitized aphids in which the B larvae had survived. Any dead aphids and mummies that did not emerge (mean \pm s.d. = 4.7 ± 2.2 ; n = 10 groups) were excluded from the analysis.

In some trials, I could judge the outcome of larval competition directly on the basis of which species of parasitoid larva was dead and which one was alive at the time the aphids were dissected. This procedure was followed for A = A. ervi and B = A. smithi (t_0 = 64 h, 72 h (\pm 12 min)) and for A = A. smithi and B = A. ervi (t_0 = 24, 48 h (\pm 12 min)). A larva was considered dead if it did not move after being prodded repeatedly with a dissecting needle; most of these larvae were opaque.

3. Results

a. Host discrimination

Females of A. smithi and A. ervi kept in gelatin capsules showed a clear oviposition preference for unparasitized aphids. They laid an egg in over 80% of unparasitized hosts (Fig. 4) but rejected a large portion of aphids parasitized by the other species (Tables 6, 7). The percentage of multiparasitized aphids varied with the interval between ovipositions, declining from about 40% to 50% for $t_0 = 0$ h to near zero for longer intervals. Females of A. smithi rejected almost all aphids containing an A. ervi egg that was aged 30 h or older.

The rejection of heterospecific-parasitized hosts was not affected by the method in which aphids were presented to wasps. For short oviposition intervals (t_0 less than or equal to two hours), wasps allowed to search for hosts in a paper cup attacked a significantly greater proportion of unparasitized pea aphids than of aphids previously struck by the other species (Fig. 5), a finding consistent with discrimination. (X^2 -test, $df=1$; for species B = A. ervi: at $t_0 = 0$ h, $X^2 = 4.699$, $P<0.05$; at $t_0 = 2$ h, $X^2 = 14.810$, $P<0.001$; for species B = A. smithi: at $t_0 = 0$ h, $X^2 = 21.275$, $P<0.001$; at $t_0 = 2$ h, $X^2 = 25.686$, $P<0.001$). For $t_0 = 24$ h, A. ervi females often examined and then rejected hosts struck first by A. smithi. The number of each host type attacked was not significantly different (X^2 -test, $X^2 = 3.815$, $df=1$, $P>0.05$), although host discrimination occurred (see below).

Table 6. Number and survival of parasitoid offspring in pea aphids parasitized first by Aphidius smithi and then by A. ervi.

Oviposition interval	Time of dissection (days) ^a	Frequencies of parasitoid offspring in dissected aphids			Condition of parasitoid larvae in multiparasitized aphids		
		n	0	1	2	1 dead, 1 live	2 live
0	4	45	5	22	18	18 ^b	0
7	4	59	2	37	20	17 ^b	3
24	5	63	3	45	15	14 ^c	1
48	7	53	4	42	7	7 ^c	0

^a Day 0 = aphid attacked by A. smithi.

^b Both larvae in first instar.

^c A. ervi larva live, A. smithi larva dead.

Table 7. Number and survival of parasitoid offspring in pea aphids parasitized first by Aphidius ervi and then by A. smithi.

Oviposition interval	Time of dissection (days) ^a	Frequencies of parasitoid offspring in dissected aphids	Condition of parasitoid larvae in multiparasitized aphids				
t ₀ (h)	n	0	1	2	1 dead, 1 live	2 live	
0	4	44	0	24	20	19 ^b	1
7	4	73	3	33	37	36 ^b	1
18	5	61	1	41	19	19 ^b	0
30	5	108	0	106	2	2 ^b	0
42	5	42	5	37	0	0	0
44	5	54	2	52	0	0	0
64	6	49	3	44	2	0	2
72	7	147	5	135	7	1 ^c	6

^a Day 0 = aphid attacked by A. ervi.

^b Both larvae in first instar.

^c A. ervi larva live, A. smithi larva dead.

Fig. 4. Percent emergence of Aphidius ervi (Ae) and A. smithi (As) from aphids attacked by one or both parasitoid species for different oviposition intervals (t_0). Column 1 shows emergence of the first-attacking wasp (species A) from single-parasitized aphids. Column 2 shows emergence of the first-attacking (open) and second-attacking (shaded) parasitoid from multiparasitized aphids. Data were pooled over all females (n=10) in each group.

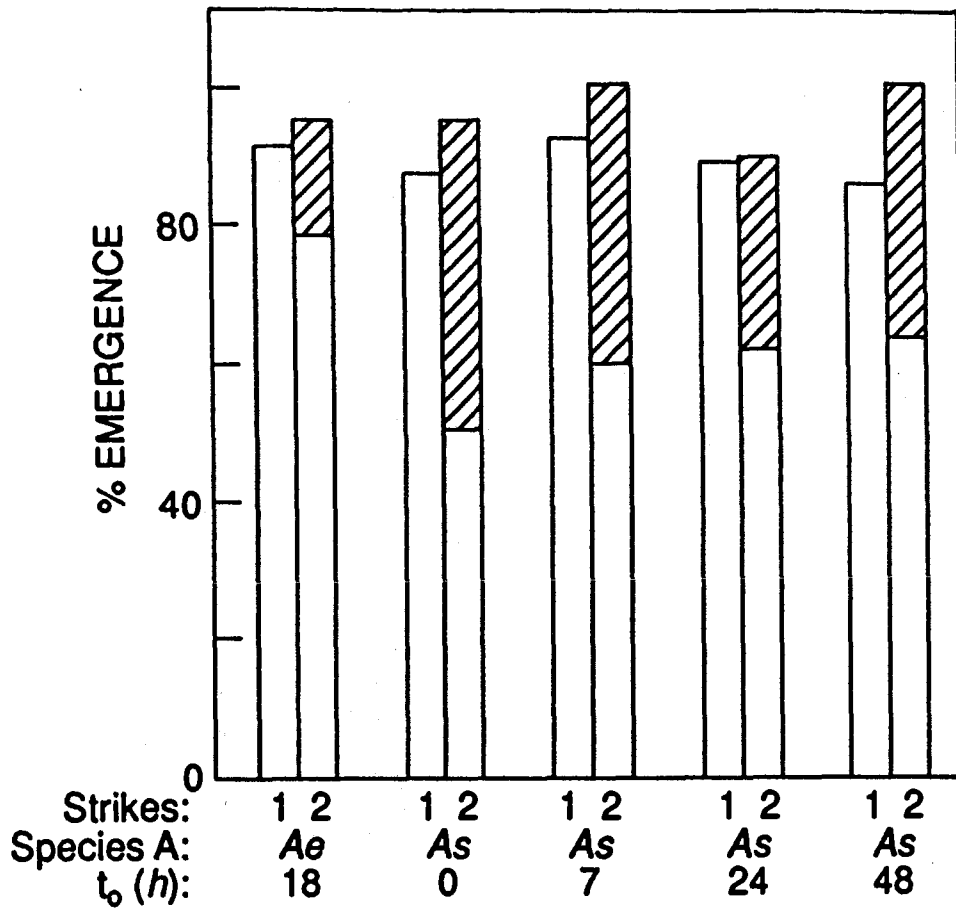
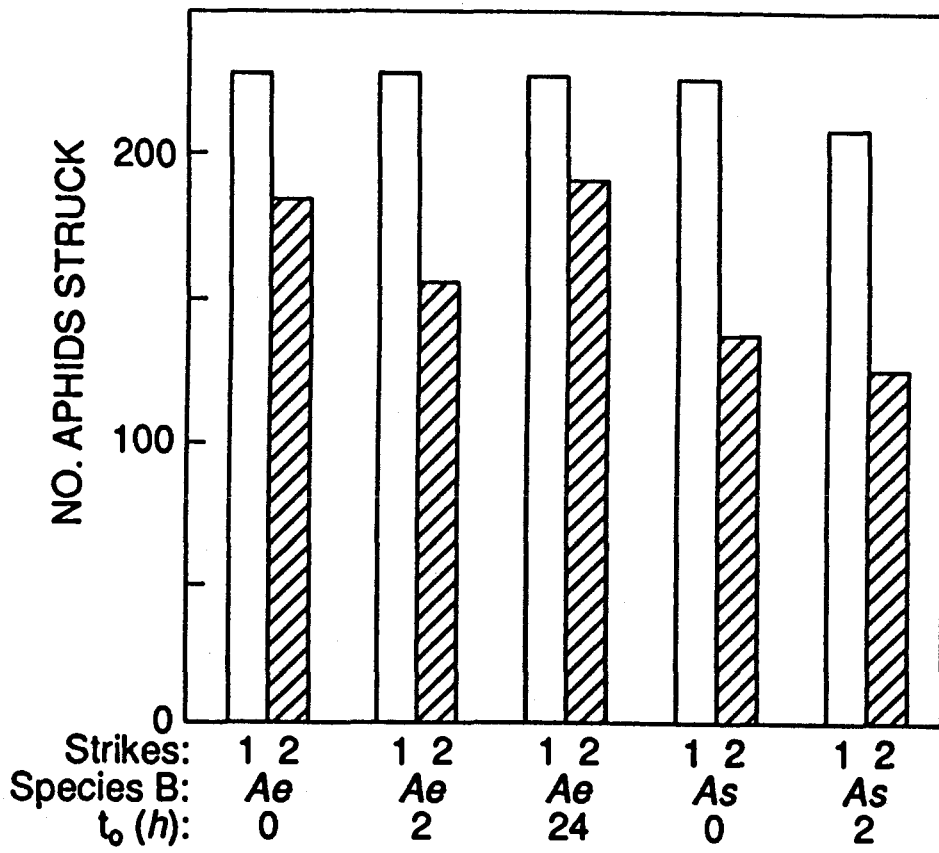


Fig. 5. Numbers of aphids attacked by Aphidius ervi (Ae) and A. smithi (As) when give a choice between unparasitized (column 1, open) and heterospecific-parasitized hosts (column 2, shaded) for different oviposition intervals (t_0). Species B was the second-attacking wasp. Data were pooled over all females (n=10) in each group.



As a further test of discrimination, I compared the observed number of multiparasitized hosts with the numbers expected if the searching wasp did not discriminate. I estimated the proportions of aphids parasitized by each species, p_1 and p_2 (Table 8), based on the assumption that females did not discriminate between unparasitized and heterospecific-parasitized aphids. For all t_0 , fewer aphids than expected were multiparasitized (Fig 6) (X^2 -test, $df=1$, $P<0.001$; for species B = A. ervi: at $t_0 = 0$ h, $X^2 = 40.196$, at $t_0 = 2$ h, $X^2 = 70.335$, at $t_0 = 24$ h, $X^2 = 195.113$, for species B = A. smithi, at $t_0 = 0$ h, $X^2 = 48.982$, at $t_0 = 2$ h, $X^2 = 46.776$). Also, when provided with equal numbers of unparasitized aphids and those struck and presumably parasitized by the other species, wasps laid more eggs in the former. For example, A. ervi females laid an average of 15.5 eggs per female (s.d. = 4.9, $n = 40$) in unparasitized aphids but only 5.2 eggs per female (± 2.2) in aphids attacked and presumably parasitized by A. smithi when t_0 was less than or equal to two hours (Student's $t = 8.59$; $df = 38$; $P < 0.001$); the corresponding values for A. smithi were 18.2 (± 6.4) eggs per wasp in unparasitized aphids and 3.9 (± 2.7) eggs per wasp in aphids attacked by A. ervi (Student's $t = 9.24$; $df = 38$; $P < 0.001$).

b. Larval competition

The numbers of dissected aphids that contained zero, one, or two parasitoid eggs or larvae are shown for aphids attacked first by A. smithi followed by A. ervi (Table 6) and for those attacked first by A. ervi followed by A. smithi (Table 7). I did not find any dead parasitoid eggs in these dissected aphids.

