Laboratory studies on the glasshouse leafhopper *Hauptidia maroccana* (Melichar) and its egg parasitoid *Anagrus atomus* (Linnaeus)

By

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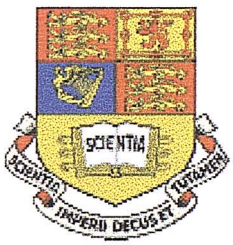

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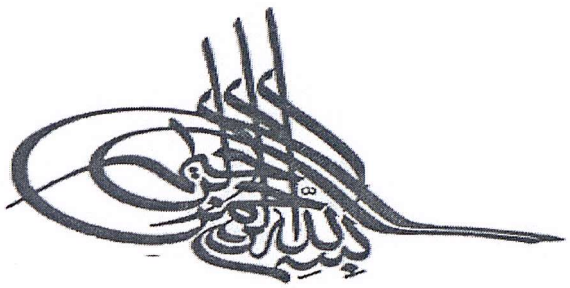
Dedication



In the name of Allah, most Gracious, most Merciful

I would like to dedicate this thesis to my beloved father and the memory of my beloved mother who passed away while I was conducting this research

I confirm that the work presented in this thesis is my own work

Signed.....

Signed.....**Supervisor**

Abstract

Biology and behaviour of glasshouse leafhopper *Hauptidia maroccana* and its egg parasitoid *Anagrus atomus* were studied in relation to temperature and host plants in the laboratory.

Immature survival and adult longevity of *H. maroccana* decrease with increasing temperature. The rate of development increased linearly over the range 17 to 25°C on different host plants. On average, *H. maroccana* laid 66±14 eggs in their 40±3 days average life time.

Preimaginal survival and adult longevity of *A. atomus* decreased with increasing temperature. Both sexes of *A. atomus* lived longer when fed with 50% honey solution. Developmental rate of *A. atomus* showed a positive linear trend with increasing temperature. Parasitoids developed pro-ovigenically with more mature eggs at low temperature.

The fecundity of *A. atomus* was highest when developed in hosts on French bean. A greater intrinsic rate of increase (r_m) and shorter doubling time (DT) were observed in Cucumber. The highest r_m and shortest DT were observed at 25°C. *Anagrus atomus* developed faster in older hosts (7-9 d old eggs) but their survival decreased. The sex ratio was always female biased especially in older hosts.

Self and conspecific host discrimination by antennal examination was observed within 12 h of parasitization. The conspecific parasitoids showed 4% ovicidal action on 72 h old parastized hosts. Superparasitism was low (2%) in self specific discrimination but comparatively high (30%) in conspecific parasitism. Oviposition experience reduced host-handling time. Searching efficiency varied significantly among different host plants.

Thelytoky was observed in *A. atomus* possibly caused by unknown micro-organisms. Thelytokous females produced male offspring with viable sperm when the parents were fed with the antibiotic Rifampicin. Those males showed successful mating.

The above studies may contribute to the understanding of tritrophic relationships and provide a guideline for mass rearing and utilization of *A. atomus* in biological control programmes.

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Chapter 1

General Introduction and Review of Literature

1.1 General Introduction

The glasshouse leafhopper, *Hauptidia maroccana* (Melichar) has been recorded as a glasshouse pest from nearly every county in England. It has been a sporadic pest of Tomato in the south of England for the last decade and the recent outbreak in the North suggests it is becoming more important. This pest has a wide host range including Tomato, Cucumber, Geranium, Nicotiana, French bean, Fuchsia, Chrysanthemum, Cotton, Primula etc. (Copland and Soeprapto, 1985). It is also a serious pest of cereals and grape vines in America, in the cotton growing areas of Central and South Africa (MacGill, 1932), and is a vector of diseases in Tomato in the west of France (Maisonneuve *et al.*, 1995). Both nymph and adult glasshouse leafhoppers feed on the leaves, never apparently attacking the stem or other parts. They suck the cell sap resulting in white bleached areas on the leaf surface. As the leafhoppers continue to feed the spots gradually become confluent and irregular white areas are found. In addition, the growth of sooty mould fungus spoils the appearance of leaves and fruits.

A control strategy is urgently needed which will be economically feasible, environmentally safe and long lasting in glasshosues. Control of this pest by chemicals is possible but it is harmful for the natural biological control agents, creates the problems of insecticide resistance, resurgence and disrupts the balance in the ecosystem. Helyer and Gill (1995) suggested that a higher dose of the growth regulator insecticide Buprofezin would be needed in the glasshouse to give the same degree of control for leafhopper as for whitefly. Buprofezin has a narrow range of insecticidal activity (Anon., 1993) and hence permits integration with biological control agents. Laboratory tests have not shown any major mortality to the egg parasitoid, *Anagrus atomus* at red egg stage that reach after 7-8 days of ovipisition, due to Buprofezin (Helyer & Gill, 1995).

There is ample scope for utilising the parasitoids for the control of glasshouse leafhopper. The mymarid wasp *Anagrus atomus* (Linnaeus) is found to be an effective parasitoid attacking the leafhopper eggs (MacGill, 1932) and has been used commercially

in the UK since 1992 (Cooper, 1993). Reports are also available on the utilisation of *A. atomus* which gives satisfactory control without application of chemicals throughout the growing season on Tomato (Maisonneuve *et al.,* 1995). Mymarids have rarely been used deliberately in biological control programmes, although they evidently exert a considerable degree of natural control.

To develop such a biological control programme, information is needed on the life table data, which comprise the development, fecundity, adult longevity of the pest and parasitoid as well. Data also needed on the abiotic factors such as, temperature, humidity, photoperiod and biotic factors such as host quality and availability, which act as constraints upon the success of a biological control programme.

1.2 Biological control

1.2.1 Introduction

Biological control has been defined as the "action of parasites, predators, and pathogens in maintaining another organism's density at a lower average than would occur in their absence" (DeBach, 1964). Van Driesche and Bellows (1996) refer to biological control as a population-level process in which one species population lowers the numbers of another species by mechanisms such as predation, parasitism, pathogenicity, or competition. Moreover, other biologically based techniques have been added to the above definition, such as utilization of pheromones, the sterile male technique and genetic manipulation or host plant resistance (Greathead, 1991). But while these behavioural approaches to pest control have a biological basis, many investigators do not consider them to be biological control. Nevertheless, biological control is one of several possible biorational techniques for use in integrated pest management programmes (Figure 1.1).

1.2.2 Techniques of biological control

Generally, biological control has been achieved by the use of one of the three basic approaches, importation, augmentation and conservation (Dent, 1995; Nordlund and Legaspi, 1995; van Driesche and Bellows, 1996).

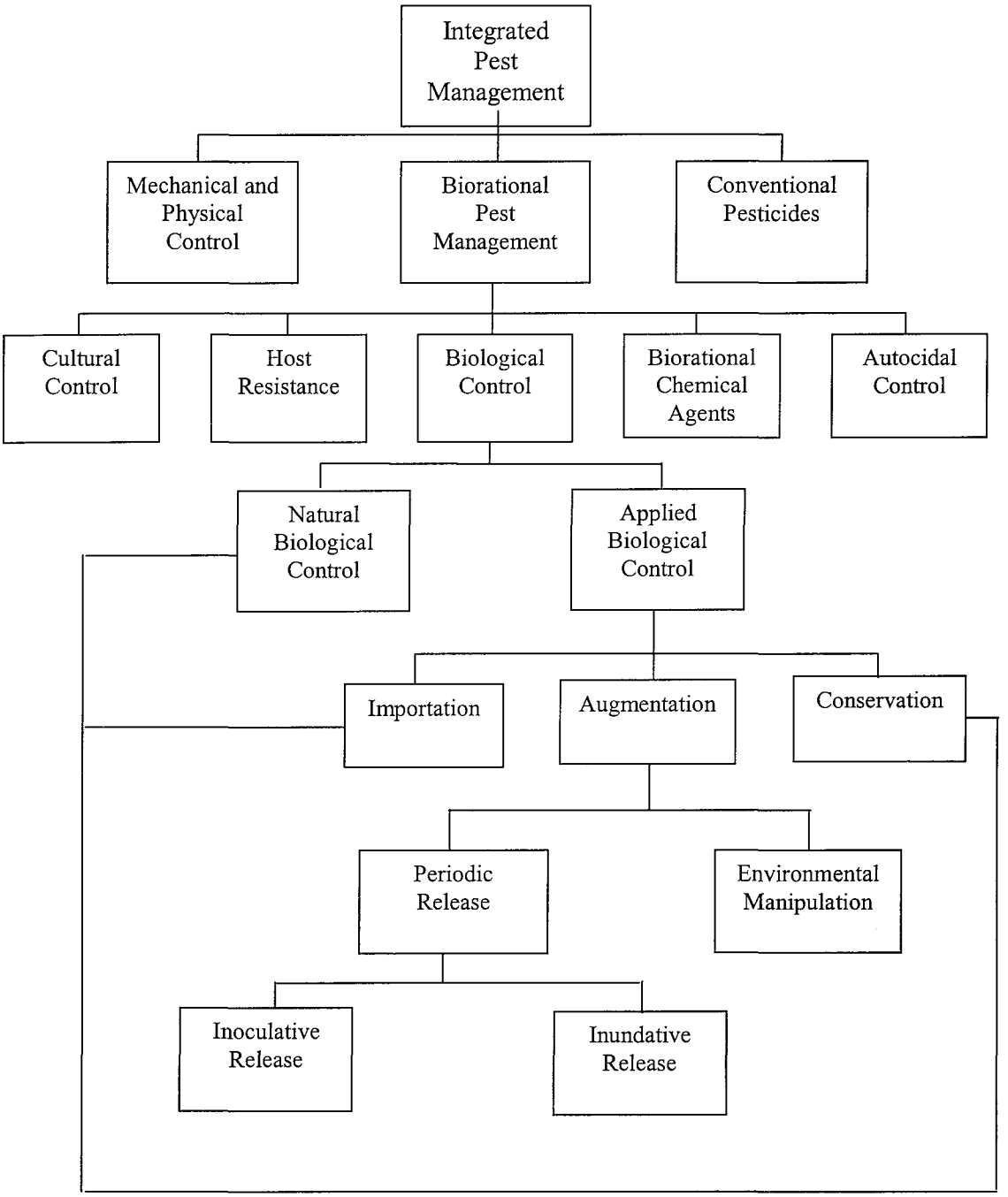


Figure 1.1 Pest management and control techniques available for use in integrated pest management programmes (after Nordlund and Legaspi, 1995)

1.2.2.1 Importation

Classical biological control is based on the importation of exotic natural enemies (parasitoids, predators, or pathogens) and their long term establishment in the new environment, a strategy which may then provide long term control of target exotic pest arthropods or weeds. In addition, once a successful programme is achieved in one location, the same natural enemies are frequently used to control the same pest elsewhere in other similar climatic locations (Hoy, 1988). Hundreds of successful projects have reduced damage caused by a wide array of exotic pests (Laing and Hamai, 1976; Clausen, 1978). The examples are the control of cottony cushion scale, Japanese beetle, European corn borer, Florida red scale, Rhodesgrass scale, spotted alfalfa aphid, gypsy moth, California red scale, olive Parlatoria scale, European red mite, imported southern red fire ant, Russian wheat aphid and boll weevil (Hoy, 1988).

Dent (1995) summarized the stages that should be involved in the introduction of a natural enemy: (i) identification of the pest and its area of origin, (ii) an exploratory expedition in the pest's region of origin, (iii) an inventory of natural enemies associated with the pest, (iv) field screening and evaluation, (v) importation, culture, possible pre-culture tests, (vi) release, and (vii) occasionally where funds permit, post release evaluation. For a classical biological control programme to be completely successful the natural enemy has to reduce the pest populations to a level when the latter no longer inflict economic damage (Kidd and Jervis, 1996).

DeBach and Rosen (1991) described three degrees of classical biological control. Complete biological control refers to applications on serious widespread target pests and their reduction to non-pest level and maintenance below the economic threshold with a nil or rare use of insecticides. Substantial biological control concerns less serious pests or less wide crop areas, or occasional use of insecticides, so that the economic benefits are of lower value. Partial biological control involves reduction of the pests, which are considerable but not consistent or not regularly below the economic threshold, so that insecticides should be used although reduced even by 50%.

Classical biological control has been actively practised for about 100 years in the United States. Worldwide, approximately 2300 introductions directed against insect pests

have provided complete biological control in about 100 cases (Ehler and Andres, 1983). Unfortunately, most classical biological control programmes probably require substantially more time and resources than other forms of biological control. However, if they were provided, both the rate of establishment and rate of success in classical biological control could probably be increased, thus providing long-term, economical and environmentally sound control of exotic pests (Tauber *et al.,* 1985)

1.2.2.2 Augmentation

Augmentation involves the collection, mass rearing and periodic release of a large number of natural enemies to obtain immediate control. This method is applicable in cases where there is no interest in long term stability but where there is a need to reduce pest numbers below economic thresholds (van Lenteren, 1986).

Two methods can be distinguished in augmentation: Seasonal inoculative and inundative releases. Seasonal inoculative release method or repeated releases involves relatively few agents and the expected control depends on the progeny of released individuals (Nordlund and Legaspi, 1995). This method can be applied for many generations depending on the duration of the crop and the number of generations of the pest per season (van Lenteren, 1986; van Lenteren *et al.*, 1996).

In inundative release or mass release methods, beneficials are collected, mass reared and periodically released in large numbers and pest control is achieved exclusively by the released individuals themselves (van Driesche and Bellows, 1996). Examples are the application of the parasitic wasp *Trichogramma evanescens* Westwood against the European cornborer *Ostrinia nubilalis* (Hubner) in Europe (Hassan, 1981), *Encarsia formosa* Grahan against *Trialeurodes vaporariorum* (Westwood) (Heinz and Nelson, 1996), *Eretmocerus eremicus* Rose and Zolnerowich against *Bemisia argentifolii* (Hoddle *et al.*, 1998).

General application of the method is restricted by the high cost, quality and field effectiveness of the reared organisms. Mass production is done by private companies only in situations where the cost of rearing is economically reasonable, or the crop has a high

cash value and less expensive alternatives are not available. More extensive applications may be possible if mass rearing of the natural enemies is done by government institutes (van Driesche and Bellows, 1996).

1.2.2.3 Conservation

Conservation involves protecting and maintaining natural enemy populations. Conservation is crucial if both native and exotic natural enemies are to be maintained in agricultural ecosystems (Hoy, 1988). Landis *et al.* (2000) described habitat management, as a form of conservation, as an ecologically based approach aimed at favouring natural enemies and enhancing biological control in agricultural systems. The goal of habitat management is to create a suitable ecological infrastructure within the agricultural landscape to provide resources such as food for adult natural enemies, alternative prey or hosts, and shelter from adverse conditions (Landis *et al.*, 2000). Most commonly, conservation involves modifying pesticide application practices so that they occur only when the pest population exceeds specific levels. In some cases, conservation of natural enemies can be achieved by changing the active ingredients, rates, formulations, timing and location of pesticide application (Hull and Beers, 1985) and by protecting the population of natural enemies by maintaining refuges (Tauber *et al.*, 1985).