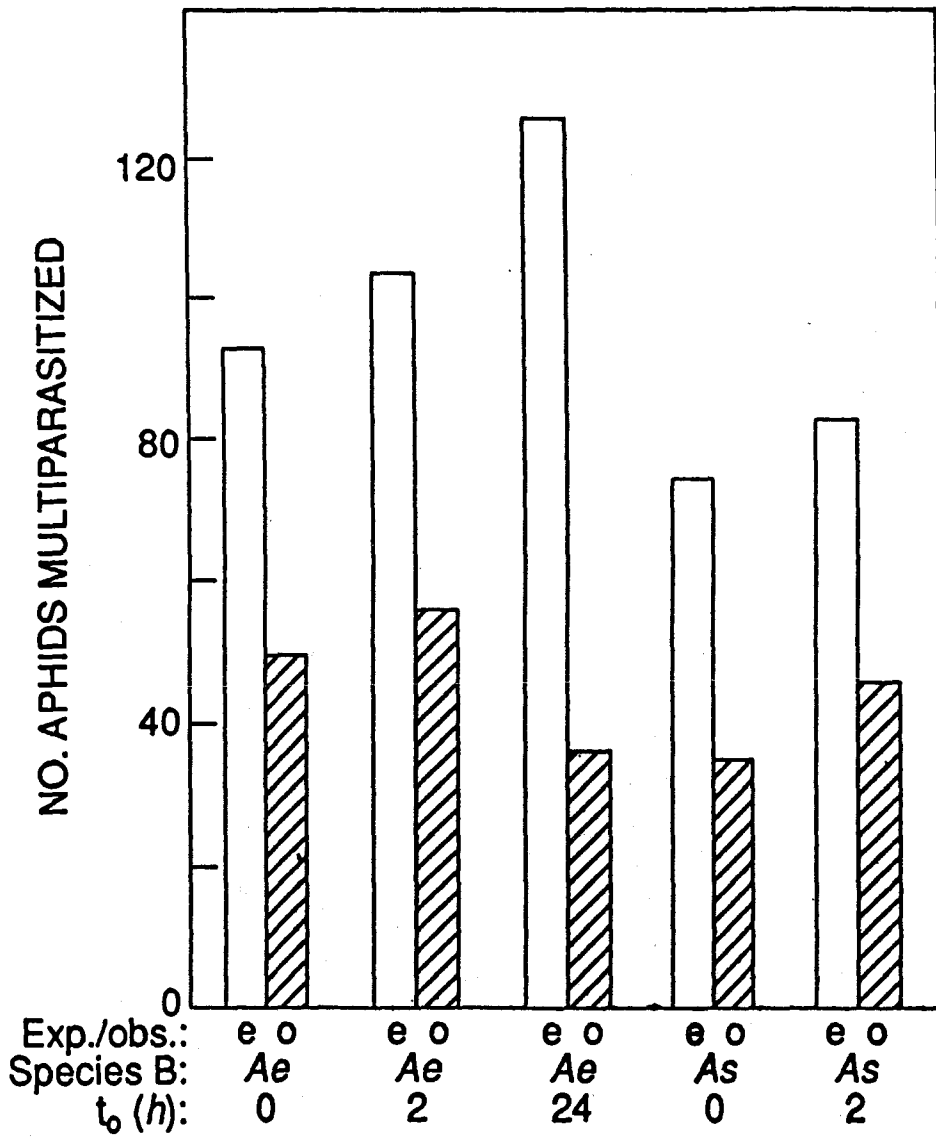
Table 8. Estimated proportions of aphids parasitized by parasitoid species A (p_1) and species B (p_2)¹.

Attack sequence (A + B) ²	Oviposition interval t_0 (h)	Oviposition	
		p_1	p_2
Ae + As	0	.70	.77
Ae + As	2	.76	.86
As + Ae	0	.83	.60
As + Ae	2	.88	.75
As + Ae	24	.91	.74

¹ Species B is assumed not to discriminate (see text for details).

² Ae, Aphidius ervi; As, A. smithi.

Fig. 6. Expected and observed numbers of aphids multiparasitized by Aphidius ervi (Ae) and A. smithi (As) for different oviposition intervals (t_0). Column "e" shows numbers expected if neither parasitoid discriminated. Column "o" shows observed numbers of aphids multiparasitized by the second-attacking wasp (species B). Data were pooled over all females in each group.



For all t_0 , fewer species A adults emerged from multiparasitized aphids than from aphids struck only by A (Fig. 4) (G-test with William's correction factor, $df=1$; for species A = A. ervi, at $t_0 = 18$ h, $G = 6.594$, $P < 0.025$; for species A = A. smithi, at $t_0 = 0$ h, $G = 35.136$, $P < 0.001$, at $t_0 = 7$ h, $G = 23.186$, $P < 0.001$, at $t_0 = 24$ h, $G = 22.080$, $P < 0.001$, at $t_0 = 48$ h, $G = 12.956$, $P < 0.001$). This indicates that A's survival was lower in the presence of B. When aphids were parasitized first by A. smithi and later by A. ervi, the number of A. smithi emerging from multiparasitized hosts was not different ($P > 0.05$) from the number expected if I assumed that A. smithi did not survive in competition with A. ervi (G-test with William's correction factor, $df=1$; at $t_0 = 0$ h, $G = 0.438$, $P > 0.10$, at $t_0 = 7$ h, $G = 1.015$, $P > 0.10$, at $t_0 = 24$ h, $G = 0.060$, $P > 0.50$, at $t_0 = 48$ h, $G = 2.267$, $P > 0.10$). However, when A. ervi oviposited 18 h earlier than A. smithi, more A. ervi adults emerged than were expected if A. ervi died in all multiparasitized aphids (G-test with William's correction, $G_{adj} = 9.642$; $df = 1$; $P = 0.002$). This indicates that A. ervi was likely to survive in multiparasitized hosts when A. smithi eggs hatched first (as explained above), but not when the eggs of both species hatched simultaneously ($t_0 = 18$ h); in the latter case neither species had a clear advantage.

Aphid dissections confirmed that A. smithi larvae normally were killed in the second instar by early first-instar A. ervi larvae (less than four days old). For example, I found a first-instar larva of A. ervi with its mandibles embedded in the tail of a second-instar A. smithi larva. Some dead A. smithi larvae had melanized wound marks on their bodies or were

grossly deformed, which is indirect evidence of physical combat.

In the interaction between A. ervi and 48-h-older A. smithi, both larvae were still alive in 16 of 18 aphids that were dissected four days after parasitization by A. ervi. However, a second sample dissected after five days contained only aphids in which the older A. smithi larvae were dead and the younger A. ervi larvae survived (Table 6; $t_0 = 48$ h). These results show that 1) a younger A. ervi larva developed at a slower rate in the presence of an older A. smithi larva and 2) fighting by A. ervi was restricted to a short interval (less than or equal to 24 h) during the first larval stage.

A. smithi females rarely accepted any aphids parasitized by A. ervi if the oviposition interval was greater than or equal to 30 h and A. ervi eggs were expected to hatch first (Table 7). The possible mechanism of competition between a young and much older larva is evident from the data for $t_0 = 72$ h (Table 7). When these aphids were dissected seven days and a second sample ($n = 83$, not shown) was dissected eight days after parasitization by A. ervi (i. e. four and five days after parasitization by A. smithi), I found a living third instar A. ervi (according to O'Donnell, 1987) and either a living or dead A. smithi larva. In all cases, A. smithi was still in the first-instar stage, which is evidence that its development was delayed in the presence of an older A. ervi. Dead A. smithi larvae showed no wound marks or other signs of physical combat. In a singly-parasitized-aphid, a four- or five-day-old A. smithi larva would normally have progressed beyond the first-instar stage.

4. Discussion

The oviposition patterns of A. ervi and A. smithi show that females discriminated between unparasitized pea aphids, which they preferred, and those parasitized by the other species. Wasps tended to reject heterospecific-parasitized aphids under all conditions tested (Tables 6, 7; Fig. 6). For short intervals (t_0 less than or equal to two hours) between attacks, evidence of oviposition restraint was consistent with response to an external rather than internal marker (Fig. 5), because wasps attacked more unparasitized hosts than those attacked by the other species. Observations on other species of aphid parasitoids (Mackauer, 1990) suggest that females use a pheromone or pheromone-like substance to mark parasitized hosts. These markers are often detected by antennation, making ovipositor insertion unnecessary. The strong rejection of aphids containing an older parasitoid embryo (t_0 greater than or equal to 24 h) indicates that A. smithi and A. ervi make use of internal cues detected with the ovipositor to recognize parasitized hosts as the interval between ovipositions increases, although this type of cue may have also played a role in the recognition of different host types at shorter oviposition intervals. Females probably detect changes in host physiology caused by the developing parasitoid when they insert their ovipositor into the aphid (Fisher, 1971; Beckage & Templeton, 1986; Strand, 1986).

Aphidius ervi was the superior larval competitor. Except when eggs of both species hatched at the same time and neither species appeared to have a competitive advantage, early first-instar larvae of A. ervi normally

attacked with their sickle-shaped mandibles and killed older A. smithi larvae. Even though first instars of A. smithi have similar mandibles (Chorney & Mackauer, 1979), I found no evidence that they physically attacked immature A. ervi, which agrees with other observations (Wiackowski, 1962; Chow & Mackauer, 1984). Instead, a "toxic secretion", probably a cytolytic enzyme, is thought to be released at egg hatch (Vinson & Itswantsch, 1980; Strand, 1986).

An older A. ervi larva generally killed a competing first instar of A. smithi. Physical combat can be excluded as a cause of death because the dead larvae showed no wound marks and fighting in A. ervi is restricted to the early first-instar stage. A. smithi probably was killed by some form of physiological suppression (Fisher, 1971; Vinson & Iwantsch, 1980) or the host's inability to support both parasitoid larvae.

A recently published study by Chua et al. (1990) attempted to examine the outcome of larval competition between A. ervi and A. smithi in the laboratory. However, these researchers did not dissect what they considered to be multiparasitized aphids and therefore did not know how many eggs were actually present inside an aphid. Since they claimed "that actual oviposition could be easily confirmed by the greater effort required by a female when withdrawing the ovipositor from the host", which is not correct, they reasoned that confirmation of oviposition by dissection was not necessary. Without this information, their method of analysis is not correct and some of their observations can be explained by the fact that the second-attacking wasp discriminated against

parasitized hosts. Also, the sample sizes of their controls (singly-parasitized aphids) were not given, but appear to have been small. They were a small percentage of the experimental groups which ranged in size from 34 to 59. In spite of all this, Chua et al. (1990) concluded that A. ervi was the better larval competitor, which agrees with the present study.

A study similar to the present one has been done using A. ervi and another solitary endoparasitoid of the pea aphid, Aphelinus asychis Walker (Hymenoptera: Aphelinidae). In that study (Bai & Mackauer, unpublished data), A. ervi females behaved in much the same way as they did in competition with A. smithi. A. ervi females discriminated between unparasitized pea aphids, which they preferred, and those parasitized by A. asychis. However, females of both species either ignored any external marks on parasitized hosts or did not recognize them, and appeared to use only internal cues for discrimination. A. ervi was also the superior larval competitor. A. asychis was killed by physical combat, which was restricted to a short period (less than or equal to 24 h) after A. ervi hatched, and by physiological suppression. Further studies on heterospecific host discrimination may reveal that it is more widespread than indicated by the few reports in the literature.

For solitary parasitoids, conditions under which hosts parasitized by a different species should be accepted or rejected have not been clearly defined. Bakker et al. (1985) have suggested that acceptance of hosts parasitized by another species is the best strategy for sympatric

parasitoids when females have a large supply of mature eggs (as is the case for A. smithi and A. ervi (Mackauer, 1971; Kambhampati & Mackauer, 1989)) and hosts are scarce. In a related paper, Turlings et al. (1985) examined heterospecific host discrimination in two solitary parasitoids of Drosophila larvae. They concluded that discrimination and oviposition restraint by only one of two competing species is not an evolutionary stable strategy. For heterospecific host discrimination to evolve, both competitors must simultaneously adopt this strategy. Although the model was based on the actual parasitization process and included important search and oviposition parameters, it permitted only one outcome in the interaction between Asobara tabida (Nees) and Leptopilina heterotoma (Thomson): a larva either survived or died in a heterospecifically parasitized host. The model did not include the possibility that previously parasitized hosts may be of lower quality than initially unparasitized ones and as a result the surviving larva may develop at a slower rate. Liu & Morton (1986) also assumed that moderate superparasitism (by Aphidius sonchi Marshall) was not harmful to the survivor.

In the field, larval competition between A. smithi and A. ervi will be avoided as long as unparasitized hosts are available. This is confirmed by field data showing that superparasitism is usually rare (Appendix I, Tables 12, 13). If unparasitized aphids are scarce, wasps must choose between laying eggs in parasitized aphids or dispersing in search of higher quality hosts. As the inferior larval competitor, A. smithi cannot gain in fitness by ovipositing in aphids already parasitized by A. ervi

and therefore is expected to discriminate. The benefits of discrimination to A. ervi are less clear. According to the ideas put forward by Bakker et al. (1985) and Turlings et al. (1985), as the superior larval competitor, A. ervi females should accept aphids already parasitized by A. smithi when few or no unparasitized hosts are available, which sometimes occurs in the field (Appendix I, Tables 12, 13; Campbell, 1973).

However, when there are qualitative differences between unparasitized and parasitized hosts, the avoidance of multiparasitism may be adaptive. As shown in this study, an A. ervi larva required longer to develop in the presence of an older A. smithi larva, even though the latter was eventually killed. It is not known whether the A. ervi larva can compensate for this lengthened growth period before becoming an adult. However, a small increase in the developmental time from egg to adult could have a significant influence on the ability of these wasps to compete for mates or hosts with earlier-emerging individuals. Slower developmental time could also increase the probability of attack by predators or hyperparasitoids. Further studies are needed to test these ideas. If correct, they could explain the evolution of heterospecific discrimination in a species such as A. ervi which is a superior larval competitor.

V. CHOOSING BETWEEN CONSPECIFIC-, HETEROSPECIFIC-, AND SELF-PARASITIZED HOSTS

1. Introduction

When given a choice between unparasitized and parasitized hosts, a solitary wasp is expected to prefer the former (see Chapter IV). However, if unparasitized hosts are not available, it may be adaptive to oviposit in parasitized ones (van Alphen et al., 1987; van Dijken & Waage, 1987; Hubbard et al., 1987). The benefits of laying an egg in an already parasitized host will be influenced by the probability that the second-attacking wasp's offspring will survive. This is largely determined by the identity of the parasitoid already inside a host. When given only parasitized hosts to choose from, a wasp could be faced with two situations:

1. choosing between hosts parasitized by a conspecific or by a female of another species (a heterospecific);
2. choosing between hosts parasitized by a conspecific or by herself.

In the first situation, a wasp is predicted to oviposit more often in those hosts where her offspring have a higher chance of survival, which are defined as higher quality hosts. No published studies have formally tested this prediction. In the second situation, a wasp is predicted to avoid self-superparasitism (*i. e.* laying two eggs in the same host), as has been shown for some parasitoid species (Hubbard et al., 1987; van Alphen & Visser, 1990; Völkl & Mackauer, 1990). Unless the presence of

two eggs belonging to the same female increases the chance that at least one of them will survive (Cloutier, 1984; van Alphen & Visser 1990; Visser et al., 1990), a solitary wasp cannot increase her fitness by laying two eggs in the same host because the offspring that completes development kills its sibling. Therefore, conspecific-parasitized hosts are considered to be of higher quality than self-parasitized ones.

Using A. smithi and A. ervi, I tested the prediction that a wasp will oviposit more often in higher quality hosts when given only parasitized hosts to chose from. When offered conspecific- and heterospecific-parasitized aphids, a wasp's oviposition decisions should be based on the probability of her offspring's survival. She should oviposit more often in (i. e. prefer) hosts in which her offspring have a higher chance of winning at larval competition. Because A. ervi is a superior larval competitor to A. smithi under most conditions (see Chapter IV), the fitness consequences of laying an egg in a host already containing the immature of a heterospecific differ between these species. Aphidius ervi should prefer hosts previously parasitized by A. smithi over those parasitized by conspecifics. In contrast, A. smithi should accept hosts parasitized by conspecifics in preference to those parasitized by A. ervi.

By offering A. smithi a choice between conspecific- and self-parasitized hosts, I tested the hypothesis that a solitary wasp will avoid self-superparasitism and oviposit more often in hosts already containing the egg of a conspecific. Under the experimental conditions, an A. smithi female cannot increase her fitness by laying two eggs in the same host,

since the presence of both eggs will not increase the probability that at least one of them will survive. There is no evidence that encapsulation of eggs is a common phenomenon in this host-parasitoid system, so the presence of the first egg will not make the probability of the second egg escaping encapsulation more likely. Also, since only one female will be searching for hosts at a time and a superparasitized host will contain at most two eggs, self-superparasitism will not increase the chance of one female's offspring emerging from a particular host. Self-superparasitism might be advantageous if a host contained three or more eggs and a larger proportion belonged to one female. Such a situation could occur if more than one female searched simultaneously for hosts or another female searched an area soon after the previous parasitoid left (Cloutier, 1984; Visser et al., 1990).

2. Materials and methods

a. Conspecific vs heterospecific superparasitism

A female of A. ervi (n=10) or A. smithi (n=10) was placed in a waxed paper cup (12 cm diameter, 6 cm height) that contained 10 conspecific- and 10 heterospecific-parasitized third-instar pea aphids. Singly-parasitized aphids were prepared as described in Chapter IV. An aphid was struck once by a wasp before placement in the arena. In each trial, one of the host classes was marked by amputation of the distal third of one antenna (Mackauer, 1972). The interval between preparation of experimental aphids and introduction of the searching wasp into the arena was 40 min (± 20 min).

Aphids were allowed to move freely in the arena and the searching wasp was observed continuously. Any aphid attacked by the parasitoid (*i. e.* struck with the ovipositor) was immediately removed and replaced by one of the same kind. All attacked aphids were reared and later dissected to verify oviposition (see below).

A trial ended when a wasp attacked 20 aphids of one kind or after 35 min, whichever came first. Wasps that attacked fewer than 10 aphids were not included in the analysis, as discussed in Chapter IV.

From each group of experimental aphids initially attacked by either A. smithi or A. ervi, a subsample of 20 to 30 individuals (= control sample)

was set aside. These aphids were later dissected to estimate the proportion which had been successfully parasitized by each species prior to placement of aphids in the arena.

The number of parasitoid eggs and/or larvae found in dissected aphids was used to evaluate oviposition restraint by the searching wasp. Two immature parasitoids inside an aphid showed a lack of restraint. However, if an aphid contained only one egg or larva, I could not determine whether it belonged to the first-attacking wasp or the searching parasitoid. These aphids were not included in the analysis.

b. Conspecific vs self superparasitism

I followed the experimental procedure described above, with the following exceptions. A female A. smithi (n = 12) was placed in a plastic Petri dish (diameter=5.5cm) containing four conspecific- and four self-parasitized third-instar pea aphids. Attacked aphids were replaced, as before. A trial ended when a wasp had attacked 20 aphids; wasps requiring more than 30 min to complete 20 attacks were not included in the analysis.

c. Statistical analysis

Some of the experimental aphids used in the choice tests were not parasitized, even though they were each struck by a parasitoid before being placed in the arena. Dissection of control samples showed that, on

average, A. ervi struck but did not lay an egg in a higher proportion of aphids (mean \pm s.d. = 0.28 ± 0.09 ; n = 665 in 10 samples) than A. smithi females (mean \pm sd = 0.15 ± 0.09 ; n = 1064 in 22 samples). This was corrected for by calculating, separately for each female in an experiment, the numbers of aphids that contained two eggs (i. e. were superparasitized) among those initially parasitized in each host class. The number of aphids in each host class that actually contained an egg of the first-attacking wasp was estimated from control samples (see above). For each experiment, I tested the null hypothesis that the mean probability of superparasitism by the searching wasps (i. e. the probability of a wasp laying an egg in a parasitized aphid given that it was attacked) was the same for each host class. This was done by fitting a log-linear model to the pooled data, using the SAS statistical software package (SAS, 1985).

3. Results

Figure 7 (clear bars) shows that A. smithi attacked more aphids previously struck by a conspecific when given a choice between these aphids and those initially struck by themselves or A. ervi (paired sample t-test, $P < 0.001$; con vs het: $t = 7.14$, $df = 9$; con vs self: $t = 5.62$, $df = 11$). Aphidius ervi avoided aphids struck by conspecifics and attacked more hosts previously attacked by A. smithi (paired sample t-test, $df = 9$, $t = 3.84$, $P = 0.004$). The same preference pattern was obtained when I compared the mean numbers of each host type superparasitized per searching wasp (Fig. 7, shaded bars). Aphidius smithi superparasitized more aphids already containing the egg of a conspecific than aphids parasitized by A. ervi or themselves (paired t-test, $P < 0.001$; con vs het: $t = 6.33$, $df = 9$; con vs self: $t = 5.29$, $df = 11$). Aphidius ervi superparasitized more aphids previously parasitized by A. smithi than by conspecifics (paired t-test, $t = 2.43$, $df = 9$, $P = 0.038$).

Some of the presumably parasitized hosts used in the choice tests were unparasitized (see section V.2.c). After the data were corrected for this (Fig. 8), the mean probability of conspecific superparasitism by A. smithi was higher when wasps had a choice between conspecific- and heterospecific-parasitized aphids ($X^2 = 7.33$, $df = 1$, $P = 0.007$). No difference in probability of superparasitism between host types was found when A. smithi females were presented with conspecific- and self-parasitized aphids ($X^2 = 2.45$, $df = 1$, $P = 0.118$). However, conspecific and heterospecific superparasitism by A. ervi occurred with equal probability once an aphid was struck with the ovipositor ($X^2 = 0.55$, $df = 1$, $P = 0.458$).

Fig. 7. Mean numbers of aphids attacked (open columns) and superparasitized (shaded columns) by Aphidius ervi (n=10) and A. smithi (n=10 for con vs het, n=12 for con vs self). Searching wasps were offered equal numbers of either conspecific- (con) and heterospecific-parasitized (het) hosts or conspecific- and self-parasitized (self) hosts. Data show mean and 1 SEM.