1.2.3 Advantages of biological control

There are some specific reasons that make growers working in glasshouses prefer biological control as mentioned by van Lenteren (2000):

1. With biological control there are no phytotoxic effects on young plants, and premature abortion of flowers and fruit does not occur.
2. Release of natural enemies takes less time than applying chemicals in humid and warm glasshouses.
3. Release of natural enemies usually occurs shortly after the planting period, when the grower has plenty of time to check for successful development of natural enemies; therefore the system is reliable for months, with only occasional checks; chemical control requires continuous attention.

4. Chemical control of some of the key pests is difficult or impossible because of pesticide resistance.
5. With biological control there is no safety period between application and harvesting fruits; in chemical control one has to wait several days before harvesting is allowed.
6. Biological control is cheaper than chemical control.
7. Biological control is permanent: once a good natural enemy, always a good natural enemy.

1.2.4 Disadvantages of biological control

1. Biological control agents are mostly host specific, less predictable and need several years of scientific research before application while a single chemical can kill a wide range of pest species and thus is more acceptable to the farmer (Jutsum, 1988; Waage and Greathead, 1988 and Mackauer *et al.*, 1990).
2. Relatively low levels of efficiency in some situations hampers the development and acceptance of biological control systems in commercial agriculture. In the long term, predator-prey relationships depend on a sufficient population of the prey surviving to sustain the predators (Winstanley, 1995).
3. In contrast, the proper utilization of biological control needs a great degree of knowledge and sophistication much above that required by pesticide technology (Perkins, 1982).
4. In some cases, biological control causes extinction of both target and non-target species and local extirpation of pests by biological control agents may be a common phenomenon (Pimentel *et al.*, 1982; Murdoch *et al.*, 1985; Murdoch and Bence, 1987).
5. Storage of biological control agents is not easy to achieve and is often impossible (Samways, 1981).

1.2.5 Desirable attributes of beneficial organisms

The desirable attributes which tend to make a beneficial species display an efficient relationship with the pest has been mentioned by several authors viz., Huffaker and Messenger (1976), van Lenteren (1980, 1986), Kidd and Jervis (1996) and van Driesche and Bellows (1996) and are described below:

a) Ecological compatibility: When searching for exotic beneficial agents for importation against native or introduced pests, it is usually important to seek species whose ecological requirements are similar to those of the intended target insects.

b) Temporal synchronisation: It is not enough for the pest and its natural enemies to be in the same place at the same time; their life cycle must be synchronised for adequate regulation to be possible. Seasonal synchrony with the host is not an important selection criterion in biological control programmes involving inoculative and inundative release, since synchrony can be achieved by the grower through the release of parasitoids when most hosts are in the susceptible stage for parasitism.

c) Density responsiveness: The most desirable biological control agents will exhibit rapid positive functional and numerical density responsiveness.

d) Reproductive potential: An important factor in density responsiveness is a high reproductive capacity, through either short generation time, high fecundity or both. In most cases, we seek parasitoids or predators with an innate potential for increase relatively greater than that of the host/prey.

e) High intrinsic rate of natural increase (r_m): Candidate species can be preferentially selected over others if they have a higher r_m under temperature conditions similar to those in the area of introduction. A natural enemy having a lower r_m than that of its host or prey can be considered as a poor biological agent, provided that the r_m is high enough to balance that part of the host's r_m that is not negated by factors other than the parasitism in question.

f) Searching capacity: The ability of a beneficial organism to find host/prey at a low population density and reduce its numbers has a significant bearing on the long term success of entomophages in more stable situations.

g) Dispersal capacity: The ability of an introduced beneficial species to rapidly disperse and have a capacity to coincide with the dispersal of its prey. This ability is closely related to the searching capacity and ecological adaptibility of the beneficial.

h) Host specificity and compatibility: Host specificity indicates a high degree of biological adaptation to the host and probably a greater degree of direct and rapid responsiveness to density changes in the population of the target host. Host specificity is also related to the compatibility or degree of biological adaptation to the defensive mechanism of their host/prey, which renders all individuals susceptible. However, the attribute of polyphagy in predators that can survive in the absence of the pest, by switching to alternative available prey cannnot be considered undesirable.

i) Food requirements and hosts: A natural enemy which can fill its food requirements using only the specific host prey against which it is released is most useful. Frequently however, suitable alternate hosts or nectar or pollen or honeydew sources must be available in order to sustain adult parasitoids. Useful parasitoids usually display the power of host discrimination such that they avoid superparasitism and thereby, in effect, increase their fertility by distributing their reproductive capacity among more hosts.

j) Ease of rearing: It is important that the entomophagous species used for pest suppression, can be easily mass reared. Usually commercial glasshouses are disinfected once a year, so that, biocontrol agents need to be re-introduced.

k) No negative effects: A negative attribute to be avoided in selecting biocontrol agents is the incidence of hyperparasitoids, detrimental diseases or predators. The selected natural enemy should not attack other beneficial organisms in the same environment.

1.3 Glasshouse leafhopper

The glasshouse leafhopper (*Hauptidia maroccana)* and the green leafhopper (*Empoasca decipiens*) are the most important leafhoppers in European glasshouses (Copland and Soeprapto, 1985; Jervis and Kidd, 1993). They were noted as pests in the UK in 1918 increasing in significance until the 1940s before returning recently as secondary pests (Fox-Wilson, 1938; Copland and Soeprapto, 1985

1.3.1 Egg

The egg is elongated (0.5-0.7 mm in length), slightly curved, white with no distinctive markings (MacGill, 1932; Fox-Wilson, 1938). Eggs are laid singly into secondary veins on the undersides of leaves with a saw-like ovipositor (Fox-Wilson, 1938; Copland and Soeprapto, 1985). Occassionaly eggs have been found on the upper side of the leaves. The egg becomes distinguishable late in development as a slight swelling (Plate 1.1) and hatches after about 17 days at 18°C. (Fox-Wilson, 1938). After one mating the female leafhopper continues to lay fertile eggs for the rest of her life. Each female lays approximately 50 eggs during a 2-3 month oviposition period, typically 1-2 eggs per day with a sex ratio of approximately 50:50. (MacGill, 1932). There is a pre-oviposition period of 7-14 days.

1.3.2 Nymphs

There are 5 nymphal instars becoming progressively more adult like. The typical feature of the nymphal stages is the presence of a number of long setae, clubbed at the distal end. Different instars can be distinguished from each other on the basis of the number of setae and wing pad size as as development proceeds (MacGill, 1932).

The 1st instar nymphs are approximately 1 mm long excluding antennae, white and transparent. The eyes are red at first, becoming black later. The head is proportionately large with long antennae, being at least three-quarters of the body length. On the dorsal surface of the head, there are six setae, four along the anterior border and two behind these near the middle line. There is a pair on each of the three thoracic segments and four on the dorsal side of the third to the ninth abdominal segments. There are also some clubbed setae on the front on the head. All the legs are of equal length and

the tarsi have two joints with well-developed pulvilli but no claws (MacGill, 1932). The middle and hind legs each have one clubbed setae on the outer side of the tibiae close to the proximal end (Plate 1.2).

The 2nd instar nymph is similar to the first, approximately 1.2 mm in length. The hind angles of meso and metathorax are slightly prolonged backward giving the definite indication of wing pads. In addition to the setae found in the preceding stage, there is a pair of clubbed setae at the interior angle of both prothorax and metathorax and one at the posterior end of the mesothoracic wing pad. The second instar nymph is still white and transparent with no sign of pigmentation (MacGill, 1932) (Plate 1.3).

Third instar nymphs are approximately 1.6 mm long. The chief difference between the nymphs of the 2nd and 3rd instar is in the length of the wing pads (Plate 1.4). In this instar, the metathoracic pads extend back as far as the second abdominal segment. The chaetotaxy of the body is similar to that of the preceding stage, but eight large setae are present on the hind tibiae (MacGill, 1932).

The 4th instar nymphs are about 2 mm in length. The wing pads are longer than in the 3rd instar. The number of setae on the dorsal surface of the insect has increased. On the thorax, in addition to those found in the earlier stages, a pair of setae is present at the hind angle of the prothorax, a pair at the proximal angles of the wing pads on the mesothorax and three setae on the inner border of each of the anterior wing pads; on the metathorax there is an additional setae on the inner border of each wing pad. The hind tibiae have now ten setae and the mid-tibiae two. The first sign of pigmentation appears in this stage, there being two patches of dark pigment on the mesothorax, one at the proximal angle of the wing-pads and the other nearer the anterior border of the segment (MacGill, 1932) (Plate 1.5).

The 5th instar nymphs are approximately 2.8 mm in length. The wing pads are now longer and reach about halfway to the posterior end of the abdomen. The pigment on the metathorax is clearer than that of the fourth instar and the patches of colour are larger. There is an additional pair of setae on the prothorax, on the posterior edge of the segment, and there are now five setae along the inner border of the mesothoracic wing pads (Plate 1.6). In the 5th instar it is possible to distinguish the sex of the insect (MacGill, 1932).

1.3.3 Adult

The adult is 3-4 mm in length, slender and pale coloured. The wings extend beyond the body when at rest with dark patches on the wings producing V-shaped bands across the insects back (Fox-Wilson, 1938; Copland and Soeprapto, 1985). The head is pale yellow with dark prominent eyes; there are two additional spots between the eyes. The thorax has two distinct spots between the bases of the forewings (Fox-Wilson, 1938) (Plate 1.7). Male and females are identical dorsally and can be differentiated on the basis of abominal features in lateral view (Plate 1.9 and 1.10).

The external genitalia of the male consists of a broad basal plate on the ventral side of the abdomen, which bears two short projecting triangular plates from its posterior end. In the female there are two pairs of long pointed plates on the ventral side of the abdomen (MacGill, 1932). Feeding behaviour may be adversely affected outside a temperature range of 14 to 30°C i.e. little feeding above or below this range, while movement to colonise new plants is restricted below it. More eggs tend to be laid at lower temperatures. Temperature affects the rate of development, 42 days at 18°C from egg to adult, reduced to 23 days at 30° but with very high mortality. Adult longevity is up to 4 months at low temperatures and although males and females are in equal proportions, females are longer-lived (Copland and Soeprapto, 1985). During copulation the male and female insects lie in the same plane facing in opposite directions (Plate 1.8).

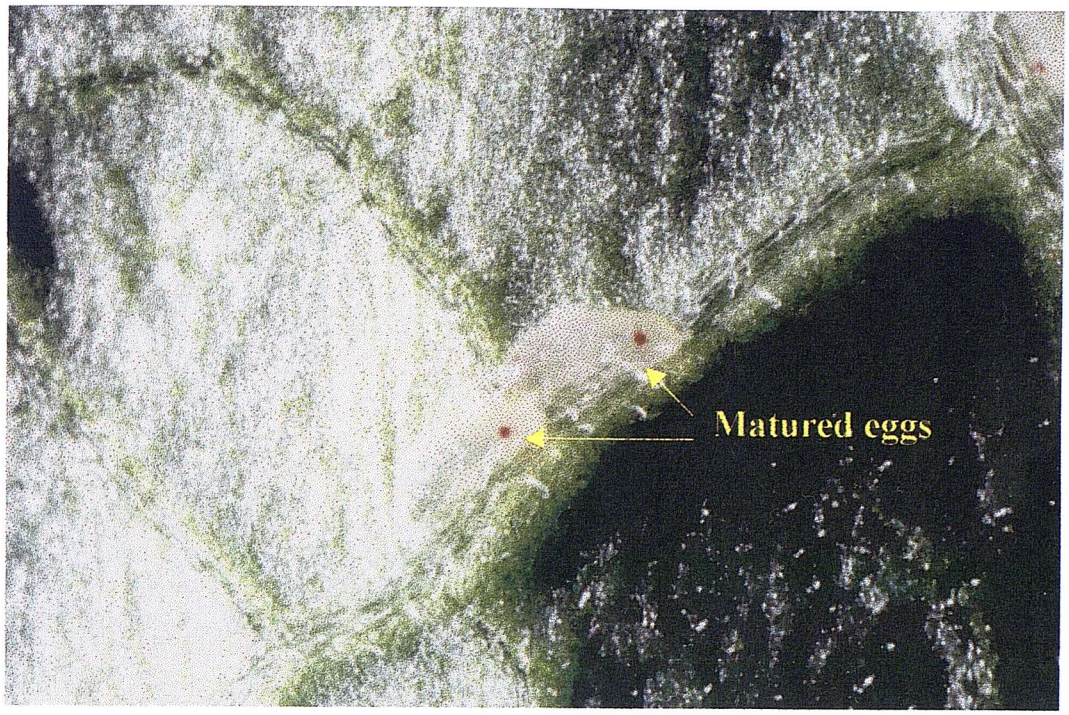


Plate 1.1: Matured eggs of *Hauptidia maroccana* laid inside leaf vein (x 30)



Plate 1.2: A first instar nymph of *Hauptidia maroccana* (x 30)



Plate 1.3: A second instar nymph of *Hauptidia maroccana* (x 30)



Plate 1.4: A third instar nymph of *Hauptidia maroccana* (x 30)

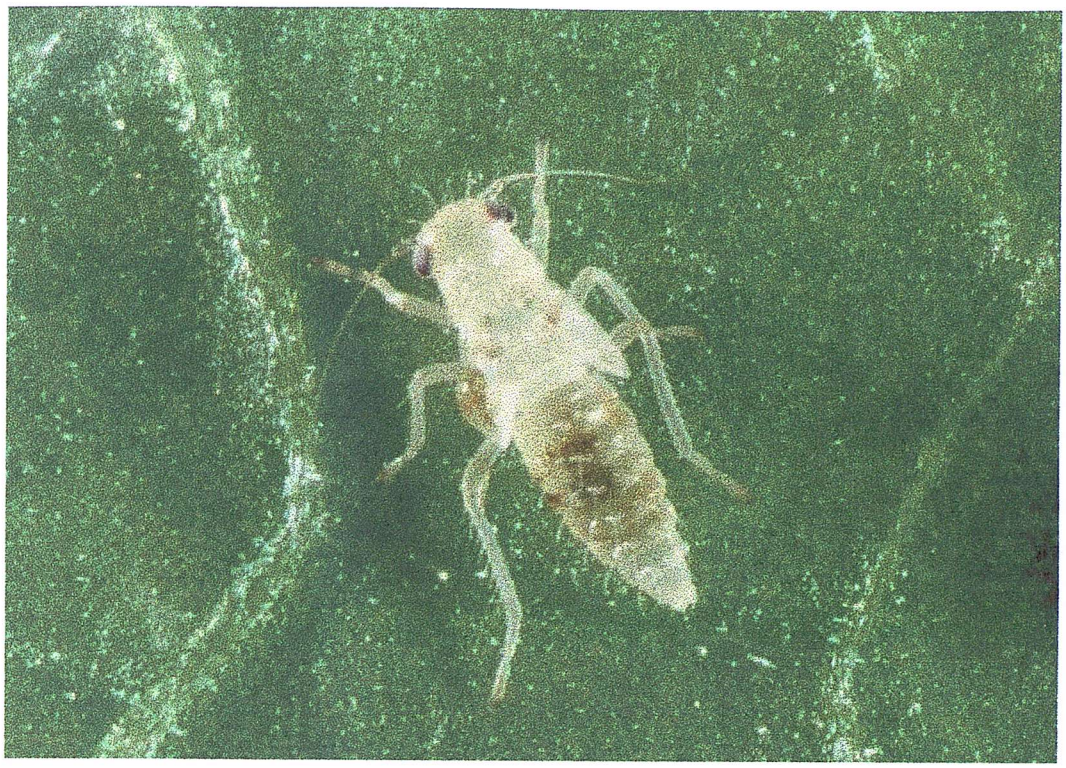


Plate 1.5: A fourth instar nymph of *Hauptidia maroccana* (x 30)