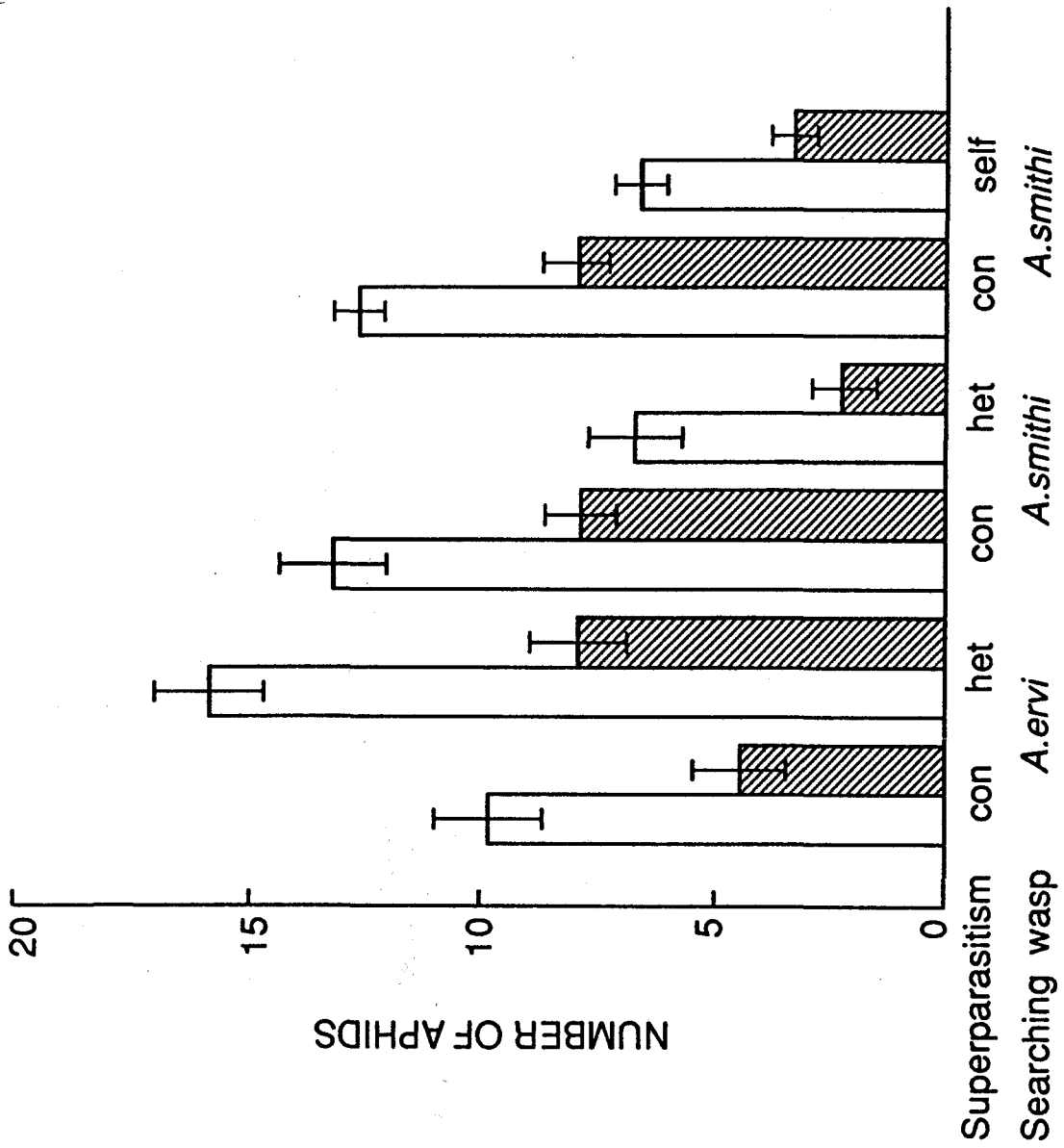
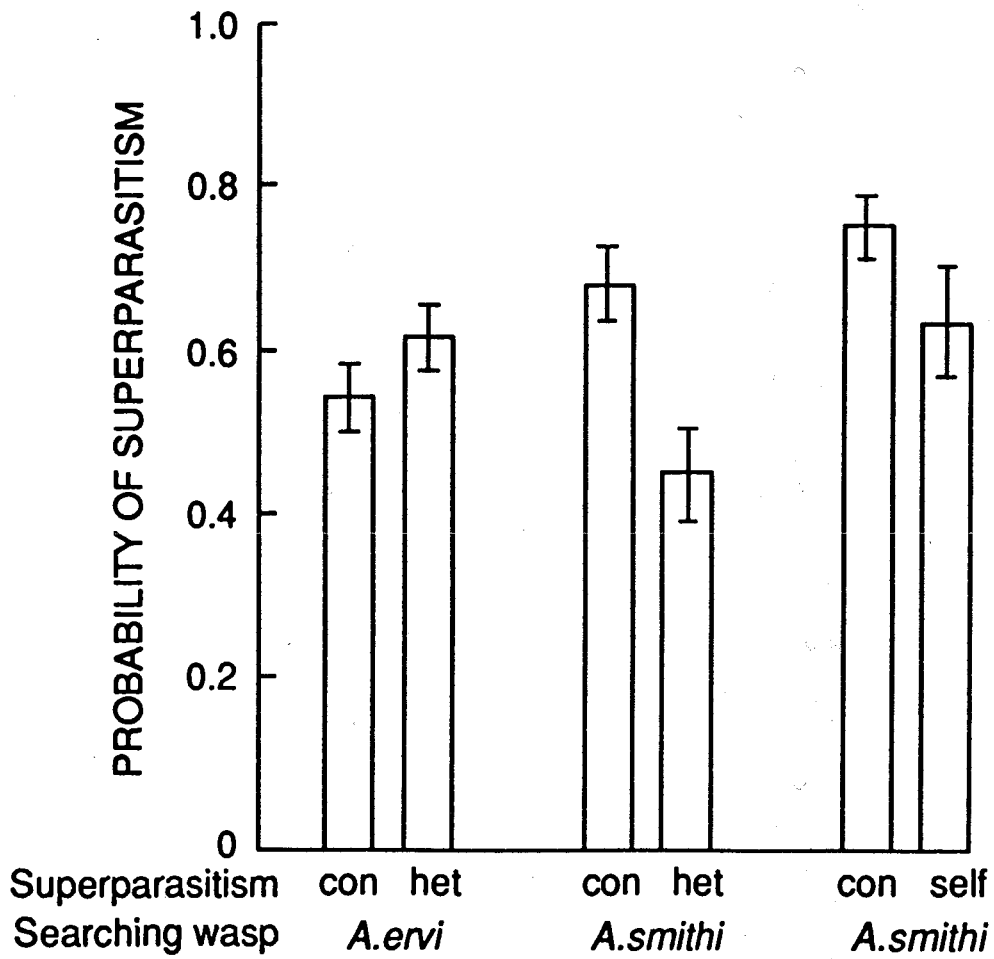


Fig. 8. Probability of a parasitized aphid in each host class being parasitized once it was attacked (i. e. probability of superparasitism) by Aphidius ervi and A. smithi in choice tests (data show mean and 1 SEM; see Fig. 7 for abbreviations). For each trial, the proportion of parasitized aphids in each host class was estimated from dissected subsamples.



4. Discussion

Aphidius ervi and A. smithi discriminated between hosts that were already parasitized by a conspecific female and those parasitized by a female of the other species; both species selectively oviposited in aphids parasitized by A. smithi. Discrimination between these two host classes did not require a wasp to probe an aphid with her ovipositor (Fig. 7). Females probably responded to an external marking pheromone detected by antennation (Mackauer, 1990). They were able to distinguish between a conspecific's marker and that of a different species and, in the case of A. smithi, between their own and a conspecific's marker. For A. smithi, rejection of heterospecific-parasitized aphids possibly involved two mechanisms: external cues as well as internal cues that required ovipositor probing (Fig. 8) (Chow and Mackauer, 1986; Steinberg *et al.*, 1987).

The observed asymmetry in host selection was predicted from the differential larval survival of A. ervi and A. smithi in conspecific- and heterospecific-parasitized aphids. Under most conditions, immature A. smithi are unlikely to survive in competition with A. ervi (see Chapter IV). However, because of developmental uncertainty, the offspring of a superparasitizing A. smithi female has an equal probability of winning or losing against a conspecific larva if the oviposition interval is short (Chow and Mackauer, 1984; Mackauer, 1990), as in the present study. The egg laid by the second-attacking wasp may develop slightly faster and hatch earlier than one laid by the first-attacking female. When two A.

smithi immatures compete, the larva which hatches first usually wins, especially when the immatures are close in age. The mechanism for eliminating supernumerary larvae is thought to be a "toxic secretion", which could be a cytolytic enzyme, released at egg hatch (Vinson & Itswantsch, 1980; Strand, 1986).

Using a similar argument, an A. ervi larva has a higher chance of survival when competing with A. smithi than with a conspecific. Under the experimental conditions, a first-instar A. ervi hatched about 18 h before A. smithi and had a high probability of killing its heterospecific competitor by physical combat (see Chapter IV). First instar A. ervi larvae also kill newly-hatched conspecifics by physical combat (pers. obs.), but the one which hatches first most likely has the competitive advantage. As explained above, the egg laid by the second-attacking A. ervi female may hatch first because of individual variation in developmental time. Thus it has an equal probability of winning or losing in competition with a conspecific immature when the oviposition interval is short.

The result that A. smithi females attacked more conspecific- than self-parasitized aphids and thus laid more eggs in the former host class was predicted. Under the experimental conditions, the second-attacking A. smithi could not increase her fitness by laying eggs in hosts that already contained her own offspring.

In spite of the low probability of her offspring surviving in aphids

parasitized by A. ervi, it may be a better strategy for a female A. smithi to oviposit in these hosts than not to lay any eggs at all. Aphidius smithi and A. ervi females emerge with only a fraction of their total egg complement and mature eggs continuously throughout life (*i. e.* they are synovigenic). These parasitoids are also classified as hydropic species, meaning their eggs lack the nutritive substances necessary for embryonic development and they must obtain nourishment from the host's fluids (Flanders, 1942; Jervis & Kidd, 1986). Newly-laid eggs are comparatively small and may be deficient in yolk or have almost no yolk (*i. e.* they are alecithal). In contrast to anhydropic species, females of hydropic species are unable to resorb eggs when hosts are scarce. Thus, a female A. smithi may increase her fitness by laying at least some eggs in low-quality hosts, once the number of mature eggs exceeds the storage capacity of the ovaries (Völkl & Mackauer, 1990). A female of an anhydropic species would be less likely to lay an egg in a lower quality host. Since she has the ability of oöSORPTION, resources may be better spent producing an egg for oviposition in a higher quality host at a later time (Bai & Mackauer, 1990).

Because A. smithi is a poor larval competitor, avoidance of heterospecific superparasitism is probably a general phenomenon in this species. This does not mean, however, that the observed avoidance demonstrates a specific response to A. ervi's marking pheromone (and vice versa). A less restrictive assumption is that both species can distinguish between self- and nonself-parasitized hosts. If correct, the strength of a wasp's rejection of nonself-parasitized hosts should vary with her physiological

state as well as the probability of her offspring's survival in such hosts (Mackauer 1990).

VI. SEX ALLOCATION: UNPARASITIZED VS PARASITIZED HOSTS

1. Introduction

The haplodiploid mechanism of sex determination in the Hymenoptera (i. e. fertilized eggs are female and unfertilized eggs are male) allows a parasitoid female to manipulate her offspring sex ratio in response to differences in host quality. Such differences are expected to reflect resource availability and/or survival probabilities of the offspring. According to a model developed by Charnov et al. (1981), in an outbreeding population of solitary parasitoids, a female is expected to control her sex ratio as a function of the available host sizes, with more males being allocated to the smaller, and thus lower quality hosts. This assumes that:

1. wasps paralyze or kill their hosts by oviposition so that the total food available for offspring growth is present in the host at the time of attack,
2. final parasitoid size is positively correlated with host size at the time of oviposition,
3. a host is only large or small relative to the other hosts being attacked,
4. large daughters gain more in terms of increased fitness than large sons, and
5. this sex ratio is determined at the egg stage and does not include sex ratio shifts caused by differential mortality of male and female eggs or larvae.

Of the five assumptions listed above, the most difficult one to validate

is the fourth one, that the fitness of daughters increases more relative to that of sons when offspring develop in larger hosts.

In many studies involving hymenopterous parasitoids, host quality has been equated with larger host size, since host size has been positively correlated with adult size of parasitoid offspring. In turn, larger parasitoid size has been positively correlated with increased female reproductive capacity, either because of higher fecundity or increased longevity, or both (Sandlan, 1979; Charnov et al., 1981; Takagi, 1985; Lui, 1985; Hurlbutt King, 1987, 1988). Larger size is also predicted to enhance host-finding ability (Charnov et al., 1981). Similarly for males, larger size has been shown to increase mating success (Grant et al., 1980; Charnov et al., 1981, Hurlbutt King, 1988). However, unless female fitness is enhanced relative to that of males, sex ratio shifts are not predicted when parasitoids are offered large (high quality) and small (low quality) hosts. Such shifts have been observed in some parasitoid species (Charnov et al., 1981; Simbolotti et al., 1987; Hurlbutt King, 1987, 1988; Griffiths & Godfray, 1988; Werren & Simbolotti, 1989), although the assumption of a relative increase in the fitness of female progeny has been more difficult to demonstrate (Hurlbutt King, 1987).