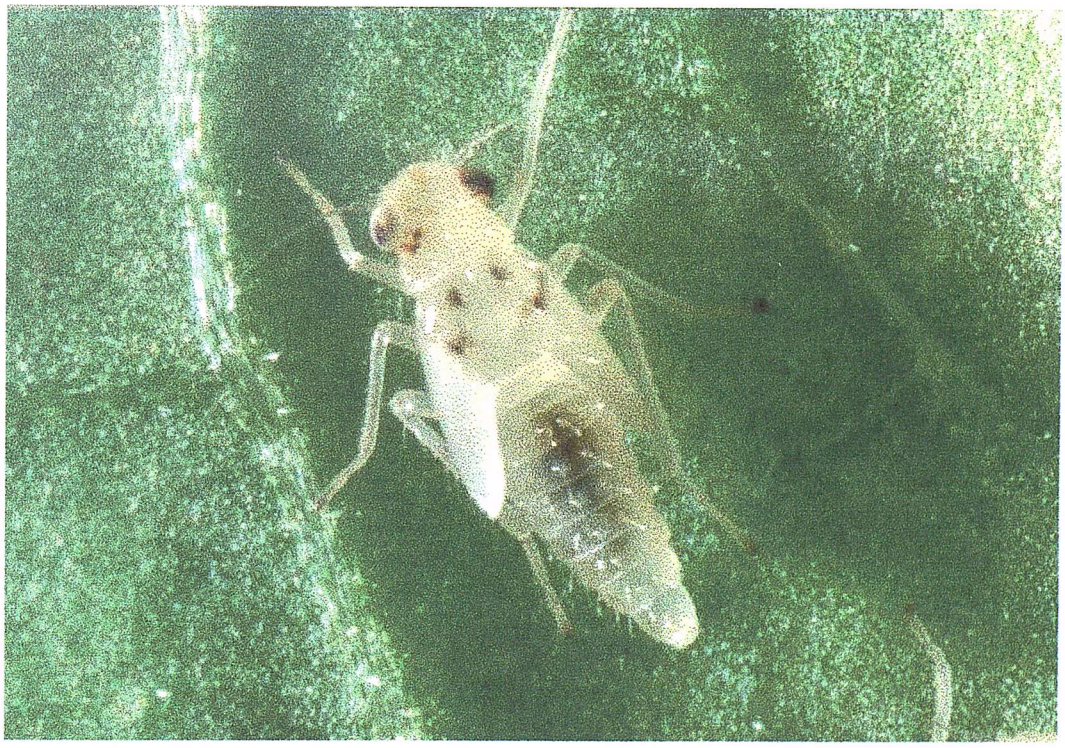


Plate 1.6: A fifth instar nymph of *Hauptidia maroccana* (x 30)



Plate 1.7: An adult *Hauptidia maroccana* (x 30)



Plate 1.8: Mating posture of *Hauptidia maroccana* (x 15)



Plate 1.9: Lateral view of an adult male of *Hauptidia maroccana* (x 15)

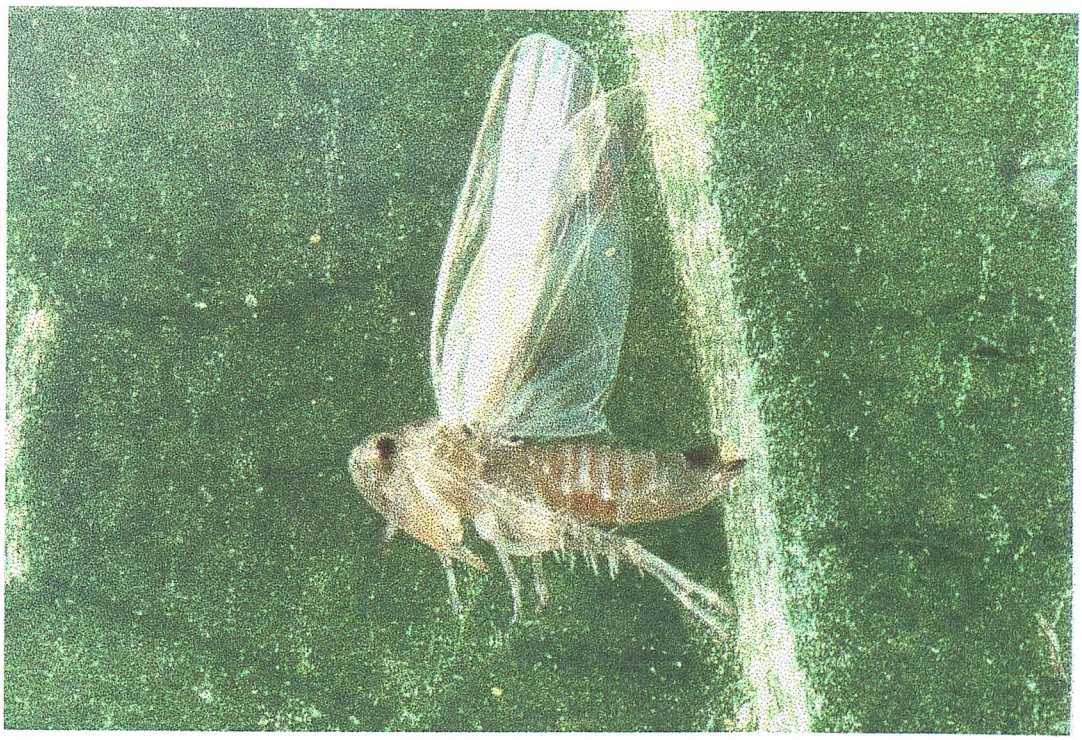


Plate 1.10: Lateral view of an adult female of Hauptidia maroccana (x 15)

1.3.4 Nature of damage

Both adults and nymphs feed on the leaves, never apparently attacking the stem or other parts. Usually the insects feed on the lower surface of the leaves producing mottled areas on the upper surface as the stylet may be pushed into the palisade tissue of the leaf (Fox-Wilson, 1938; Copland and Soeprapto, 1985). The characteristic damage appears as white spots on leaf surface where the chlorophyll is removed. As the leafhopper continues to feed the spots gradually become confluent and irregular white areas are found (Plate 1.11). In a severe attack, the whole leaf becomes bleached white, reducing growth or killing the plant (Copland and Soeprapto, 1985). When a plant becomes badly infested the leaves are much soiled by the fluid excreted by the nymphs, which serves as a very suitable medium for the growth of moulds (MacGill, 1932).

1.3.5 Host range

Hauptidia maroccana attack a wide variety of vegetables and ornamental crops. The vegetable crops attacked include Sweet Pepper, Cucumber, Tomato, French bean and Cotton. (Copland and Soeprapto, 1985). In addition they also attack some ornamental plants, such as *Geranium*, *Chrysanthemum, Fuchsia, Primula* and *Salvia* (MacGill, 1932; Copland and Soeprapto, 1985). Attacks are more severe in long season crops where there is time for a number of generations to be completed (Jervis and Kidd, 1993). Several overlapping generations occur throughout the summer and the leafhopper can overwinter on weeds such as chickweed (MacGill, 1932). All growth stages may be found on a crop at any given time (Fox-Wilson, 1938).

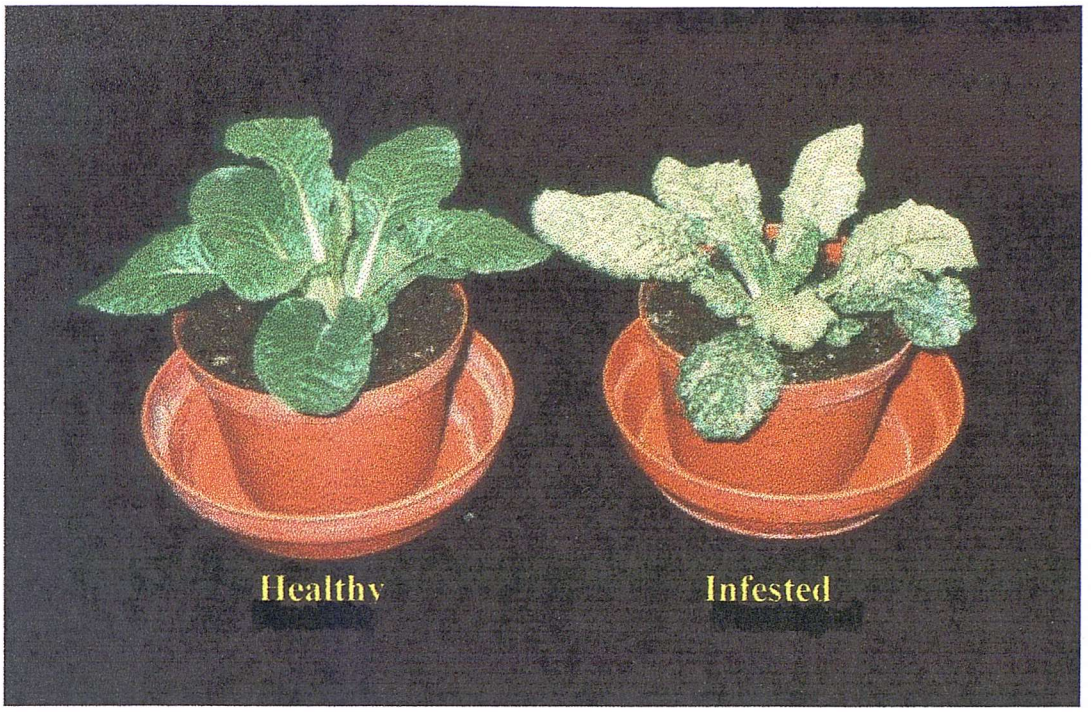


Plate 1.11: A healthy and a *Hauptidia maroccana* infested *Primula* plant.

1.4 Parasitoid

1.4.1 Mymaridae and the Genus *Anagrus*

The family Mymaridae contains 1,300 species in 89 genera, including some of the smallest known wasps; hence their common name of fairy flies (Cronin and Strong, 1990). Mymarids are distinctive in appearance, having slender bodies and long wings that are fringed with setae (Huber, 1986).

The hosts of mymarids are primarily Homoptera; others include Odonata, Lepidoptera, Coleoptera, Neuroptera, Heteroptera and Psocoptera (Clausen, 1940; Cronin and Strong, 1990). They are true egg parasitoids, attacking the host egg before the embryo has developed to any significant extent (Waloff and Jervis, 1987). As egg parasitoids, mymarids may be described as idiobionts laying eggs into a non-feeding host stage (Jervis and Copland, 1996). *Anagrus* represents one of the largest mymarid genera (Waloff and Jervis, 1987). They are able to parasitize a broad range of species in the leafhopper genera. However, different species of *Anagrus* are likely to have some kind of

preference not only for their leafhopper hosts but also possibly even a stronger preference to the host plant (Triapitsyn, 1998).

Reproduction is generally arrhenotokous i.e. diploid females developing sexually from fertilised eggs and haploid males developing pathenogenetically from unfertilised eggs *Anagrus* species are pro-ovigenic but with inter and intraspecific variation in potential lifetime fecundity.

Males emerge before females but stay near the emergence site to mate (Waloff and Jervis, 1987). In all *Anagrus* species studied, mating takes place immediately after emergence. The female releases a species-specific pheromone, which attracts the male. The pattern of oviposition is qualitatively the same for all species of *Anagrus*. The majority of the eggs laid throughout a female's lifetime are deposited within the first 2 days after emergence (Cronin and Strong, 1990).

There may be up to 5 generations per year with overwintering as pupae or prepupae; it is unknown whether these stages undergo a period of diapause. Increased temperature greatly reduces the longevity of the adult stage. Narrow host ranges are typical; ovipositor length and olfactory stimuli are thought to determine this range. The foraging behaviour of mymarids is characterised by walking quickly over plant surfaces and drumming with their club-like antennae (Waloff and Jervis, 1987).

1.4.2 *Anagrus atomus* (Linnaeus)

Anagrus atomus is an egg parasitoid of leafhopper species including the commercially important glasshouse leafhopper *Hauptidia maroccana*. It is generally believed that *A. atomus* represent a species complex but seperation in to different species has so far not been attempted. Biology of a native species of mymarid wasp as described by MacGill (1934) is mentioned below.

The general adult appearance is typical of a mymarid. Adults are approximately 0.6 mm in length as are the forewings i.e. the width of the insect with wings extended is about the same as its total length (Plate 1.12 and 1.13).

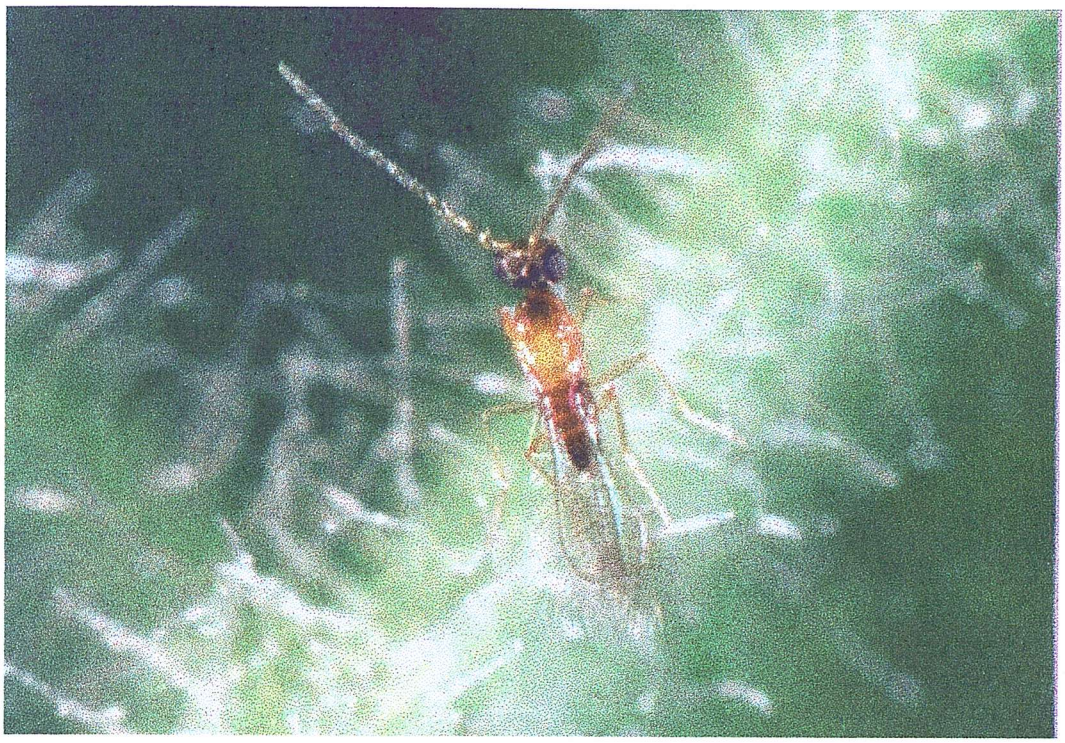


Plate 1.12: An adult male of *Anagrus atomus* (x 75).

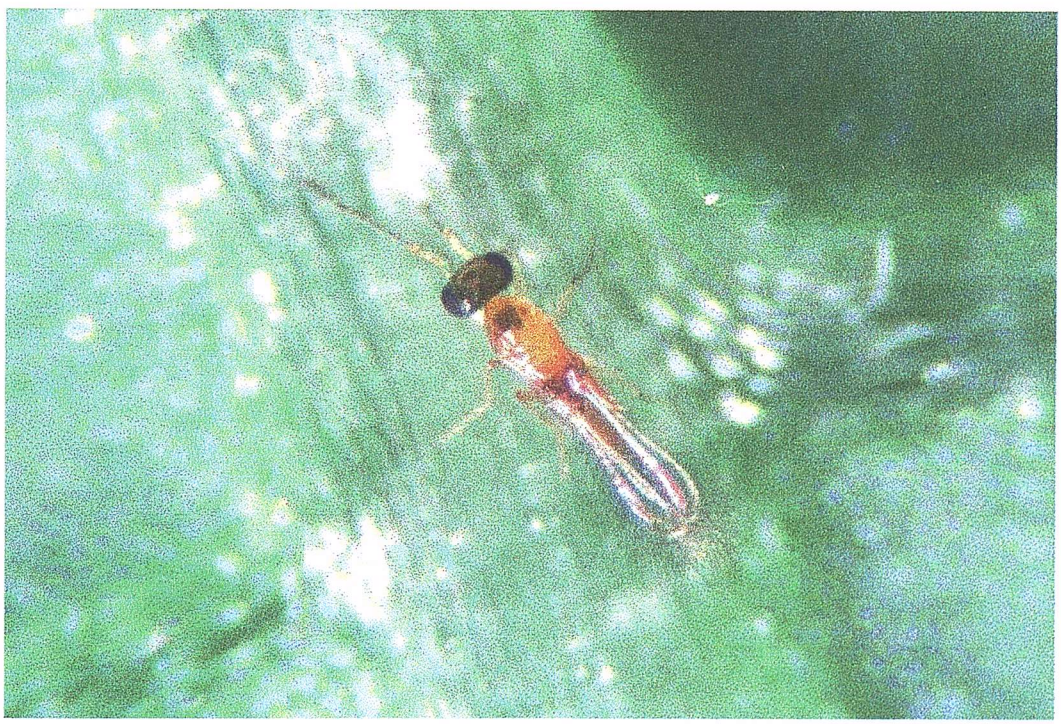


Plate 1.13: An adult female of *Anagrus atomus* (x 75).

The head is characterised by black eyes, which have a slight keel along the inner margin. Female antennae have nine segments, shorter than the body with the bases widely separated. The scape of the antennae is twice as long as the pedicel while the first funicular segment is very short. The last antennal segment is much enlarged giving the antennae a club like appearance. The male antennae have 13 segments, the scape is only slightly longer than the pedicel and the first segment of the funicle is not significantly smaller than the other segments. The final segment of the antennae is pointed at the tip, not club like.

The metathorax is prolonged backwards over the first segment of the abdomen and bears a stout curved seta at its posterior end. The posterior tibiae have an apical plumose spur. The forewings are bent so that the apex is directed towards the head, the setae on the distal half of the wing are very long. The surface of the wing has short setae distributed over it. The hind wings are linear with long strong setae on the posterior margin; the setae on the anterior margin are not so strong.

The abdomen is broadly sessile with the ovipositor emerging from the posterior end of the third segment on the ventral side, the end of the ovipositor being just visible beyond the apex of the abdomen. The ovipositor is composed of two straight blades serrated at the tip and is enclosed within a sheath when not in use. Apart from the genitalia the main difference between the sexes are the antennae.

1.4.3 Biology of *Anagrus atomus*

Oviposition takes place shortly after emergence from the pupae. The male starts mating immediately after emergence. Host eggs appear to be detected by drumming of the antennae on the leaf surface. An egg may be examined several times before the female raises its body and inserts its ovipositor at a right angle to the egg. The egg laid is oval, about 0.12 mm x 0.06 mm, with a narrow stalk about half as long as the egg at one end. Oviposition takes approximately 3 minutes. More than one egg may be laid per host egg i.e. superparasitism, although all the eggs may hatch only one adult emerges from each

parasitised host egg. The first larval stage is transparent apart from a pair of large curved sclerotised mandibles (typical of protopod type larvae). There are six bosdy segments including the last segment, which is much larger and composed of undifferentiated segments. The head bears a pair of unknown ventral appendages; it is possible that these have some sensory role possibly corresponding to antennae. In addition there is a pair of stout ear shaped appendages on the abdomen becoming gradually smaller as the larvae develops. This stage is immobile, therefore food and oxygen uptake is probably by diffusion (Clausen, 1940).

Later instar larvae are 0.7mm in length, typically bright red in colour (allowing easy recognition of parasitised leafhopper eggs) and contain large amounts of fat. This is an active stage producing wiggling, rolling etc. motion in order to circulate the contents of the host egg to aid feeding and gaseous exchange (Waloff and Jervis, 1987).

The pupae are slightly smaller than the mature larvae and are straight not curved. The pupae become darker as the insect takes on the adult form, the eyes are red at first and can be seen through the chorion of the host egg. The adult emerges by cutting a neat hole at one end of the egg with its mandibles.

The complete life cycle takes 14.1 days from egg to adult at 26°C with the green leafhopper (*Empoasca decipiens*) as the host. The thermal constant is 252-day degrees with a lower threshold of 4°C. The red second larval stage can be seen after 7-8 days with the adult emerging 7-10 days after this. Adult longevity is 5 days at 26°C (Jervis and Kidd, 1996a).

1.5 Insects and host plant relationship

1.5.1 Host plant morphological features and insect

The temporal and spatial diversity of host plants represent a variable microhabitat setting a selective pressure to the inhabiting fauna on the plant (Schultz, 1983). Similarly, these morphological and chemical variations between plant species can bring about changes in the insect population (Edmunds and Alstad, 1978).

Plant surface plays an important role in changing the behaviour of insect pests. The leaves of many plants are covered by short hair, or trichomes and these may impede insect movement, feedings and oviposition on a particular plant or leaf. In both natural and agro-ecosystems, the trichomes of several plant species are known to contribute to defence against many pests. Thus trichomes can be an important factor in plant resistance to insects (Gregory *et al.*, 1986). Many authors have highlighted the importance of glandular trichomes in the resistance mechanisms of various commercially grown crops such as tomato, potato and alfalfa. Gibson and Turner (1977) showed the resistance mechanisms of glandular trichomes of *Solanum berthaultii* Hawkes towards *Myzus persicae* (Sulzer). Variation in trichome type, structure and density occurs in many plant species and can have positive, negative or no effects on insect plant relationship of herbivores colonizing a specific plant species (Webster, 1975). Hoffman and McEvoy (1985) showed that the feeding of spittle bug *Philaenus spumaria* was greatly reduced in the early instar if the plant was hairy, but the bug readily fed on the plants when the hairs were shaved off. Therefore this evidence suggests that trichomes can influence not only the movement of insects on the surface of plants, but also their feeding to a large extent (Lyman and Cardona, 1982).

Plant surface characteristics may affect parasitoid movement by altering walking speeds (Hulspas-Jordaan and van Lenteren, 1978), affecting searching rate (Keller, 1987), and reducing adult survival (Obrycki and Tauber, 1984). Treacy *et al.* (1984) mentioned that the rate of parasitization by *Trichogramma pretiosum* Riley varies according to the leaf trichome density on cotton, suggesting that trichomes affect the walking speed of female. Differences in leaf surface morphology and chemistry can potentially cause large variation in parasitization rates of eggs associated with different plant species.

Gardner and Dixon (1985) also found that plant structure can influence the foraging behaviour of the aphidiid *Aphidius rhopalosiphi* (DeStefani-Perez), which was found to parasitize less successfully on aphids feeding on the ears of wheat than on the leaves. This was because the grains protected the aphids from parasitoid attack and also because the parasitoids were unable to search intensively on ears due to the presence of hairs. Keller (1987) indicated that *Trichogramma exiguum* Pinto and Planter walked faster on maize, soybean and tomato but slowest on woolly melon leaves because of the presence of trichomes.

The thickness and texture of the bark of the plant is particularly important to some Hemiptera and can be a formidable barrier for the insect to pierce and locate the vascular bundles. Undoubtedly the plant surface structures play an important role in sap sucking insects (Moran and Southwood, 1982; Southwood, 1987). Phloem feeders were found to prefer and aggregate in succulent plants where vascular bundles are easily accessible (Gibson, 1972; Kidd, 1976).

Most plant species produce secondary metabolites, which contribute in significant ways to the fitness of those plant species that accumulate them. Terpenoids are the most common secondary metabolite contributing to differential insect behaviour (Harrewijn *et al.*, 1995). Sometimes, some of the insect species synthesize terpenoids, which act as defences against predators (Hallhan *et al.*, 2000). Aphids have the capacity to synthesize cyclopentanoid nepetalactones, which are the constituents of the sex pheromone and act as attractants for aphid parasitoids (Poppy *et al.*, 1997; Powell *et al.*, 1998).

1.5.2 Host plant effects on insect development

Plants can possess some properties that enable a plant to avoid, tolerate or recover from insect injuries that would cause greater damage to other plants of the same species under similar environmental condition, termed as host-plant resistance (Kogan, 1975). Three mechanisms of plant resistance to insects are termed antixenosis, antibiosis and tolerence. Antixenosis describes the inability of a plant to serve as a host to an insect; antibiosis is the mechanism that describes the negative effects of a resistant plant on the biology of an insect (eg. adverse effect on development, reproduction and survival); tolerence is the degree to which a plant can support an insect population that under similar conditions would severely damage a susceptible plant (Dent, 1991). However, the three mechanisms of resistance to insects do provide a useful basis for exploring the population dynamic consequences of resistance, because they can be related to particular life-history parameters of insect pests, such as survival, growth, fecundity and oviposition (Thomas and Waage, 1996). Among the three mechanisms, antibiosis could potentialy influence pest fecundity, juvenile or adult mortality, or development time. Thus in slow-growing populations, increasing development time or increasing juvenile mortality reduces population increase. Antibiosis, for example, can act directly on those pest stages that feed on the plant. The effects of antibiosis are likely to be cumulative within a generation of a pest such as an aphid which cause damage as nymphs and adults. Thus different plant resistance factors can influence pest biology in different ways, and the effectiveness of individual mechanisms in suppressing pest population growth rate depends critically on the pest life history. Moreover, certain kinds of pests are likely to be more strongly affected by particular resistance mechanisms than others because of the cumulative effects of resistance across generation and the presence of vulnerable stages within generations in the same species (Thomas and Waage, 1996).

Nutritional quality of the plants as well as their morphology, anatomy and phenology also play a vital role in the development of insects, which they feed on. The toxicity of plants is one example of nutritional quality that affects insect development (Way and Murdie, 1965). The protein content, which varies to a great degree in different plant species, is a more limiting factor for insect development. For example, the quantity of protein on a dry weight basis is 4% on reedmace (*Typhis*) and 38% on peanut (*Arachis*) (Southwood, 1972). Thus the variation in the nutritional quality is closely related to the variations in the distribution and abundance of herbivorous insects.

Reports are available on the effect of host plants on insect development. Hsieh *et al.* (1974) reported that the development rate of alfalfa weevil larvae, *Hypera postica* (Gyllenhal) varied with the host plant species. McNewbery *et al.* (1983) and Backwith (1976) reported similar information on the development of the coccid *Icerya seychellarum* (Westw) and Douglas-fir tussock moth *Orgyia pseudotsugata* McDunough respectively.

1.5.3 Host plant effect on insect fecundity and oviposition

Insect oviposition was found to be influenced greatly by the host plant species (Schotzko and O'Keeffe, 1988). They mentioned that the number of eggs laid by pea leaf weevil *Sitona lineatus* (L.), was significantly higher when the weevils fed on pea foliage than on alfalfa or lentil leaves. Al-Munshi *et al.* (1982) reported that *Lygus hesperus* Knight produced significantly more nymphs on green beans and wheat than on mustard and tomato. Low fecundity of *Haltica lythri* Aube was observed after forced feeding on senescent leaves of *Epilobium hirstum* L. (Philips, 1976). However, Beckwith (1976) showed that plant varieties had no influence on the fecundity level of Douglas-fir Tussock moth, *Orgyia pseudotsugata* McDunnough.

The reduced ovipositional rate attributes to poor quality of host plant and this demonstrates the importance of host plant on the early development of the reproductive system. Many insects exhibited retarded reproductive development when they were placed in adverse environments resulting in low reproductive successe. The importance of physical properties of the plant and the range of factors employed in decision making by the insect, is well illustrated in a study of oviposition behaviour by the onion fly *Delia antiqua* Meigen (Harris and Miller, 1984). They mentioned that the fecundity was influenced by the colour, shape and orientation of the host. Yellow leaves induced more oviposition than leaves of the other colours, tall narrow leaves were more effective than any other shape and upright leaves than those at any other angle.

1.6 Temperature and insect development

Temperature is one of the most important environmental factors that influence the rate of development of a particular insect pest. Temperature is known to be the pre-eminent determinant of poikilotherm developmental rate (Andrewartha and Birch, 1954; Kitching, 1977). The relationship between temperature and the rate of development of poikilotherms is an important aspect of ecological studies and basic to the development of a pest management programme.