Waage (1982) has argued that size-dependent sex ratios are expected to occur only in parasitoids that oviposit in non-growing host stages (e.g. eggs or pupae) or that paralyze their hosts (e.g. many larval ectoparasitoids). However, these shifts should not be observed in parasitoids that develop in a growing stage (egg-larval or larval

endoparasitoids), since host size at oviposition is not a good predictor of larval resources. Hurlbutt King (1989) has reviewed Waage's (1982) hypothesis and has suggested that it does hold for some host-parasitoid systems, particularly for parasitoids using only one host species, but not for all parasitoids of growing hosts.

Instead of looking at size-dependent sex ratio shifts, Waage (1982) suggested a more general test of the prediction that relative differences in host quality result in sex allocation changes. He proposed that the effect of superparasitism on sex ratio is an alternative way to view this strategy. Larval resources are expected to be reduced for the second-attacking female's offspring, resulting in offspring with smaller size or reduced growth rate (Strand, 1986). Also, survival probabilities in previously-parasitized hosts may be decreased, which also reduces host quality. Few reported studies have examined sex allocation between unparasitized and parasitized hosts, but of the species examined, a few show evidence of sex ratio shifts (Wylie, 1966, 1973, 1976; Holmes, 1972; van Alphen & Thunnissen, 1983), while others have not (Suzuki et al., 1984; Orzack & Parker, 1986; van Dijken et al., 1989).

Using A. smithi and A. ervi, the hypothesis was tested that a female wasp allocates a higher proportion of daughters to unparasitized hosts and more sons to parasitized ones. Mated A. ervi females were offered a choice between unparasitized aphids and those parasitized by A. smithi. Since A. ervi larvae are superior competitors to A. smithi (see Chapter IV), A. ervi offspring have a high probability of successfully emerging from

aphids already parasitized by A. smithi. However, the size or growth rate of offspring developing in these hosts may be reduced compared to their siblings reared from unparasitized hosts, resulting in decreased fitness.

By increasing the time interval between oviposition by A. ervi and A. smithi, it may be possible to further reduce the host quality of parasitized aphids relative to unparasitized ones. In this situation, the A. smithi larva will have consumed more host tissue by the time an A. ervi larva has killed it, leaving fewer resources for the developing A. ervi.

2. Materials and Methods

Prior to adult emergence, mummified A. ervi and A. smithi were individually isolated in gelatin capsules (size 00). Virgin A. smithi adult females were collected from the capsules and later used in experiments. These females could lay only male (= unfertilized) eggs. When A. ervi adults emerged, males and females were kept in separate cups and 24 h later each female was individually mated with one male; that male was then discarded and not used for future matings.

A mated A. ervi female (n = 11) was placed in a waxed-paper cup arena (12 cm diameter, 6 cm height) containing 10 unparasitized third instar pea aphids and 10 aphids parasitized less than one hour earlier by virgin A. smithi. To obtain aphids parasitized by A. smithi, I placed a third instar aphid in a gelatin capsule (size 00) containing a virgin A. smithi. After the wasp had struck the aphid once, I removed the aphid from the capsule. Unparasitized aphids were marked by amputation of the distal third of one antenna (Mackauer, 1972).

Aphids were permitted to disperse freely in the arena. I removed any aphid struck by the searching A. ervi female immediately and replaced it with one of the same type. A trial was completed after 30 minutes had elapsed or the wasp had struck at least 30 aphids previously parasitized by A. smithi. Females that attacked fewer than 15 aphids were excluded from analysis.

All struck aphids were separated according to their original type (initially unparasitized or parasitized by A. smithi) and counted. They were then reared on bean stalks, still separated according to original host type, until parasitoid emergence. The species and sex of the parasitoid offspring were recorded. Any mummies which did not yield adults were dissected five days after all others had emerged and the sex of the parasitoid was determined if possible.

From each group of aphids initially parasitized by A. smithi, I set aside a subsample (= control) of 20 to 30 aphids. These aphids were reared and later dissected to estimate the proportion of aphids struck by A. smithi that actually contained an egg.

For each trial, I calculated the proportion of female A. ervi adults that emerged from each host class. Data were transformed using the Arcsine transformation and then comparisons were made between host classes using a paired t-test.

The above procedure was repeated with one exception. An A. ervi female (n = 13) was placed in an arena with unparasitized aphids and those parasitized 24 h (\pm 20 min) earlier by virgin A. smithi females. When hosts parasitized by A. smithi were prepared for the arena, I used nymphs that were 24 h younger than those used in the first experiment. These aphids were then reared on bean stalks for 24 h, which ensured that aphids in the arena were the same age for both host classes.

3. Results

The numbers of A. ervi females emerging from initially unparasitized aphids and those parasitized by A. smithi less than one hour previously and 24 h earlier are shown in Tables 9 and 10, respectively. When A. ervi females were offered unparasitized aphids and those parasitized less than one hour earlier by A. smithi, there was no difference in the sex ratio of A. ervi offspring emerging from each host class (paired-sample t-test after Arcsin transformation, $df=10$, $t = 0.95$; $P = 0.37$). The result was similar when parasitized aphids were attacked 24 h earlier by A. smithi (paired sample t-test after Arcsin transformation, $df=12$, $t = 0.05$; $P = 0.96$). All female A. ervi used in the experiments were mated, since each one produced female offspring. Although there was a wide range in the sex ratio of offspring produced by individual A. ervi, the overall values were slightly female-biased (ranging between 63% and 70% female offspring from each host class; see Tables 9 and 10).

Dissection of control groups showed that a high proportion of aphids struck by A. smithi were actually parasitized. Controls for data in Tables 9 and 10, respectively, showed that 91% ($n=268$) and 93% ($n=278$) of aphids struck once by A. smithi were expected to contain an egg.

These data confirm results of host discrimination studies done in Chapter IV. Figure 9 shows that A. ervi females attacked unparasitized aphids more often than those parasitized less than one hour earlier by A. smithi (X^2 -test, $X^2=26.568$, $df=1$, $P<0.001$). However, this was not the case when

A. ervi had a choice between unparasitized hosts and those parasitized 24 hours earlier by A. smithi. In this situation, there was no significant difference between the number of each host type attacked (Fig. 9; χ^2 -test, $\chi^2=3.358$, $df=1$, $P>0.05$).

Table 9. Number of Aphidius ervi females emerging from initially unparasitized aphids and those parasitized less than 1 hour earlier by A. smithi.

Trial	initially unparasitized		parasitized by <u>A. smithi</u>			
	Ae struck	Ae emerged females	Ae struck	As emerged	Ae emerged	Ae females
1	20	14	16	3	13	12
2	29	20	14	2	12	6
3	26	23	20	5	9	8
4	30	26	16	10	6	5
5	34	21	23	9	9	4
6	42	27	28	9	11	8
7	31	21	30	17	9	4
8	39	33	24	9	15	15
9	30	23	26	9	14	9
10	18	14	6	2	4	2
11	32	24	8	1	7	3
TOTAL	331	246	211	76	109	76

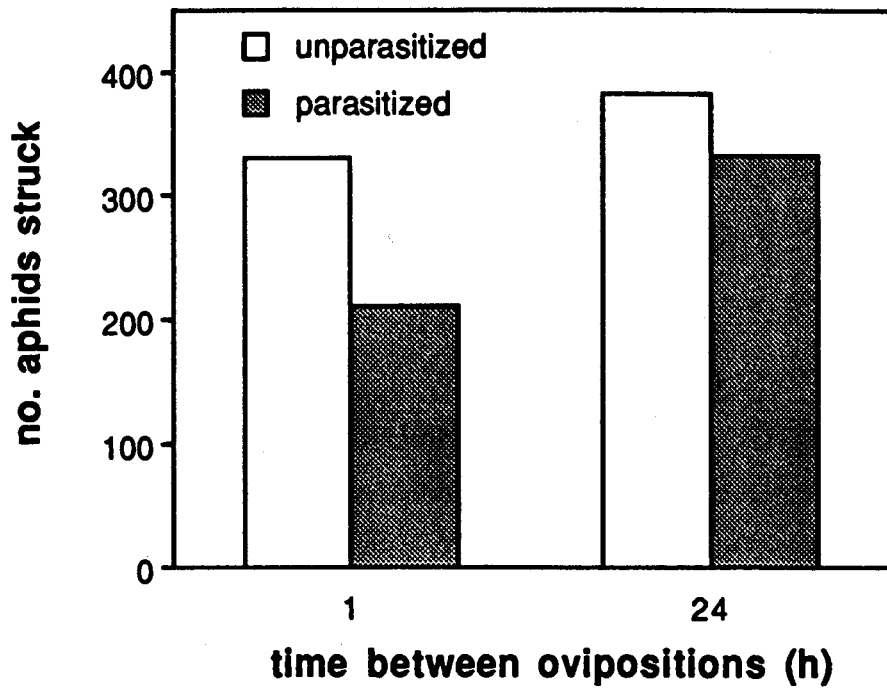
Ae = A. ervi; As = A. smithi

Table 10. Number of Aphidius ervi females emerging from initially unparasitized aphids and those parasitized 24 hours earlier by A. smithi.

Trial	initially unparasitized		parasitized by <u>A. smithi</u>			
	Ae struck	Ae emerged females	Ae struck	As emerged	Ae emerged	Ae females
1	25	9	25	19	2	2
2	44	33	30	9	16	11
3	26	22	30	14	3	2
4	42	37	28	24	2	0
5	51	27	33	15	14	10
6	35	22	35	24	5	1
7	7	5	7	4	2	2
8	16	14	11	2	8	6
9	25	13	14	3	5	0
10	41	34	36	23	10	8
11	28	18	27	11	3	2
12	17	14	25	19	4	1
13	25	16	32	20	9	7
TOTAL	382	264	333	187	83	52

Ae = A. ervi; As = A. smithi

Fig. 9. Numbers of aphids attacked when Aphidius ervi was given a choice between unparasitized aphids (open columns) and those parasitized by A. smithi (shaded columns). The time interval between ovipositions (t_0) by A. smithi and A. ervi was less than 1 hour or 24 hours. Data were pooled for all A. ervi females (n=11 for $t_0 < 1$ h; n=13 for $t_0 = 24$ h).



t_0

4. Discussion

Under the conditions tested, A. ervi females allocated equal proportions of fertilized eggs to unparasitized hosts and those parasitized by A. smithi. The result was the same regardless of whether parasitized hosts contained an A. smithi embryo that was less than one hour old or one that was 24 h old. Results gave no indication that survival of female A. ervi immatures differed from that of males. Under the experimental conditions, A. ervi females did not behave as predicted. It is possible that host quality differences between unparasitized hosts and those parasitized by A. smithi were not great enough to warrant differences in progeny allocation.

In larval competition between A. smithi and A. ervi (see Chapter IV), a decrease in larval growth rate of A. ervi was not recorded until $t_0 = 48$ h (Table 6) (i .e. A. smithi oviposited 48 h before A. ervi). When the older A. smithi larva was killed by first-instar A. ervi, the former was in the late second instar and had likely used up a considerable amount of host resources. If $t_0 = 48$ h had been chosen to test the sex allocation hypothesis, a difference in the sex ratio of A. ervi emerging from unparasitized and parasitized hosts may have been detected. However, at $t_0 = 48$ h, A. ervi females have a strong tendency to avoid oviposition in already parasitized hosts. Such an experiment would have data analysis problems due to small sample sizes.

The decreased larval growth rate of A. ervi caused by the presence of a

48-h-older A. smithi may not translate into a longer adult developmental time. During later growth stages, A. ervi could compensate for its initially slower growth rate and emerge from the host as quickly as conspecifics developing in unparasitized aphids. This would probably depend on how easily the dead A. smithi larva is converted into digestible material by A. ervi.

The problem of allocating female and male progeny between unparasitized and parasitized hosts has not received as much attention as sex ratio shifts involving small and large hosts. Only a few authors have reported evidence of male progeny being allocated more often to parasitized hosts (Wylie, 1966, 1973, 1976; Holmes, 1972; van Alphen and Thunnissen, 1983). Wylie (1966), for example, did find such evidence using Nasonia vitripennis (Walk.) (Hymenoptera: Pteromalidae) parasitizing housefly (Musca domestica L.) pupae. Female N. vitripennis laid a smaller percentage of female (= fertilized) eggs on previously attacked pupae than on unattacked ones. Wylie's conclusion that N. vitripennis laid fewer fertilized eggs on parasitized hosts was later supported by Holmes (1972), who used genetically-marked strains of N. vitripennis. In a later paper, Wylie (1973) showed that N. vitripennis females laid more unfertilized eggs on house fly pupae previously parasitized by their own species, or by Muscidifurax zaraptor K. & L., or by Spalangia cameroni Perk. (Hymenoptera: Pteromalidae) than on unparasitized hosts.

Observations on host discrimination from Chapter IV (Fig. 5) are verified by data presented in Tables 9 and 10. A. ervi females struck more

unparasitized hosts than those parasitized less than one hour earlier by A. smithi (Fig. 9). This is consistent with recognition of parasitized hosts using an external marker, which is also shown by data in Fig. 5. Recognition of aphids parasitized 24 h earlier by A. smithi seemed to require A. ervi to first probe the host with the ovipositor (Fig. 9), indicating the external marker was probably no longer effective at that time. This is also shown in Fig. 5.

Further studies are needed to determine the criteria used by superior larval competitors, such as A. ervi, to assess quality differences between unparasitized hosts and those parasitized by an inferior larval competitor. Under the conditions tested, any differences in quality between unparasitized and parasitized hosts did not translate into differential benefits for male and female offspring.

VII. GENERAL DISCUSSION

Competitive interactions between the solitary endoparasitoids A. ervi and A. smithi were examined in the laboratory. Since only one parasitoid offspring normally emerges from each host, oviposition decisions are predicted to be influenced by the relative increase in fitness which results from choosing one available host type over another. Compared to other accessible host types, a higher quality host is assumed to be a better source of food for a parasitoid's offspring (Waage, 1986). In the present study, one index chosen to measure quality was whether the host had been previously parasitized by a female of another species. Wasps are predicted to prefer unparasitized hosts, particularly if they are inferior larval competitors. Even for the larva which kills its competitor and completes development, fitness may be reduced relative to a wasp emerging from an unparasitized host.

Further choice tests examined parasitoid behaviour when females were offered two different types of parasitized hosts. Host classes were defined according to the identity of the wasp which attacked each aphid before presentation of these hosts to a searching parasitoid in an arena. The aphids were initially struck either by a conspecific female, a female of another species, or herself. Wasps were predicted to prefer host classes in which their larvae had a higher chance of survival. When choosing between conspecific- and self-parasitized hosts, they were expected to prefer the former, since females could not increase their fitness by laying two of their own eggs in the same aphid.

Before these studies were undertaken, the potential for competitive interactions between A. smithi and A. ervi was assessed. This was done by determining whether females of each species display a similar pattern of host instar preference. If competitive interactions are likely to occur between A. smithi and A. ervi, then females of each species should do this. Both parasitoid species can develop in all four instars and the adult of the pea aphid. Potential for interaction between these two species is higher if both tend to attack the same instar and lower if one is more likely to oviposit in younger hosts while the other selects older ones. In the latter case, interaction between parasitoid species will result from a female of one species encountering an older aphid instar containing a relatively advanced immature of the other parasitoid species.

While the results of host instar preference studies are influenced by the experimental design (Mackauer, 1973, 1983), the use of one method to compare two different parasitoid species will indicate if fundamental differences in parasitoid behaviour exist. A parasitoid's instar preference is a combination of:

- 1) the fitness consequences of selecting a particular instar for oviposition,
- 2) aphid behaviour which decreases the incidence of parasitization for some instars, and
- 3) the experimental design used to test preference, including the parasitoid's previous experience with hosts.

Results of the host instar preference study showed that A. smithi and A. ervi have the same pattern of instar preference. When presented with equal numbers of all four instars simultaneously, females wasps parasitized more second instars of the pea aphid, while there was no difference among parasitization rates of the other three instars. Wasps were caged on a bean stalk with these aphids for four hours. A shorter time interval would probably not be sufficient to obtain high enough rates of parasitism to obtain meaningful results, while exposure for a longer time interval could result in unacceptable levels of superparasitism and masking of any preference pattern as all host types become parasitized. Exposure of the wasp to all four instars simultaneously more closely approximates a field situation than offering only one instar at a time, or giving wasps only two instars to choose from (i. e. paired comparisons).

For A. ervi and A. smithi, parasitoid offspring emerging from third and fourth instar aphids were heavier than those emerging from first and second instars aphids, with females being heavier than males in all instars. If final parasitoid weight is used as a simple index of fitness, then older aphid instars produce parasitoid offspring with a higher fitness. Parasitoid size has been positively correlated with attributes such as fecundity and longevity, and hence is assumed to be an indirect measure of fitness (e.g. Charnov et al., 1981; Hurlbutt King, 1987). However, some advantage could be associated with being small, such as decreased developmental time (Hurlbutt King, 1987). Using A. ervi developing in pea aphids, Sequeira & Mackauer (unpublished data) have

shown that host quality is not a linear function of host size at oviposition in this particular system. Since parasitized aphids continue to grow, the relationship between the parasitoid and the host as a food source changes as both host and parasitoid develop. All parasitoid traits (e.g. fecundity, developmental time, longevity, sex ratio, larval development) are not optimized in any one host instar. Determination of which instar actually produces the parasitoid offspring with the highest fitness will involve analysis of a number of different parasitoid attributes.

Female parasitoids may select third and fourth instar aphids for oviposition, but because these instars can better defend themselves against parasitoid attack than younger aphids (Gerling et al., 1990), they may escape parasitization. Second instars may be selected over first instar aphids because they are easier to find due to their larger size. The pattern of instar preference seen in any given situation in the field will be influenced by these differences in behaviour between aphid instars, as well as the density and spatial distribution of each aphid instar on the host plants, and the searching wasp's previous experiences.

The influence of aphid behaviour on parasitization rates of each instar were further investigated in the laboratory. Differences in the response of each aphid instar to searching A. ervi females showed that aphid behaviour does influence parasitism rates. At a high aphid density (20 aphids per stalk), percent parasitism of second instars tended to be

highest, while at a low density (5 aphids per stalk), larger instars were more heavily parasitized. At the high density, second instar aphids behaved more like older instars; more of them dropped off the stalk in response to parasitoid disturbance than remained on the plant. At the lower density, second instars behaved more like first instar aphids, escaping parasitism by remaining hidden on the plant. Second instar aphids were too numerous at the higher density for all of them to use this strategy, and as a result were often parasitized.

Observations of aphid responses to a searching wasp in the laboratory demonstrate that host selection in the field is a complicated process to measure. The observed patterns of parasitization when a wasp is offered only unparasitized aphids are influenced by a wide range of parameters, as discussed above. This will be further complicated when hosts are scarce and previously parasitized aphids are encountered more frequently, which sometimes happens in the field (Appendix I, Tables 12, 13). Host selection can be reliably studied only in the laboratory when conditions are controlled. However, collecting parasitized aphids in the field does give valuable information such as identification of species present, and values of percent parasitism and super/multiparasitism. These data show the incidence of larval competition and which parasitoid species are in direct competition for hosts (see Appendix I).

In the laboratory, females of A. smithi and A. ervi were offered a choice between unparasitized aphids and those parasitized by females of the other species. Wasps reduced competition between their progeny and offspring

of another female by ovipositing more often in unparasitized aphids. Parasitized hosts were recognized by an external cue detected by antennation, most likely a pheromone or pheromone-like marker (Mackauer 1990), for at least two hours after oviposition by the first-attacking wasp. Later internal cues, probably caused by physiological changes associated with the developing embryo and detected by probing with the ovipositor (Fisher, 1971; Beckage & Templeton, 1986; Strand, 1986), were more important.

The ability to distinguish unparasitized hosts from those parasitized by conspecifics is a widespread phenomenon among hymenopterous parasitoids (van Lenteren, 1981), including A. smithi (Chow & Mackauer, 1984) and A. ervi (B. Bai, unpublished). Heterospecific host discrimination has been reported less often (Wylie, 1970; Chow & Mackauer, 1984; Vet et al., 1984) and according to Bakker et al. (1985) and Turlings et al. (1985) is not expected to evolve in sympatric species that are not egg-limited. They propose that heterospecific host discrimination is not an evolutionary stable strategy and it is unlikely that both competitors will simultaneously adopt this behaviour. Vet et al. (1984) have suggested that this type of host discrimination depends on the degree of relatedness between the parasitoid species. Even if host markers are assumed to be species-specific, a wasp will recognize a marker belonging to a closely-related species because it is similar to her own, but she will not be able to distinguish it from a conspecific's marker.

If females of A. ervi and A. smithi fail to discriminate

interspecifically, larval competition occurs. Under most conditions, A. ervi is the superior larval competitor. Young first instar A. ervi kill older A. smithi larvae by physical combat, while older A. ervi eliminate first instar A. smithi by physiological suppression. If eggs of both species hatch at approximately the same time (at 21°C, when A. ervi oviposits 18 h before A. smithi), neither species appears to have a competitive advantage.

Immature parasitoids use the same mechanisms to eliminate supernumerary larvae regardless of the potential competitors' identity (Mackauer, 1990). However, methods which kill conspecifics are not necessarily effective against immatures of another species. When a host contains two immatures of the same species, the older larva generally defeats the younger one, either by physical combat or physiological suppression (Salt, 1961; Fisher, 1961, 1971), although sometimes a young first instar can kill an older conspecific by physical combat (Chow & Mackauer, 1984, 1986). In many species of solitary hymenopterous parasitoids (including A. ervi and A. smithi), first instar larvae have sickle-shaped mandibles which can be used to physically attack potential competitors (Salt, 1961; Clausen, 1962). It is not known why first instar A. smithi larvae do not appear to use their mandibles for physical combat, while A. ervi does.

When immature parasitoids are similar in age (*i. e.* the oviposition interval is short), the chronologically older larva may not win at competition. Due to individual variation in developmental times, the egg laid by the second-attacking wasp may grow faster and be the first to

reach the critical stage at which it is able to eliminate competitors (Mackauer, 1990).

Only one mechanism used by immature A. smithi to eliminate supernumerary larvae appears to have any effect on A. ervi larvae. Several authors have suggested that when an A. smithi egg hatches, a "toxic secretion", perhaps a cytolytic enzyme, is released which kills all younger parasitoids and begins dissolution of host tissue (Vinson & Iwantsch, 1980; Strand, 1986). Parasitoid eggs apparently must hatch or at least be at a very advanced embryonic stage before they are killed. If oviposition intervals are adjusted so that eggs of A. smithi and A. ervi hatch at approximately the same time, A. smithi probably kills A. ervi if it hatches first. Otherwise, A. ervi has a better chance of winning, likely killing A. smithi by physical combat, a method used against first-instar conspecifics (pers. obs.).