Different species, or even populations of a species have different temperature ranges, however, all conform to the following basic model (Campbell *et al.*, 1974). In Figure1.2, the relationship can be represented by a straight line within temperature range in B. The linear response of development over this range is not understood as enzymes, which should influence the rate of development increase exponentially not linearly with increasing temperature (Jervis and Copland, 1996). This linear trend is a more common phenomenon in laboratory studies at constant temperatures. There may be a problem in relating the results of constant temperature studies to the fluctuating conditions in the field where the insects are employed. There is no firm evidence to indicate that the development rate at fluctuating temperatures is not closely equivalent to that of a corresponding constant temperature (Howe, 1967).

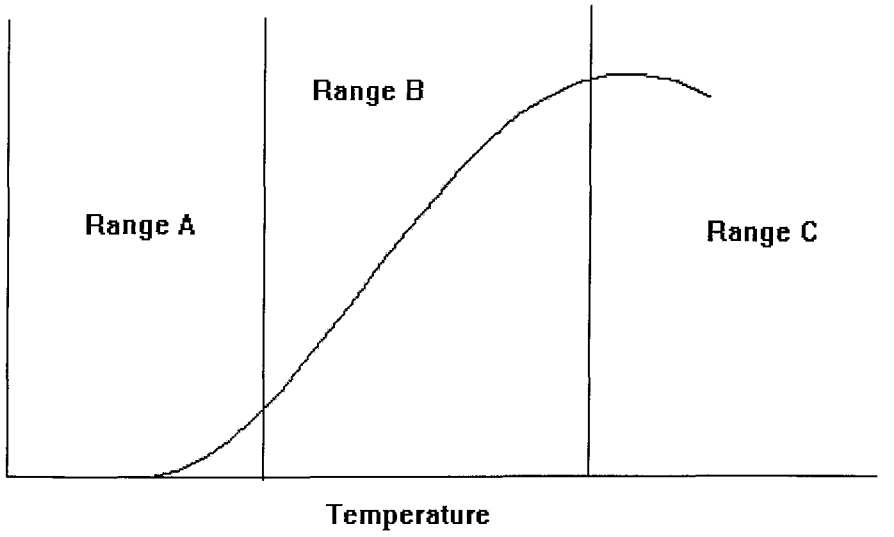


Figure 1.2 **The relationship between the rate of development and temperature, showing the nonlinear portion A, C and the linear portion B, used to estimate the threshold of development by extrapolation (after Campbell *et al.*, 1974)**

The deleterious effect of high temperature depends on how long the insect is exposed to it. Campbell *et al.* (1974) pointed out that the deleterious effect of high temperature occurs only if the temperature is held constant in the range C or fluctuates about an average value within the range. If the temperature fluctuates about a daily average in the range B and the daily maximum reaches range C, no deleterious effect is observed. It is not practical to observe the rate of development at temperature in the range A, close to the lower threshold, because of the considerable insect mortality and time involved.

Several functions may be used to describe the relationship between temperature and development of poikilotherm species (Wagner *et al.*, 1984). Degree-day models based on simple linear regressions of development rate and temperature are used widely for predicting and forecasting insect development within the range of temperatures typically encountered in the field (Campbell *et al.*, 1974). The duration of development is calculated by adding up the number of thermal units (e.g., degree hours or degree-days) contributed at each temperature. This method is widely used because it requires minimal data for formulation, is easy to calculate and apply, and often yields approximately correct values (Eckenrode and Chapman, 1972; Obrychi and Tauber, 1981).

The typical relationship between temperature and the rate of development can be represented by linear regression equation of the form $\mathbf{y = a+bT}$ where, y is the rate of development (reciprocal of development time) at temperature T, a and b are constants. The thermal constant (K) or the total quantity of thermal energy required to complete the development is calculated from the reciprocal of the slope of the regression line i,e, $K = 1/b$ (Jervis and Copland, 1996). The lower threshold or the developmental zero below which no development occurs can be calculated by extrapolating the straight line back so that it crosses the y-axis i,e. $t = -a/b$ (Campbell *et al.*, 1974). Similarly, there is also an upper threshold above which, development is retarded, metabolic process interrupted and subsequent death of organisms occur (Sharpe *et al.* 1977). These temperature thresholds may be used to predict an insect's distribution and abundance.

However, the linear approximation has been criticised for two reasons. the assumed relationship is known to hold for an approximation of the median temperature range only and the lower temperature threshold, on which the model is based is

determined theoretically by extrapolation of the linear line i.e. ignoring any possible curve (Jervis and Copland, 1996). This model has been found to overestimate development rates close to the lower threshold and underestimate higher temperatures.

At the lower thermal limit the development rate curve asymptotically approaches the point of zero development because insects often survive for long periods at cold temperatures with little or no development. For this reason, the temperature at which development first occurs (i.e. the threshold) is difficult to measure accurately. As temperature increases from the lower limit, development rates become proportional to temperature, and a linear response curve results in the mid region. Development begins to slow as the optimal temperature (i.e. the temperature associated with the fastest rate) is reached; therefore development falls off sharply here after.

In general the method used to predict development under fluctuating conditions should be selected to match the accuracy of actual temperature readings (Howe, 1967). Polynomial regression analysis was found to give an excellent, empirical description of development rates at different constant temperatures for the McDaniel spider mite (Tanigoshi *et al.*, 1975). In view of the curvilinear nature of the temperature-development relationship, it was felt that polynomial regression analysis y= a+bx+cx2+dx3+..... might give a more accurate basis for thermal simulation than the straight line used in the day degree concept (Jervis and Copland, 1996). Development rates of three encyrtid mealybug parasitoids, *Anagyrus pseudococcidae* (Girault), *Leptomastix dactylopii* Howard and *Leptomastidea abnormalis* (Girault) were examined under constant, cycling and glasshouse temperatures and the use of polynomial regression was found to better cope with extreme temperature than day-degrees (Tingle and Copland, 1988).

Where extended periods at extreme temperatures are involved the day-degree method under-predicts at high temperature, and over-predicts at low temperatures. This occurs due to the variation of the temperature/development relationship from the linear at extreme temperatures. The extent of the errors incurred here is dependent on the proportion of the temperature/development relationship selected for linear regression analysis. The polynomial method however, remains reasonably consistent under all conditions and should thus prove useful for predicting development in temperate

glasshouses, where night temperature during winter and midday temperatures during the summer reach opposite extremes (Tingle and Copland, 1988).

1.7 Parasitoids' dietary requirements

Many parasitic insects differ from other parasitic organisms in that the adults of parasitic insects are free living, a limited number of progeny develop per host and the host does not survive the encounter. These parasitic insects have been referred to as parasitoids, which may live externally (ectoparasitoids) or internally (endoparasitoids). Over the past decade, parasitoid feeding behaviour has become a focus of attention not only of biological control workers (Kidd and Jervis 1989; Jervis *et al.*, 1996), but also of behavioural ecologists and population biologists (Heimpel and Collier 1996; Jervis and Kidd ,1996b; Jervis *et al.*, 1993, 1996). In many parasitoid-host systems, parasitoid species have been found to use the same host individuals for both feeding and oviposition, termed as `concurrent' host feeding, which usually does not cause the host to die (i.e. is `non-destructive'). However, the majority of host feeding parasitoids use different host individuals for feeding and oviposition. This behaviour is termed `non-concurrent' host feeding, and usually does cause the host to die (i.e., is`destructive') (Jervis and Kidd, 1986; Jervis *et al.*, 1996).

Host feeding leads to a clear cost in terms of lost reproductive opportunity, but also yields two major potential benefits; increased fecundity and longevity. It has long been known that nutrients obtained by host feeding are used to mature eggs (Jervis and Kidd, 1986; van Lenteren *et al.*, 1987; Collier, 1995). Indirect evidence of a connection between host feeding and egg production can be found in studies that show a positive correlation between the amount of host feeding and fecundity (van Lenteren *et al.*, 1987; Jervis and Kidd, 1991).

Pro-ovigenic parasitoids emerge with their full lifetime complement of eggs and therefore do not require materials for egg production. However, they are likely to require carbohydrate to supply their maintenance requirements. Synovigenic parasitoids mature eggs during adult life, so they require materials for egg production as well as maintenance (Jervis and Kidd 1986; van Lenteren *et al.*, 1987; Jervis *et al.*, 1996). Without exception,

parasitoid species known to host feed are 'synovigenic' (Jervis and Kidd, 1986) although some synovigenic species do not host feed (Donaldson and Walter, 1988; Volkl and Mackauer, 1990). In contrast, there are no known cases of host feeding by parasitoids that are pro-ovigenic.

In laboratory experiments carried out on a wide variety of species, females given sugar-rich non-host foods were usually found to be considerably more fecund and longer lived than adults that were given water and no food (van Lenteren *et al.*, 1987). However, fecundity and/or longevity of insects given certain honeydews were not as high as in insects given other non-host foods (Leius, 1961; Avidov *et al.*, 1970). A depressive effect of honeydew on longevity and fecundity was also recorded in females that were able to feed on hosts (Leius, 1961). These negative effects are possibly attributable to the presence of harmful oligosaccharides such as melezitose (Leius 1961; Avidov *et al.*, 1970; Foster and Ruesink 1974; Lim, 1982; Idoine and Ferro 1988). Maternal diet is another factor that may affect a female's offspring sex ratio. Female *Bracon hebetor* (Say) feeding on hosts and honey produce significantly higher male offspring sex ratios than do females feeding only on hosts, may be due to the increased longevity of females given honey: in this species, older females produce exclusively sons (Rotary and Gerling, 1973).

1.8 Host selection by insect parasitoids

Host selection has been viewed as a sequence of behavioural responses to environmental and habitat cues which bring the adult female parasitoids into an appropriate habitat and from there into contact with the host (Bailey and Ridsdill-Smith, 1991). In a given host-parasitoid interaction, the age of the host, the rearing temperature and the host plant are among several factors that may influence the frequency of encapsulation responses by the host (Blumberg and Debach, 1981; Blumberg, 1988). A number of steps are necessary for the success of the parasitoid-host relationship, e.g. (a) host habitat location, (b) host location, (c) host acceptance, and (d) host suitability and (e) host regulation (Vinson, 1975). Once the host is located and then encountered, female parasitoids respond to physical and chemical stimuli associated with the host.

The size, shape, movement, sound, surface texture, and external and internal chemicals of an exposed host all or in part provide stimuli influencing host acceptance by a parasitoid (Boethel and Eikenbary, 1979). Although plant shape and colour can play an important role in host selection (Vinson, 1985; Wackers *et al.*, 1998), volatiles emanating from plants play a critical role (Vinson, 1998). Vinson (1976) mentioned the importance of plant volatiles emanating from the host's food or food plant and food odours in host habitat location for a number of hymenopterous parasitoids as well as several of the dipterans. Parasitoids receive these stimuli via receptors on their antennae, tarsi and ovipositors (Vinson, 1976). However, it is clear that parasitoids are preferentially attracted to damaged plants, and there are many examples demonstrating the significantly enhanced response of female parasitoids to volatiles released by herbivore-damaged plants (Vinson and Williams, 1991; Boeve *et al.*, 1996; Turlings *et al.*, 1998).

Knowledge of the host selection mechanism is indispensable for the efficient use of parasitoids both for mass rearing and biological control of pests. Nutritional suitability and environmental conditions of the host often have a profound effect on sex ratios, size, developmental time, fecundity and longevity of the parasitoid (Vinson and Iwantsch, 1980a).

1.8.1 Effect of host stage on oviposition preference

Host quality is one of many factors influencing the oviposition decision by insect parasitoids. Decision making by the ovipositing female wasps are determined largely by the competing demands of parents and offspring fitness (Harvey *et al.,* 1998). Hosts of different size may differ in their profitability for a parasitoid. Therefore, choice of host may reflect interaction among host size, handling time, clutch size, sex ratio and offspring size, development time and survival of the parasitoid. Encounter rate, handling time, success of attack, developmental time, size and probability of survival of offspring may all depend on host size (de Jong and van Alphen, 1989; Sait *et al.*, 1997; Heinz, 1998). Once parasitoid oviposition has occurred, the fate of the progeny depends upon the food quality of the host, whether or not the host insect rejects the parasitoid egg or larvae or the presence of host pathogens (Vinson and Iwantsch, 1980b).

To maximise the success of parasitoids, the host should be at a suitable stage or age for a successful parasitoid attack, their survival, growth and development, and progeny sex distribution. For example, certain aphid parasitoids attack early instar aphids preferentially because late instar and adult aphids exhibit better defensive behaviours than do early instars (Gerling *et al.*, 1990; Weisser, 1995; Tang and Yokomi, 1996). Many parasitoids preferably attack and oviposit in or on the largest available hosts in which offspring development rate and survival is greatest (Fuester and Taylor, 1991; Sequeira and Mackauer, 1993).

The size of many parasitoids is also related positively to host size (Hardy *et al.*, 1992; Croft and Copland, 1995; Reitz and Adler, 1995; Wen *et al.*, 1996). The larger size benefits the parasitoid by living longer and having the potentiality of laying more eggs than do smaller ones (Hardy *et al.*, 1992; Visser, 1994; Lampson *et al.*, 1996).

1.8.2 Effect of host stage and size on parasitoid sex ratio

Progeny sex ratio is an integral part of the host selection behaviour. Host quality has an important effect on the fitness of the ovipositing females with haplo-diploid sex determination. Most Hymenoptera exhibit arrhenotokous parthenogenesis in which males develop from unfertilized haploid eggs and females from fertilized diploid eggs. This haplo-diploid sex determination mechanism allows the insect to precisely control the sex of their offspring by fertilizing or not fertilizing eggs as they are laid (Charnov, 1982).

Waage (1982) and King (1993) suggested that size dependent sex allocation is unlikely to occur in koinobiont parasitoids, which lay eggs in growing hosts, because at oviposition a female wasp is unable to determine the eventual size of a host. On the other hand, in gregarious idiobiont species that attack hosts in various instars, smaller hosts often receive single male eggs; mid sized hosts, single female eggs; and large hosts may receive several eggs (van Driesche and Bellows, 1996; Heinz, 1998). The model of King (1989) predicts that a greater proportion of female eggs will be laid in large than in small hosts by maternal manipulation of offspring sex ratio. Several studies have shown that female (fertilized) eggs are oviposited more often on large hosts, and male (unfertilized) eggs are oviposited more often on small hosts (Tillman and Cate, 1993; Croft and Copland, 1995; Urano and Hijii, 1995, Wen *et al.*, 1995; Lampson *et al.*, 1996; Bertschy

et al., 2000). Egg parasitoids like *Trichogramma* sp. and *Uscana lariophaga* Steffan, can manipulate the sex ratio of their offspring (van Huis and Rooy, 1998).

As the female parasitoids are larger than males in most species, the former may have greater energy requirements, which can be met in larger hosts. Therefore, it is selectively advantageous for some species to produce a male egg if the host is small or a female egg if the host is large. This may be explained by differential fitness consequences: parasitoid size is positively correlated with the host size, and female fitness may increase more strongly with size than males (Charnov, 1982; King, 1993).

A positive relationship between parasitoid size and longevity and fecundity of females has also been reported extensively (Visser, 1994; Croft and Copland, 1995; Reitz and Adler, 1995; Urano and Hijii, 1995; West *et al.*, 1996).

1.8.3 Effect of host stage or size on parasitoid survival and development

The fitness of the progeny of any parasitoid is generally measured by development time, larval and progeny survival, progeny size, mating ability, fecundity and progeny sex ratio (Pandey and Singh, 1999). The successful development of the parasitoid into an adult depends on the suitability of the host. Host size and quality regulates the development of immature stages and eventually affects the development and successful emergence. Therefore, the costs associated with parasitism may vary between the instars of the same host and some stages are more profitable in terms of fitness than others (Godfray, 1994). Such costs may involve increased mortality of the hosts or parasitoids, lengthening development time and reduced parasitoid size and fecundity (Sait *et al.*, 1997). In the aphid parasitoid, *Lysiphlebia mirzai* Suja-Uddin, females developed faster in large hosts (Mackauer, 1986 and Srivastava and Singh, 1995).

1.9 Factors affecting parasitism

1.9.1 Effects of temperature on parasitism

Knowledge of developmental rates of host and parasitoid is useful in analysis of population level impacts of parasitoids where total numbers entering different stages must be determined from stage frequency data (Bellows and Hassel, 1988), or where trends of percentage parasitism are to be interpreted in terms of underlying causal processes such as differential developmental periods of host and parasitoid (van Driesche and Bellows, 1996). For poikilothermal organisms, basic information on development and reproduction characteristics as affected by temperature and host plant are needed for estimates of parameters that are used in population models (Gutierrez *et al.*, 1977). Temperature is the most important factor affecting development in poikilotherm organisms (Sharpe and DeMichele, 1977). When the rate of development (reciprocal of development period) is plotted against temperature, the result is usually a sigmoid curve with a straight line at the intermediate temperatures (Campbell *et al.*, 1974; Wagner *et al.*, 1984; Higley *et al.*, 1986).

1.9.2 Effects of host plant on insect parasitism

Any attempt to understand the factors involved in population changes of an insect pest would be incomplete if the relationship between plant and insect is excluded. The plant is another environmental factor that may affect host selection in several ways (Vinson, 1976). A parasitoid may orient towards its host's food plant utilising factors different from those used by the host in locating a plant. Thus a host may have a wide range of plants on which it develops, but the parasitoid may only respond to a certain number of these plants but not to another (Clausen, 1941; Smith, 1957; Zwölfer and Krous, 1957). Chemicals appear to play a major role at almost every level of the host selection process.

There is a tritrophic relationship between plants, host and most hymenopterous parasitoids of herbivorous insects (Vet and Dicke, 1992). Plant characteristics such as hairiness, waxes, venation, plant structure and plant chemicals are important factors in determining the quality of a habitat and ultimately foraging strategy of natural enemies (Caster *et al.*, 1984). 'Infochemicals', the chemicals from host and host plants play a

crucial role in the interaction between two individuals. This chemical information can originate from the host food plants, phytophagous host, from host by products, as well as the plant-insect complex (Zhang *et al.*, 1998). These chemicals regulate the foraging behaviour of parasitoid (Turling *et al.*, 1991). Moreover, herbivore-induced plant volatiles are the most reliable indicators of the presence of a host (Nordlund *et al.*, 1977; Dicke, 1994; Mattiacci *et al.*, 1994) and attract the natural enemies of herbivores (Agelopoulos and Keller, 1994a, 1994b; Takabayashi *et al.*, 1994; Potting, 1995). Vinson (1999) summarised the tritrophic relationship of the interaction between the plant and parasitoid, the plant and the herbivore, and the parasitoid and herbivore (Figure 1.3).

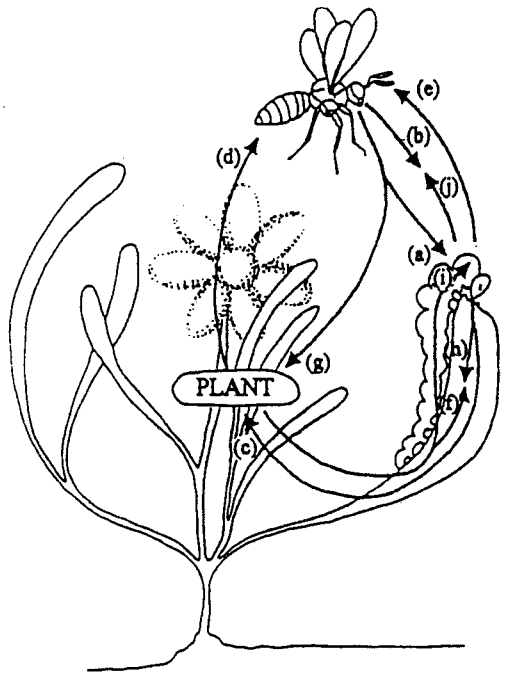


Figure 1.3 **The tritrophic relationship between plant, herbivore and parasitoid where the herbivore depends on the factors from the plant (f) that include specialist herbivore attractants, specific nutrients, precursor compounds, and sometimes even secondary plant-defensive compounds. The herbivore develops some resistance or tolerance (h) to the plant defensive compounds and induces the plant (c) to release parasitoid attractants (d) and increase internal defensive compounds (f). The attracted parasitoid attack (a) the herbivores which benefits the plant (g), and the progeny of the parasitoid are able to develop to adults (e) because the plant defensive compounds are capable of being rendered ineffective (i) against the parasitoid. Although herbivores can defend themselves against parasitoids (j), the parasitoid can counter (b) but do not locate and attack all herbivores because host marking ensures that all herbivores are not eliminated (after Vinson, 1999).**

Parasitoid searching time, pattern and efficiency are governed by the host plant characteristics and by the parasitoid's innate behavioural pattern. Tingle and Copland (1988) noticed that the degree of parasitism varies with the plant species. The searching efficiency is affected by the length, density and alignment (erect, procumbent, etc.) of the leaf pubescens. Trichome diversity in plants is well known and the function of plant hairs is manifold: i) climbing, ii) shedding water, iii) preventing dessication and reducing transpiration, iv) providing optical qualities and v) hampering and trapping insects (Jeffree, 1986). Insects may be trapped by the sticky exude of glandular trichomes, leading to slower walking speed and poor encounter rates. Vertically oriented long trichomes impair normal searching pattern and sometimes irritate the insect leading to it abandoning the surface and flying away from the prey. Secondary plant chemicals may also influence the searching behaviour of natural enemies.

1.10 Sex determination in insect parasitoids

The most common mode of reproduction in hymenopteran parasitoids is arrhenotoky, where male progeny develop parthenogenetically from the unfertilized (haploid) eggs and females from the fertilized (diploid) eggs (Jervis and Copland, 1996). The other forms of reproduction are thelytoky and deuterotoky. In thelytoky, unfertilized eggs give rise to diploid females. But with deuterotoky, occasional males may be produced (Jervis and Copland, 1996). These males are produced when the maternal females have been exposed to high temperature, but are usually considered as nonfunctional. In some cases they are not only capable of mating but also able to pass on their genes to progeny, which reproduce thelytokously (Jervis and Copland, 1996). Thus the distinction between deuterotoky and thelytoky has become increasingly ambiguous and thelytoky is frequently applied to cases that traditionally have been termed as deuterotoky (Luck *et al.*, 1993).

The study of sex ratio in haplodiploid insects has proved to be one of the most successful areas of experimental evolutionary biology. The haplo-diploid sex determination system of most parasitoid wasps provides females with a means of controlling the offspring sex ratio by adjusting the proportion of fertilized eggs at oviposition (King, 1987).

Females of a given species have a life time optimum sex ratio, many species lay different sex ratios at different times of their lives. Sometimes, fertilized females produce significantly more males soon after mating, and the proportion of females increases gradually thereafter (Quicke, 1997). However, in many and perhaps the majority of species, females can and do lay a high proportion of fertilized eggs immediately after mating, and later in a female parasitoid's life, at least under laboratory conditions females tend to lay an increasing proportion of male eggs (King, 1987; Quicke, 1997). Two different sex ratios are defined in parasitic wasps, the primary sex ratio as the ratio of unfertilized to fertilized eggs laid, and the secondary sex ratio as the sex ratio of emerging adults (Quicke, 1997). Primary sex ratio (male: female) in Hymenoptera can be determined through the females manipulating fertilization of their eggs or changes in sperm availability. Furthermore, secondary sex ratio may differ from the primary due to differential survival of the sexes in small or superparasitized hosts (Charnov, 1982; Waage, 1986).

1.10.1 Evolutionary models on sex ratio

Sex allocation theory deals with the impact of natural selection on the allocation of resources to male compared to female reproductive function. This allocation to male versus female function can often be shown to maximize the fitness gains through male function multiplied by the gain through female function. From this product, the classical result of sex ratio theory in populations of parasitoid wasps can be derived, such as 'the equal resource into each sex' of Fisher (1930) and a variable allocation of reproductive resources, biased to one sex or the other (Hamilton, 1967; Taylor, 1981; Charnov *et al.*, 1981).

Fisher (1930) stated the general principle that a random mating, sexually-reproducing population should devote equal reproductive resources to the production of male and female offspring. Thus the sex ratio in the population is in equilibrium (usually implying 50:50 primary sex ratio) when the totals of effort spent producing the two sexes are equal. If the totals are not equal, a fitness advantage will be awarded to parents producing the other sex, which over time will tend towards equality (van Driesche and Bellows, 1996).

The evolutionary models which provide potential explanations of adaptive primary sex ratio in parasitic Hymenoptera includes Local Mate Competition theory (LMC) (Hamilton, 1967, 1979) and host size distribution models (Charnov, 1979, Charnov *et al.*, 1981).

In Hamilton's (1967) model, three factors are proposed as the cause of sex ratio bias: local mate competition, sib mating and inbreeding. LMC predicts that in a system where parasitoids occur in small mating populations with low male dispersal, female biased sex ratios should evolve to reduce local competition among male siblings. In patches in which only one female oviposits, only as many males should be produced as are physically needed to mate all sisters. In practice, parasitoids may use particular sequences of sex allocation in order to ensure the presence of males in each host patch (van Driesche and Bellows, 1996). In strictly solitary species, for instance, progeny allocation becomes the crucial variable. At the other extreme, in thelytokous (all female) species sex allocation is constant and progeny allocation is the important variable. Certainly the most complex reproductive strategy arises in parasitoids which are gregarious and arrhenotokous, such as *Trichogramma* which can allocate different numbers of offspring to a single unit of resource and which experience different levels of local mate competition (Waage and Ng, 1984).

The host-size distribution models (Charnov, 1979; Charnov *et al.*, 1981) predict that the sex ratios of the progeny emerging from smaller hosts tend to be male-biased and those from larger hosts tend to be female-biased. Charnov's model (1979) predicts a threshold host size below which only males and above which only females are laid. It also assumes that the rate of gain of fitness with increasing host size is greater for females than males. Females of idiobiont parasitoids can potentially assess the size of their hosts since they are not growing after parasitization and lay male or female eggs accordingly.

Many factors interfere with the effect of LMC on the sex ratio, e.g. host density, male or female wasp density and superparasitism (Charnov, 1982). LMC declines with increased spacing of the broods, brought about by either decreasing the number of wasps or increasing the number of hosts. Therefore, the proportion of males increases to equality (for a fixed number of wasps) as the number of hosts increases or (for a fixed number of hosts) as the number of wasps decrease (Charnov, 1982). Local mate competition predicts

an extremely female biased sex ratio with few foundresses (small local breeding population) and an equal sex ratio with many foundresses. If sons and daughters are of equal cost, this later situation equilibrates the resource in each sex, rather than the sex ratio itself. Different population structures alter the expected shape of the relation between group size and sex ratio (Charnov, 1982).

In solitary wasps, a change in sex allocation may arise from increased superparasitism by a conspecific, as the second female visiting a host patch is expected to bias the sex ratio of its offspring towards the sex that is the better competitor (van Dijken and Waage, 1987). In gregarious parasitoids, LMC declines if superparasitism occurs because mating then takes place among the broods of two or more females. However, sex ratio is affected mainly because the second female usually lays a smaller clutch than the first one (Charnov, 1982).

1.10.2 Sex ratio distortion in insects

The causes of thelytoky are not well understood, but it is assumed to arise from four possible causes e.g. genetic, interspecific hybridisation gynogenesis and infection with extrachromosomal elements (Luck *et al.*, 1993). Several examples of microbe-induced thelytoky have been reported in insect parasitoids (Stouthamer *et al.*, 1990; Zchori-Fein *et al.*, 1992, 1995; Arakaki *et al.*, 2000).

Many species of insects are the host to some microorganisms that are inherited through the female lines only, and that kill the infected male progeny during embryogenesis. Microorganisms exhibiting this male-killing behaviour are found predominantly in insect species where there was either antagonistic interaction between siblings, or the unhatched eggs were consumed by siblings (Hurst and McVean, 1998). Under these circumstances, male-killing can be an adaptive behaviour of the bacterium, as the death of male hosts (which cannot transmit the bacterium) increases the survival of the sisters of these hosts, which bear the same bacterium, and transmits it into their progeny. Intracellular bacteria of the genus *Wolbachia* have been reported in some hymenopteran species, such as *Trichogramma* (Stouthamer *et al.,* 1990; Pintureau *et al.*, 2000; Silva *et al.*, 2000), *Aphytis* (Zchori-Fein *et al.*, 1995) and *Telenomus sp.* (Arakaki *et al.*, 2000), which induce thelytoky. *Wolbachia* allows these females to produce diploid

daughters parthenogenetically by causing gamete or chromosomal duplication early in the first mitotic division (Stouthamer and Kazmer, 1994).

Microbe-associated thelytoky in many parasitoid species can be treated by feeding the female parents with antibiotics or by heat treatments. Thelytoky was successfully reverted to arrhenotoky by feeding the females with antibiotics (Zchori-Fein *et al.*, 1992, 1995; Arakaki *et al.*, 2000). The thelytokous *Telenomus nawai* Ashmead was reported to produce a considerable proportion of males when exposed to a high temperature (35°C) during the pupal stage (Akaraki *et al.*, 2000). This transition from thelytoky to arrhenotoky might be caused by inactivation or damage to symbiotic microorganisms through exposure to high temperature.