Results of larval competition studies between A. smithi and A. ervi clearly showed why A. smithi is expected to prefer unparasitized hosts and avoid oviposition in those parasitized by A. ervi. As the inferior larval competitor, A. smithi cannot gain in fitness by laying eggs in hosts already parasitized by A. ervi. However, the benefits of A. ervi avoiding hosts parasitized by A. smithi are less clear. According to ideas put forward by Bakker et al. (1985) and Turlings et al. (1985), A. ervi should accept aphids already parasitized by A. smithi when unparasitized hosts are scarce, as sometimes occurs in the field (Appendix I, Tables 12 and 13).

Clearly A. ervi prefers unparasitized hosts to those already parasitized by A. smithi. This is expected if there is a chance of A. ervi losing at larval competition. This study also showed that a younger A. ervi larva required longer to develop in the presence of an older A. smithi larva, even when the latter was eventually killed. Further experiments are needed to determine if this translates into longer developmental time from oviposition to adult emergence or whether later larval instars of A. ervi compensate for the reduced growth rate after the older A. smithi is dead. A small increase in developmental time from egg to adult could have a significant influence on the ability of these wasps to compete for mates or hosts with earlier-emerging individuals from unparasitized hosts. It is also possible that an A. ervi larva developing in a multiparasitized host may attain a smaller adult size because an immature A. smithi used up a portion of the available host resources before being killed.

If development in a parasitized host reduces fitness of female parasitoids relative to that of males, solitary wasps are expected to allocate more female (= fertilized) eggs to unparasitized hosts (high quality) and more male eggs to parasitized ones (low quality) (Charnov et al., 1981, Waage, 1982). Offspring growing in parasitized hosts may have a smaller adult size, longer developmental time, or a lower probability of survival because of poor competitive abilities. There was no evidence in the present study that female A. ervi allocated more female eggs to hosts previously parasitized by A. smithi. This suggests that under the experimental conditions, both sexes were equally affected by any decreases

in fitness associated with developing in an already parasitized host. Alternatively, for A. ervi there may not be a cost associated with growing in a host already parasitized by A. smithi, as long as A. ervi survives. Further studies are needed to determine what the disadvantages are for a superior larval competitor, such as A. ervi, to develop in a host already parasitized by an inferior larval competitor.

Given a choice between aphids previously attacked by conspecifics or A. ervi, A. smithi females attacked more of the former host type. Given the same choice, A. ervi also attacked more aphids previously struck by A. smithi (Fig. 7). This indicated that searching wasps used external cues detected by antennation to recognize conspecific- and heterospecific-parasitized hosts. Given that a parasitized host was attacked, the probability of an A. smithi female laying an egg in a parasitized host was higher for conspecific- than for heterospecific-parasitized aphids (Fig. 8). Under the same conditions, A. ervi oviposited with equal frequency in each host type. This suggests that A. smithi also made use of internal cues detected with the ovipositor to recognize conspecific- and heterospecific-parasitized hosts. Whether or not A. ervi females were able to do this is not clear from the data.

When adults had only parasitized aphids to choose from, oviposition decisions reflected the fitness consequences associated with the possible host choices. Both A. smithi and A. ervi showed preference for aphids previously attacked by A. smithi. This result was predicted from the probabilities of A. smithi and A. ervi larvae surviving in each host

class. The offspring of a superparasitizing A. smithi was expected to do poorly in competition with A. ervi. However, it did have an equal chance of winning or losing in competition with a conspecific when the time interval between ovipositions was short, as explained earlier.

Females of A. smithi attacked more conspecific-parasitized pea aphids when given a choice between these and self-parasitized hosts, which was predicted. Under the experimental conditions, an A. smithi female could not gain in fitness by laying eggs in hosts that contained her own offspring. Acceptance of a conspecific-parasitized host by an A. smithi female was the same as acceptance of a low quality host (Völkl & Mackauer, 1990). Under these conditions, conspecific-superparasitism was adaptive, since A. smithi females are not egg-limited and are not capable of oösrption (Mackauer, 1971; Kambampati & Mackauer, 1989), and the second egg had an equal probability of winning or losing at competition (Chow & Mackauer, 1984).

In some situations, self-superparasitism could be advantageous (van Alphen & Visser, 1990). For example, Cloutier (1984) suggested that when several females search simultaneously, self-superparasitism could increase a female's fitness. If conspecific superparasitism has a high probability of occurring, self-superparasitism could increase the chances that a particular female's offspring will survive larval competition. There will be more of her eggs present in one host and the chance of at least one completing development is increased. Data to support such a claim show that female Leptopilina heterotoma (Hymenoptera: Eucoilidae) spent more

time searching for hosts in a patch when conspecifics were present than when individuals searched alone, and the level of superparasitism was higher in the former case (Visser et al., 1990). However, these data do not show that a female increases her fitness by self-superparasitism because the fate of each egg through to adult emergence was not followed. This is difficult to do unless each mother's offspring can be identified.

Females of A. smithi and A. ervi appeared to use an external marker to distinguish hosts parasitized by conspecifics from those parasitized by the other species and, in the case of A. smithi, between their own and a conspecific's marker. Aphidius smithi may have also rejected heterospecific-parasitized aphids based on internal cues detected after probing a host with the ovipositor. There are no other reported cases of female parasitoids distinguishing between conspecific- and heterospecific-parasitized hosts when offered both host types simultaneously. Generally, external markers are assumed to be species-specific (Bakker et al., 1985; Turlings et al., 1985), and internal cues are regarded as non-specific host quality changes associated with the developing parasitoid embryo (Mackauer, 1990). Recent studies have demonstrated that external markers do vary among conspecific females, at least in some species (Hubbard et al., 1987; Völkl & Mackauer, 1990).

Aphidius smithi and A. ervi are not egg-limited parasitoids in the laboratory (Mackauer, 1971; Kambhampati & Mackauer, 1989), but this may not be relevant in the field if parasitoids die before realizing their full fecundity. Low encounter rates with suitable hosts when they are

scarce and the importance of effectively using available foraging time may have more important effects on parasitoid behaviour. Thus the ability to discriminate between hosts of varying quality and to allocate a greater proportion of eggs to higher quality hosts is important for foraging A. smithi and A. ervi females.

In this study, progeny allocation by searching wasps reflected the survival probabilities of parasitoid offspring in different host types. Given a choice, female wasps preferred to oviposit in higher quality hosts. Unparasitized hosts were preferred over those already parasitized by a female of the other species. Differences in quality between conspecific- and heterospecific-parasitized aphids depended on which wasp species was searching for hosts. Aphidius smithi, the inferior larval competitor, preferred conspecific-parasitized aphids, while A. ervi, the superior larval competitor, preferred heterospecific-parasitized ones. When offered a choice between conspecific- and self-parasitized hosts, A. smithi females selected more conspecific-parasitized aphids. Under the experimental conditions, their fitness could not increase by self-superparasitism.

This study of oviposition decisions and larval competition between A. smithi and A. ervi is unique because two apparently similar species have larvae which behave very differently from one another. The first-instar larvae of A. ervi are aggressive and kill competitors by physical combat, while those of A. smithi show no evidence of having the ability to do so. First-instar larvae of both species are morphologically similar. All have

sickle-shaped mandibles that can potentially be used to bite competitors. Differences in larval behaviour translate into differences in adult behaviour when searching wasps are confronted with two types of parasitized hosts. An aphid containing the egg of A. smithi is a higher quality host for both A. smithi and A. ervi, while one previously parasitized by A. ervi is avoided more often. Since larval behaviour affects oviposition decisions made by the adult female, the two should be considered as part of the same process.

Both A. ervi and A. smithi were introduced into North America during the late 1950's and early 1960's for the biological control of the pea aphid (Mackauer & Bisdee, 1965; Angalet & Coles, 1966; Mackauer, 1971; Halfhill et al., 1972; Angalet & Fuester, 1977; Mackauer & Kambhampati, 1986). Aphidius smithi originally came from India, while collections of A. ervi for release in North America were made in various locations throughout Europe. Initially, A. smithi spread throughout pea aphid-infested areas in North America, and A. ervi remained at relatively low levels in most regions. However, by 1972, A. smithi was no longer found in collections of pea aphid parasitoids made in Ontario, and it has since declined in numbers throughout the eastern United States (Angalet and Fuester, 1977; Mackauer & Kambhampati, 1986). A similar decrease in the abundance of A. smithi in western North America has also been described (Kambhampati & Mackauer, 1987). At the present time, A. ervi is the dominant pea aphid parasitoid in North America (Angalet & Fuester, 1977; Mackauer & Kambhampati, 1986). Several hypotheses have been discussed to account for the decline of A. smithi in North America (Campbell & Mackauer, 1973;

Angalet & Fuester, 1977; Mackauer & Kambhampati, 1986; Kambhampati & Mackauer, 1989).

The results obtained in this study show that competition between A. ervi and A. smithi could have contributed to the numerical decrease of A. smithi in North America and its subsequent replacement by A. ervi. In the laboratory, A. ervi was the superior larval competitor under most conditions. If hosts are limiting in the field, which sometimes happens (Campbell, 1973; Appendix 1 Tables 12 and 13), A. ervi will have a competitive advantage. Chua et al. (1990) have suggested that A. ervi is better at searching for pea aphids than is A. smithi. If correct, this would also contribute to the superiority of A. ervi in the field.

The elimination of A. smithi as a dominant pea aphid parasitoid in North America after it had apparently become established support the concept that biological control agents should be carefully screened before release into the field. The release of one species at a time is more desirable than introducing two or more simultaneously, particularly if little is known about how these species will interact (Turnbull & Chant, 1961; Force, 1974; Miller, 1977; Ehler, 1979; Ehler & Hall, 1982; Miller, 1983). Most likely, A. smithi could have survived in the absence of A. ervi.

APPENDIX I. SUPER/MULTIPARASITISM IN THE FIELD

Aphids were collected weekly or biweekly (Table 11; Fig 10) from a 16 acre alfalfa field (variety = Trumpeter) at the Agriculture Canada Station near Kamloops, B.C. from 9 May to 28 August 1985. The purpose of these collections was to determine the level of multi/superparasitism in the field. During that growing season, the alfalfa field was cut on 7 June, 18 July, and 29 August. On each sampling date, mean alfalfa height (Fig. 10) in the field was measured as described by Campbell (1973). Alfalfa stem density (mean \pm s.d.) on 9 May 1985 was 52 stems/sq ft \pm 11 stems (n = 40 1-ft square quadrats).

During the first sampling trip (9 May), alfalfa plants were sampled by beating them with a stick so that aphids fell on to a tray (0.47 m X 0.32 m). Fifty to 70 plants were beaten to collect one sample; 10 samples were taken at least 10 m from the field edge and at least 10 m from each other. Of the 40 aphids collected, 36 (4 adults, 2 fourth instars, 13 third instars, 14 second instars, and 3 first instars) were killed immediately in 70% ethanol and later dissected. One adult aphid contained an Aphidius larva. Four were reared until parasitoid emergence; one A. ervi male emerged.

On all other trips, aphids were sampled by alfalfa tip sampling and sweep net sampling. One hundred alfalfa tips (300 and 200 tips on 16 May and 28 Aug respectively) were collected as described by Campbell (1973). Aphids were separated according to instar (Table 11) (except on 16 and 23

May, when aphids of each instar were counted after collection but were not reared in separate cages). All were kept alive (except on 16 and 23 May, when 1/3 were killed immediately in 70% ethanol and later dissected). Approximately 1/2 were dissected 48 to 72 h later and the rest were kept alive until parasitoid emergence. By rearing aphids before dissection, I was able to detect any parasitoid eggs which had been laid shortly before sampling occurred. This was not possible if aphids were killed immediately. Dissection results and number of each parasitoid species collected in the alfalfa tip samples are shown in Tables 12 and 14 respectively. Table 16 shows the number of super/multiparasitized aphids containing Aphidius only or both Aphidius and Praon immatures. All super/multiparasitized aphids contained only Aphidius larvae, except for the two exceptions noted in Table 16. A Praon and Aphidius immature were found in a third instar aphid collected on 29 May and in a fourth instar aphid collected on 21 August.