Males obtained by heat treatments in thelytokous species of *Trichogramma* were reported as non-functional and Stouthamer *et al.* (1990) showed that even the males obtained by feeding the females of *Encarsia formosa* Gahan with antibiotics, were not sexually functional (Zchori-Fein *et al.*, 1992). But in some cases, successful induction of male offspring from the uniparental lines of *T. nawai* when treated with Rifampicin produced viable sperms and successfully transferred to the female spermatheca (Akaraki *et al.*, 2000).

1.11 Host discrimination and super parasitism by insect parasitoid

The ability of a parasitoid to distinguish between unparasitized and parasitized hosts is termed host discrimination and allows the attacking parasitoids to avoid exposing their progeny to competition by not ovipositing during an encounter and is common in insect parasitoids (van Lenteren, 1981; van Alphen and Visser, 1990). The behaviour is termed as self-discrimination when a female recognizes hosts previously parasitized by herself, and conspecific-discrimination when it concerns hosts parasitized by another female of the same species (Mackauer, 1990).

Discrimination between parasitized and unparasitized hosts allows a parasitoid to decide to oviposit in or reject parasitized hosts depending on the circumstances (Jacques *et al.*, 1990). Because superparasitism is associated with both costs and benefits, both of which are condition dependent, the benefits exceed the costs when hosts are scarce and

the parasitoid may oviposit in already parasitized hosts. However, when high quality hosts are abundant, the costs exceed the benefits and the parasitoids are then predicted to avoid ovipositing in already parasitized hosts, which might cause possible loss of offspring and waste search time (van Lenteren, 1981).

It is difficult to assess whether individual females superparasitize because of their failure to recognize a previously parasitized host (Ueno, 1999). Host discrimination usually requires some sort of physical contact between parasitoid and host. Antennae may be used to detect the external marking or in some parasitoid wasps, ovipositor insertion is necessary for recognizing prior parasitism. Such internal host recognition was well demonstrated by several authors (van Lenteren, 1981; Mackauer, 1990; Ueno, 1994). Chemosensors located at the tip of the ovipositor may respond during ovipositor insertion to chemical changes associated with parasitism in the host, which can act as internal markers. In contrast, some parasitoid wasps can distinguish hosts that she has previously oviposited or rejected from those that were unparasitized or parasitized by other conspecifics on the basis of external examination of the hosts with their antennae (Ueno and Tanaka, 1996).

The decision of a parasitoid to oviposit in previously parasitized hosts may be influenced by both host availability and quality (van Lenteren, 1981; van Dijken and Waage, 1987), external and internal markers (Chow and Mackuer, 1986), the physiological condition of the parasitoid including the supply of mature eggs (Volkl and Mackuer, 1990; Minkenberg *et al.*, 1992), and the probability of offspring survival (Chow and Mackauer, 1986; Waage, 1986).

Several gregarious parasitoids respond to a parasitized host by depositing mostly male eggs (Werren, 1980; Strand, 1988; King, 1993) or by laying a small clutch of eggs (Strand and Godfray, 1989). In these cases, sex allocation and clutch size can be used to assess whether a superparasitizing female recognizes prior parasitism.

The time interval since the previous oviposition may also influence the parasitoids decision to oviposit in parasitized hosts (Chow and Mackauer, 1986). The external marking of hosts during oviposition may not remain effective for a particular period of time and the female fails to detect the internal marker due to the deterioration in marker

quality during the growth of the parasitoid larva within the host. These external and internal markers may be detectable by both the same individual and by conspecific parasitoids (Volkl and Mackauer, 1990).

In some cases, female parasitoids destroy some eggs of the first female with the ovipositor before laying eggs and this is known as ovicide. This phenomenon is well described by Strand and Godfray (1989). They expected ovicide to occur in ectoparasitoids and found that (a) ovicide is more likely to occur when the time to kill eggs is short, (b) when it becomes increasingly difficult to destroy the next egg, (c) the frequency of ovicide increase with increasing travel time or when an increasing proportion of the hosts are already parasitized and (d) the likelihood of ovicide increases with an increasing competitive advantage of the first clutch.

Ovicide, superparasitism and host rejection are alternative reproductive tactics facing female parasitoid wasps encountering an already parasitized host (Mayhew, 1997). Ovicide is rarely reported but is common from the Pteromalidae, Aphelinidae, Ichneumonidae, Braconidae and Bethylidae (Godfray, 1994). It appears to be most wide spread in Bethylidae, being reported from *Goniozus triangulifer* Kieffer (Legaspi *et al.*, 1987), the aphelinid *Encarsia formosa* Gahan (Netting and Hunter, 2000), and the eupelmid *Eupelmus vuilleti* (Crawford) (van Alebeek *et al.*, 1993).

Mayhew (1997) speculated that ovicide should be favoured in ectoparasitoids with large, easily located eggs that can be found quickly and killed. Ovicide could also occur either in other endo-parasitoids that lay their eggs in a precise location within their hosts, even when the difference in volume between the egg and the host is great (Netting and Hunter, 2000).

Ovicide has interesting implications for understanding the oviposition behaviour of the parasitoids. Females of *Encarsia formosa* do not just assess host quality, but actively improve the quality of the parasitized hosts by eliminating competitors (Netting and Hunter, 2000).

1.12 Life table parameter study of the parasitoid

The construction of life tables is an important component in the understanding of the population dynamics of a species (Southwood, 1978). Several criteria and diverse approaches exist for evaluating and selecting the best agents for controlling a pest (Waage, 1990). The effectiveness of a parasitoid species as a biological control agent is linked to the quality of an individual parasitoid's fitness (Ridgway and Morrison, 1985). The life table enables one to best visualise the relative contributions of survival and reproduction to the mean fitness of a population, so that the more comprehensive the life table, the more accurately can these contributions be described. The key life history traits are fecundity, the age at first reproduction and the timing of peak reproduction, the interaction of reproductive effort with adult mortality and the variation in these traits among an individual's progeny (Stearns, 1976). The age of the female parasitoids may influence their fecundity and sex ratio, and knowledge of sex ratio is important in their use in biological control programmes (Luck and Podoler, 1982; Waage, 1986).

Fecundity can be measured as potential or realised fecundity. The numbers of eggs present in the ovary is a useful conservative estimate of the potential fecundity for many species, particularly for pro-ovigenic insects and termed as egg load. In pro-ovegenic parasitoids, which emerge as adults with full lifetime complement of mature eggs, the egg load is simply a decreasing function of egg laid, and there is thus no distinction between egg-load limitation and fecundity limitation. In contrast, synovegenic parasitoids continue to mature eggs throughout their lifetime, often in conjunction with host feeding, and may thus experience short-term egg load limitation without incurring lifetime fecundity limitation (Lane *et al.*, 1999). The lifetime fecundity or reproductive potential of a parasitoid has frequently been cited in the biological control literature as an important characteristics of successful biological control agents. High fecundity is considered necessary for parasitoids to be able to reproduce more rapidly than the pest population and to respond to changes in the abundance of the pest (Waage and Hessel, 1982; Waage, 1990; Ehler, 1995

Fecundity can be used in assessing a parasitoid's fitness and is usually correlated to the size of the parasitoid (van den Assem *et al.*, 1989; LeMasurier, 1991; Rosenheim

and Rosen, 1992). Female sizes, which may reflect the number of eggs present in the oviduct, can be used as a measure of potential reproductive output of many insects. There are discrepancies in defining the size of an individual (Leather, 1988), depending on whether one uses weight or body length. However, the size of a parasitoid can most easily be estimated by either measuring the head width (Liu, 1985; van den Assem *et al.*, 1989) or the hind tibia length (Heinz and Parella, 1989). As the synovigenic parasitoid emerges with fewer eggs than her full complement, dissection of ovaries at a certain age could only provide an estimate of egg-holding capacity of the parasitoid at that age and may not reflect potential or real fecundity of the parasitoid (Leather, 1988). Life table parameter study can provide the following information:

i) the egg holding capacity of the parasitoid at a certain age as a measure of their potential fecundity at that age;
ii) age specific survival and progeny production pattern of the parasitoids and
iii) the intrinsic rate of population increase (r_m) of the parasitoids under laboratory conditions.

The rate of population increase of insects may be expressed as the intrinsic rate of increase (r_m). The intrinsic rate of increase depends on fecundity, rate of development and survival. Population changes, on the other hand, are caused by many factors including parasitism, predation, food availability and diseases.

Temperature and host plants also play an important role in population growth. Iheagwam (1980) showed that the mean generation time of cabbage white fly *Aleyrodes brassicae* Walker was shorter at a higher temperature than at a lower one. He also mentioned an increased intrinsic rate of increase with increasing temperature. Soultani (1997) showed an increase in development rate of the scale insect *Unaspis euonymi* (Comstock) with increasing temperature. Mean generation time of *Aleyrodes brassicae* was found to be shortened with increasing temperature and at lower temperature below 15°C, there was a very long developmental time coupled with the fact that eggs were laid more slowly early in the life of the insect (Iheagwam, 1980). This supports the conclusion of Birch (1948) as one of the major effects of temperature on the intrinsic rate of increase of insect population.

Temperature simultaneously affects several component variables in the calculation of r_m. An increase in temperature within the limit of observed temperatures, consistently lowers both the survival rate and the oviposition period of *Trialeurodes ricini*, while on the other hand, an increase in temperature increases mean total and mean daily fecundity up to a peak of 30°C. Above 30°C, the acceleration of egg output at the onset of oviposition continues; however at such a high temperature, age specific survival (l_x) declines rapidly and limits the r_m. The lowest r_m values observed can be attributed to a low oviposition rate at 15°C and the high mortality at 35°C (Shishehbor and Brennan 1996).

The size and growth stages also affect the life history parameters of insect parasitoids. The faster development of daughters in large hosts decreases generation time (T), which is inversely related to the intrinsic rate of increase (r_m) of the parasitoid population (Tripathi and Singh, 1990).

Effect of host plants on the life history parameters of insects was reported by many authors (Steenis and El-Khawass, 1995; Drost *et al.*, 1998; Leather *et al.*, 1998). Marked differences in fecundity and adult life span of pine beauty moth *Panolis flammea* Denis and Schiffermüller were attributable to a host plant effect (Leather, 1985; Leather *et al.*, 1998).

Life-tables of an insect can be constructed using the survival data of a specific age class (l_x) and the female offspring produced per female in each age class (m_x). On the basis of l_x and m_x value, the following population parameters were calculated (Messenger, 1964)

a) Gross reproductive rate (*GRR*) is the mean total number of eggs produced by a female over its life time, **($GRR = \Sigma m_x$)** measured in female eggs/female/generation.

b) The net reproductive rate (R_o) is the number of times a population will multiply per generation, **($Ro = \Sigma l_x m_x$)** measured in females/female/generation.

c) Finite rate of increase (λ) is the number of times the population will multiply itself per unit of time, $(\lambda = e\ )$ measured in females/female/day.

Hence the finite rate of increase (λ) is the natural antilogarithm of the intrinsic rate of increase. Therefore, any statement about the rate of increase of a population is incomplete without reference to the age distribution of that population, unless every female in it is producing offspring at the same rate at all ages, and at the same time is exposed to similar chances of dying at all ages (Birch, 1948).

d) The mean generation time (*T*): The comparison of two or more population by means of their net reproductive rates may be quite misleading unless the mean length of the generation is the same.

The relation between numbers and time in a population growing exponentially is given by $N_T = N_0\ e^t$. When T= the mean length of the generation, and then from the definition of net reproductive rate $N_T/N_o = O_r$, hence $O_r = e^{rt.}$ and $T = \log_e O_r/r_m$. measured in days.

It follows that an accurate estimate of the mean length of a generation cannot be obtained until the value of r_m is known. Therefore, for estimation purposes, T may be calculated as

$T = \Sigma x\ l_x\ m_x/\Sigma l_x\ m_x$.

e) The doubling time (*DT*) is the time required for a given population to double its number, $(DT = \log_e 2/r_m)$, measured in days.

f) The capacity for increase (r_c) is an approximation for r_m. It is calculated as follows: $r_c = \log_e R_o/T_c$, where T_c is the cohort generation time, defined as the mean age of maternal parents in the cohort at birth of the female offspring, $Tc = \Sigma l_x\ m_x/R_o$

1.13 Objectives of the study

The present study was initiated to evaluate the potential of *A. atomus* for the biocontrol of *H. maroccana* with emphasis on the following aspects of biology and behaviour of both *H. maroccana* and *A. atomus.*

1) Effect of different constant temperature and important host plants on different biological aspects of the leafhopper, *H. maroccana.*
2) Effect of different constant temperatures and host plants on different biological aspects of the egg parasitoid, *A. atomus.*
3) Host selection behaviour of *A. atomus.*
4) Effect of temperature and host plants on fecundity and life table parameters of *A. atomus.*
5) Effect of different diets on the longevity of adult *A. atomus.*
6) Oviposition behaviour of *A. atomus*
7) Thelytokous reproduction in *A. atomus.*
8) Effect of physical and chemical plant characteristics on the host searching behaviour of *A. atomus* and
9) Host discrimination and superparasitism behaviour of *A. atomus.*

Chapter 2

General materials and methods

2.1 Raising of Plants

The following five plant species were grown inside the cubicles in a glasshouse of Imperial College at Wye to use in different experiments:

1) Cucumber, *Cucumis sativus* L. (var. Bushy dwarf)
2) Tomato, *Lycopersicon esculentum* Mill. (var. Ailsa Craig)
3) French bean, *Phaseolus vulgaris* L. (var. The Prince)
4) Primula, *Primula vulgaris* Huds. (var. mixed)
5) Sweet Pepper, *Capsicum annum* L. (var. Worldbeater)

Organic mixed pastel F1 hybrid Primula plants were collected from a nearby nursery and were grown in plastic pots (12 cm diameter) in the glasshouse. Seeds of Cucumber and French bean were sown directly into the pots (12 cm diameter). Seedlings of Tomato and Sweet pepper were raised in a plastic tray and then individual seedlings were planted in the pots (20 cm diameter) with compost when they were 25 days old. The plants were planted at regular intervals. Regular watering and care was taken to keep the plants healthy and free from other insects.

2.2 Mass culture of the leafhopper *Hauptidia maroccana*

Hauptidia maroccana attacks a wide variety of crops, which includes Sweet pepper, Cucumber, Tomato, Geranium, French bean, Cotton, *Chrysanthemum, Fuchsia, Primula* and *Salvia* (Copland and Soeprapto 1985; MacGill, 1932). Therefore, *H. maroccana* were mass cultured on Primula, French bean, Sweet Pepper, Tomato and on Cucumber plants in an insulated shipping container with a computer controlled environment. Temperature was kept constant at 21°C with 80-85% relative humidity, 16L:8D photoperiod and 4.2 watts/m^2 light intensity.