Aphids were collected in a sweep net (diameter = 32 cm, length of handle = 91.5 cm). One hundred sweeps were taken as I walked diagonally across the alfalfa field. Collected aphids were dropped immediately into 70% ethanol and later dissected. Sweep net samples were taken immediately after alfalfa tip sampling was finished. Dissection results for aphids collected in the sweep net are shown in Table 13. I dissected sweep net samples only for selected dates when I wanted an increased sample size to confirm results found in alfalfa tip samples. The number of super/multiparasitized aphids in sweep net samples containing Aphidius only or both Aphidius and Proan immatures is shown in Table 17. All

super/multiparasitized aphids contained Aphidius immatures only, with the following exceptions. Two adult aphids collected on 17 July contained both Praon and Aphidius. Of the 84 super/multiparasitized adult aphids collected on 21 Aug (see Table 13), 42 contained Aphidius only, 41 contained both Aphidius and Praon, and one contained Praon only. Ten of the 17 super/multiparasitized fourth instar aphids from the same collection were parasitized only by Aphidius, while the remainder contained immature of both Aphidius and Praon. Seventeen of 20 multi/superparasitized adult aphids and eight of 10 multi/superparasitized fourth instar aphids collected on 28 August (see Table 13) were parasitized only by Aphidius; the other three adult aphids and two fourth instars from the same collection contained both Aphidius and Praon immatures.

Mummified aphids were collected in the study site by walking through the field for one hour and picking alfalfa leaves to which mummies were attached. The mummies were later placed individually in gelatin capsules (size 00). Primary parasitoids that emerged were classified according to species and sex (Table 15).

Fig. 10. Total number of pea aphids collected by alfalfa tip sampling (100 alfalfa tips) from 9 May 1985 (day 1) to 28 August 1985 and alfalfa stem height (mean \pm 1 SEM; n=10) in an alfalfa field at the Agriculture Canada Station near Kamloops, B.C.

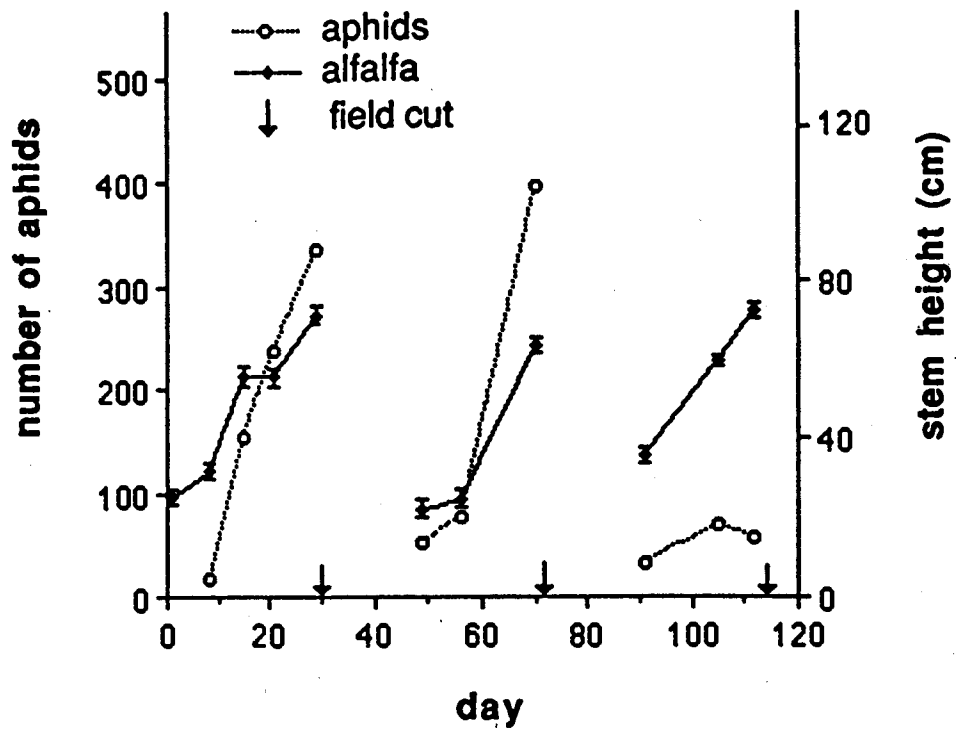


Table 11. Number of pea aphids collected on 100 alfalfa tips near Kamloops, B.C.

Date (1985)	Day ¹	Aphid instar					Total
		Adult	4th	3rd	2nd	1st	
CROP 1							
16 May ²	8	2	2	3	3	7	17
23 May	15	30	30 (5)	30 (1)	32	31	153
29 May	21	32 (3)	44 (4)	52 (1)	40	69	237
6 June	29	68 (8)	35 (16)	42 (10)	50	141	336
CROP 2							
26 June	49	8	3	4	19	17	51
3 July	56	17 (4)	7 (1)	21	17	15	77
17 July	70	82 (2)	24 (2)	45	77	169	397
CROP 3							
7 Aug	91	4 (1)	1	5	6	15	31
21 Aug	105	30	18	3	5	14	70
28 Aug ³	112	13	7	12	10	14	56

Numbers in parentheses indicate number of alates present

¹Day 1 = 9 May 1985

²number of aphids on 300 alfalfa tips/3

³number of aphids on 200 alfalfa tips/2

Table 12. Numbers of parasitized and super/multiparasitized aphids collected in alfalfa tip samples as determined by aphid dissection'. Samples were collected near Kamloops, B.C.

Date (1985)	Adult aphids		4th instar		3rd instar		2nd instar		1st instar						
	n ²	par' s/m	n	par s/m	n	par s/m	n	par s/m	n	par s/m					
CROP 1															
29 May	17	2	0	22	4	0	27	8	1	17	2	0	19	0	0
6 June	29	1	0	17	1	0	22	3	0	27	10	0	64	1	0
CROP 2															
26 June	4	0	0	1	0	0	1	0	0	10	1	0	8	0	0
3 July	8	0	0	4	1	0	9	3	0	8	0	0	4	0	0
17 July	51	5	0	10	3	1	20	8	1	42	15	0	98	5	1
CROP 3															
7 Aug	2	1	0	0	0	0	3	2	0	3	0	0	7	0	0
21 Aug	15	10	0	9	6	2	2	2	1	3	3	2	5	0	0
28 Aug	9	2	1	7	4	1	12	8	0	9	1	1	14	2	0

¹sample size = 100 tips, except 28 Aug sample size = 200 tips ²n = number of aphids dissected
³par = number of parasitized aphids (containing one or more immature parasitoids)
⁴s/m = number of super/multiparasitized aphids (containing two or more immature parasitoids)

Table 13. Numbers of parasitized and super/multiparasitized aphids collected in sweep net samples near Kamloops, B.C.

Date (1985)	Adult aphids		4th instar		3rd instar		2nd instar		1st instar						
	n'	par' s/m'	n	par s/m	n	par s/m	n	par s/m	n	par s/m					
CROP 1															
16 May	20	4	1	12	1	0	7	0	0	8	1	1	2	0	0
CROP 2															
26 June	165	35	2	81	15	1	66	2	0	53	0	0	26	0	0
17 July*	100	18	2	100	30	0	100	19	1	not dissected	not dissected	not dissected	not dissected	not dissected	not dissected
CROP 3															
7 Aug	190	70	6	102	22	1	60	6	0	36	0	0	26	0	0
21 Aug	543	384	84	264	162	17	68	22	7	27	0	0	5	0	0
28 Aug	149	109	20	79	37	10	74	14	3	49	1	0	11	0	0

'n = number of aphids collected and dissected
 'par = number of parasitized aphids (containing one or more immature parasitoids)
 's/m = number of super/multiparasitized aphids (containing two or more immature parasitoids)
 *a subsample of collected aphids was dissected

Table 14. Adult parasitoids emerged from aphids collected in alfalfa tip samples near Kamloops, B.C.

Date (1985)	<u>A. ervi</u>		<u>A. pisivorus</u>		<u>P. pequodorum</u>		<u>Unemerged mummies</u> <u>Aphidius Praon</u>		Hyperparasitoids'	Total
	f	m	f	m	f	m				
CROP 1										
16 May	0	1	0	0	0	1	0	0	0	2
23 May	6	7	0	0	1	0	1	0	0	15
29 May	15	8	1	0	1	1	1	0	1	28
6 June	16	9	0	0	1	0	2	1	0	29
CROP 2										
26 June	0	0	0	0	0	0	0	0	0	0
3 July	0	0	0	0	0	0	1	0	0	1
17 July	16	7	2	1	1	0	3	0	0	30
CROP 3										
7 Aug	2	0	0	0	0	0	0	0	0	2
21 Aug	2	3	1	0	3	1	0	1	2	13
28 Aug	3	6	1	0	0	0	2	1	2	15
TOTAL	60	41	5	1	7	3	10	3	5	135

f=female; m=male; 'emerged from Aphidius mummies

Table 15. Adult parasitoids emerged from mummies collected in 1 h mummy search near Kamloops, B.C.

Date (1985)	<u>A. ervi</u>		<u>A. pisivorus</u>		<u>A. smithi</u>		<u>P. pequodorum</u>		<u>Hyperparasitoids¹</u>		<u>Unemerged mummies</u>		Total
	f	m	f	m	f	m	f	m	A	P	A	P	
CROP 1													
23 May	18	15	0	0	0	0	0	1	8	0	0	0	42
29 May	11	5	0	0	0	0	1	1	2	0	1	0	20
6 June	23	16	1	0	0	0	4	4	115	0	17	0	177
CROP 2													
26 June	5	4	0	0	0	0	0	0	0	0	1	0	10
3 July	5	3	1	0	0	0	0	0	2	0	0	0	11
17 July	290	175	6	2	5	3	7	7	90	0	44	1	632
CROP 3													
7 Aug	84	73	9	0	0	0	11	11	92	7	34	1	320
21 Aug	62	31	0	0	0	0	6	6	79	3	19	1	203
28 Aug	50	26	5	0	0	0	0	0	82	5	42	2	213
TOTAL	548	348	22	2	5	3	30	30	470	15	158	5	1628

f=female; m=male; A=Aphidius mummy; P=Praon mummy

¹Each hyperparasitoid emerged from an Aphidius or Praon mummy.

Table 16. Number of superparasitized aphids in alfalfa tip samples containing Aphidius only or Aphidius and Praon immatures. Aphids were collected near Kamloops, B.C.

Date (1985)	No. aphids dissected ¹	No. super-parasitized aphids ²	No. of immature parasitoids/aphid	
			Aphidius only No. of immatures 2 more than 2	Aphidius and Praon No. of immatures 2
CROP 1				
23 May	89	1	1 0	0
29 May	102	1	0 0	1
CROP 2				
17 July	221	3	3 0	0
CROP 3				
21 Aug	34	5	2 2	1
28 Aug	51	3	3 0	0

¹ includes all nymphal instars and the adult stage of the pea aphid
² determined by aphid dissection

Table 17. Number of superparasitized aphids in sweep net samples containing only Aphidius, only Praon, or both Aphidius and Praon immatures. Aphids were collected near Kamloops, B.C.

Date (1985)	No. aphids dissected ¹	No. super- parasitized aphids ²	No. of immature parasitoids/aphid			
			Aphidius only No. of immatures 2 more than 2	Aphidius and Praon No. of immatures 2 more than 2	Praon only No. of immatures 2	
CROP 1						
16 May	49	2	1 1	0 0	0	0
CROP 2						
26 June	391	3	2 0	1 0	0	0
17 July	300	3	1 0	1 1	0	0
CROP 3						
7 Aug	414	7	6 1	0 0	0	0
21 Aug	907	108	57 2	38 10	1	1
28 Aug	362	33	22 6	5 0	0	0

¹ includes all nymphal instars and the adult stage of the pea aphid
² determined by aphid dissection

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