A plastic tray 500 x 500 x 60 mm (length x width x depth) was lined with capillary matting covered with black polythene sheet to prevent excessive evaporation and condensation. Holes were cut in the plastic sheet to allow the base of the plant pots to

come into contact with the matting. A large Perspex cage (620 x 400 x 540 mm) with door and ventilation areas of polyester gauze, was placed onto the tray over the capillary matting (Plate 2.1). Additional ventilation was provided through a 100 mm diameter opening at the back of the cage covered by 30 mesh/cm polyester cloth. These cages were placed in the container with the above mentioned environmental conditions.

Potted plants of different plant species were placed in each cage. Adult leafhoppers *Hauptidia maroccana* supplied from BIOWISE, West Sussex, UK were released into the cages. Plants with leafhopper eggs were transferred to new cages to become adults to have a large enough population for experimental purposes. Fresh plants were placed in the cages whenever necessary and the plants were watered through the capillary matting as required. This stock served as a continuous supply of leafhopper throughout the experimental period.



Plate 2.1 **Mass culture of *Hauptidia maroccana* inside Perspex cages in the controlled temperature shipping containers.**

2.3 Mass culture of the egg parasitoid *Anagrus atomus*

A mass culture of *A. atomus* was established at 21°C to ensure a continuous supply throughout the study period. Initially about 200 parasitized eggs of *H. maroccana* at the red egg stage were supplied from BIOWISE, West Sussex, UK. The small pieces of leaves containing the parasitized *H. maroccana* eggs were placed into 0.5ml Eppendorf microcentrifuge tubes with a piece of wet filter paper. These tubes were placed in a plastic sandwich box in the incubator in continuous light until adult emergence. The humidity inside the boxes was maintained at 85% r.h. using a saturated KCl salt solution. The tubes were checked everyday for the emergence of adult *A. atomus*. The emerged adults were used to establish a mass culture.

Leafhopper eggs were produced in the leaves by placing Primula plants with fully expanded leaves inside the mass culture of leafhopper. Leaf discs were made from the leaves containing leafhoppers egg and placed on agar (8g/litre) in 50 mm Petri-dish cages, lower surface facing uppermost.

Two freshly emerged mated female *A. atomus* were introduced into each Petri-dish containing the leaf disc. Using a fine tipped painting brush, 50% honey solution was smeared over the leaf disc, which served as food for *A. atomus*. The adult females were allowed to lay eggs for 24 h and then removed from the arena. The Petri-dishes were placed in temperature controlled illuminated incubators at 21°C in continuous light condition to allow the eggs to develop. Ten days after laying eggs by *A. atomus*, leaves were cut into small pieces containing individual parasitized eggs (those which turned red) and the same procedure was followed to produce the adult parasitoids and used in different experiments. The sex of the emerging parasitoids was determined by the shape of the antennae.

2.4 Experimental tools and equipment

2.4.1 Perspex-sandwich cages

The cage was made of Perspex sheet, 75 x 38 x 8 mm (length x width x thickness), with a 20 mm diameter hole in the middle. This was sandwiched between a 75 x 38 mm glass slide and a 3 mm thick, 75 x 38 x 3 mm Perspex sheet with a 10 mm hole in the middle covered with a 50 mesh nylon cloth, all held in place by rubber bands. Leaf discs of 20 mm diameter were placed on agar medium (8g/litre) in the cage (Plate 2.2). These cages were used to study the development of immature stages and the survival of the different growth stages of *H. maroccana*.

2.4.2 Petri-dish cages

These cages were made of 50 to 70 mm diameter Petri-dishes according to the purposes of different experiments. Each dish had a 20 mm diameter hole in the middle of the lid, which was covered by 40-50 mesh nylon cloth to provide ventilation (Plate 2.3). Agar medium was poured into them and a leaf disc of the required host plant was placed over it. These Petri-dish cages were used in different experiments.

2.4.3 Aspirator or pooter

In order to suitably handle the leafhoppers a pooter was constructed using a piece of flexible tube approximately 5 mm in diameter. A second piece of tube approximately 80 mm long and relatively larger in diameter was taken and the first tube was inserted into the second one. A piece of fine mesh nylon cloth was placed inbetween to prevent the insects escaping into the mouth.

2.4.4 Fibre optic lamp

The illumination for the microscope was provided by a fibre optic lamp (Schott cold-light source KL 1500-T), which contains two long easy movable arms. The angle and light intensity could be adjusted. A halogen lamp was used to provide 2.9 to 60 watt/m^2 light with minimum radiating heat.

2.4.5 Micromeasure system

A computer program 'Inchworm' developed at Imperial College at Wye (Varley *et al.,* 1994) was used to study the searching behaviour of the egg parasitoid *A. atomus* on the leaves of different host plants.

2.4.6 Video camera

A JVC video camera fitted with 50 mm SLR macro lens fixed on a stand was used to record the searching behaviour of the egg parasitoid *A. atomus* on the leaves of different host plants.

2.4.7 Microscope

A stereo microscope fitted with a graticule eyepiece (calibrated with a precision scale) was used in the study of the developmental stages of *H. maroccana* and *A. atomus*.

2.4.8 Balance

An electronic balance (Sartorious 4503 MP6) was used to weigh the 1st instar nymphs and the adults of *H. maroccana* to calculate their relative growth rate. This balance records a minimum weight of 0.001 mg.

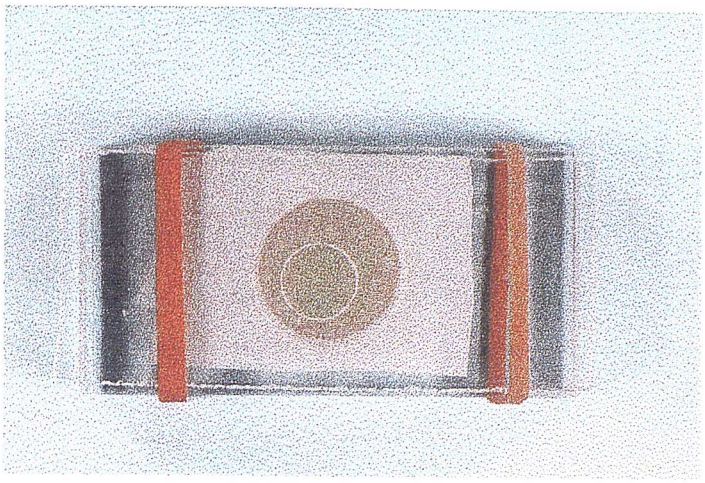


Plate 2.2 **Perspex sandwich cages used in the study of the development of *Hauptidia maroccana*.**

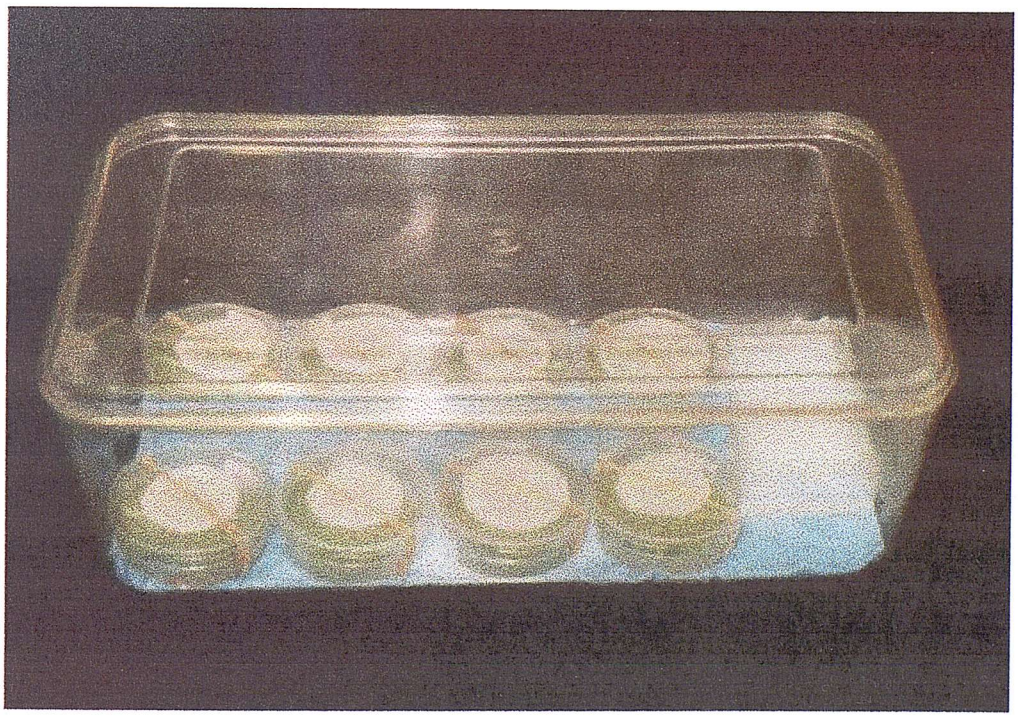


Plate 2.3 **Petri-dish cages used in the study of the development of *Hauptidia maroccana* and *Anagrus atomus*.**

2.4.9 Agar media

The agar medium was used as a source of nutrient as well as a source of moisture for the leaf-discs used in different experiments. Compared to moistened cotton wool and wet filter paper, agar medium supports the leaf disc longer and was more practical for conducting the experiment. The agar media was prepared with 8g Bacteriological agar (No. 1) (Oxoid Ltd.) per litre of distilled water. The solution was autoclaved for 20 minutes at 121°C, at a pressure of 1.4 bar.

2.5 Experimental conditioning

2.5.1 Temperature maintenance

Different temperatures were needed for different sets of experiments. The leafhopper mass culture was maintained in the controlled temperature (CT) containers where the temperature was continuously monitored by a computer. A blower fan was used to ensure uniform conditions inside the container. The fluctuation varied within a range of ±1°C.

Different biological studies on leafhopper were conducted in Gallenkamp Compenstat cooled incubators supplied with control thermostats that enabled control of the temperature and photoperiod to the condition required for each experiment. Lighting in the incubators was supplied by six flourescent tubes, which provided the light intensity of 7-15 W/m^2. Due to the effect of illumination and the presence of a cooling plate, a variation of ±1°C was found in the incubators. A maximum-minimum thermometer was kept inside the incubators to check the variation in temperature.

2.5.2 Relative humidity maintenance

The relative humidity in the CT rooms ranged from 80-85%. In the incubators, the relative humidity for each experiment was maintained at 85% by placing the rearing cages in closed transparent plastic boxes (270 x 150 x 85 mm), with a small box containing saturated KCl salt solution (Greenspan, 1977). The relative humidity was measured using an electronic hygrometer with an accuracy in the region of ±5%.

Chapter 3

Nymphal survival, development, growth rate, fecundity and adult longevity of *Hauptidia maroccana* on different host plants at different constant temperatures

3.1 Introduction

Temperature and host plant play an important role in determining insect population growth. As a poikilotherm animal, the developmental rate of insects depends on the environmental temperature to which they are exposed (Andrewartha and Birch, 1954; Kitching, 1977) and hence, the development threshold and the thermal constant may be useful indicators of an insect's abundance and distribution (Messenger, 1959). The relationship between temperature and the developmental rate can be described using linear or non-linear techniques (Campbell *et al.*, 1974; Wagner *et al.*, 1984). It is usually plotted as a developmental rate (i.e., inverse of development time) against a series of constant temperatures. Developmental rate increases with temperature to a maximum and then decreases as the upper lethal temperature is approached. This relationship is curvilinear at extreme temperatures, but at moderate temperatures it is approximately linear (Campbell *et al.*, 1974). The linear portion of the curve is often used to estimate the development threshold and the day degree requirement of the species, and as such can be used to estimate the number of generations that can develop per unit time. The relationship between temperature and the rate of development of poikilotherms is an important aspect of ecological studies and basic to the development of a pest management programme. To determine the development pattern of insects, the life history parameters are usually studied over a range of temperatures in laboratory condition (Hughes *et al.*, 1984).

Similarly, the nutritional quality of the plants as well as their morphology, anatomy and phenology play a vital role in the development of insects which feed on them (Way and Murdie, 1965). The surface structure of the plants also influences different developmental processes of insects (Moran and Southwood, 1982; Southwood, 1987). Thus the variation in the nutritional quality is closely related to the variations in the distribution and abundance of herbivorous insects. For poikilotherm organisms, basic information on development and reproduction characteristics that are affected by

temperature and host plant are needed for estimates of parameters that are used in population models (Gutierrez *et al.*, 1977). The glasshouse leafhopper was reported to attack a wide range of host plants. However, the role of plant species and different constant temperatures on the developmental rate, temperature threshold, survival and age specific fecundity of *H.maroccana* has rarely been studied. Therefore, a thorough understanding of the biology and ecology based on the effect of temperature and host plants on development, survivorship and reproduction of the pest is essential to develop an effective pest management programme.

3.2 Objectives of the experiment

In order to obtain a better knowledge of the biology and ecology of *H. maroccana,* efforts were made to examine some aspects of life history in relation to four constant temperatures and five host plants. The following were studied:

a) incubation period
b) developmental duration of each larval instar
c) total development duration for male and female
d) determination of development threshold and degree day requirements
e) survival of different nymphal instars
f) relative growth during nymphal development
g) adult longevity
h) fecundity and life table parameters

The null hypothesis:

1) Temperature and host plant has no effect on egg incubation, development duration, immature survival, relative growth rate of *H. maroccana.*
2) Temperature does not affect adult survival and fecundity of *H. maroccana.*

3.3 Materials and Methods

Different biological studies on *H maroccana* were conducted at four different constant temperatures, i.e. 17, 21, 25 and 27°C on the following five host plants

1) Cucumber, *Cucumis sativus* L. (var. Bush champion), Crucifereae
2) Tomato, *Lycopersicon esculentum* Mill. (var. Alisa Graig), Solanaceae
3) French bean, *Phaseolus vulgaris* L. (var. Prince), Leguminoseae
4) Primula, *Primula vulgaris* Huds. (var. Mixed colour), Primulaceae
5) Sweet pepper, *Capsicum annum* L. (var. Worldbeater), Solanaceae.

Gallenkamp Compenstat cooled incubators or similar cabinets with computerised temperature and photoperiod control were used to achieve the condition required for each experiment. The relative humidity for each experiment was controlled at 85% r.h. using a saturated KCl salt solution (Greenspan, 1977).

3.3.1 Egg incubation period of *H. maroccana*

Polystyrene Petri-dish cages (50 to 70 mm diameter) with ventilation in the lid were used to study the incubation period of leafhopper eggs on different host plants at different temperatures. Plants with fully expanded leaves were placed in the mass culture to allow the leafhoppers to lay eggs. After 24 hours, the leaves containing eggs were detached and leaf discs (50-70 mm) were placed in the Petri-dish on agar medium (8g/litre), the lower surface facing uppermost. These Petri-dishes were placed in plastic boxes and kept in the incubator at different constant temperatures. To ensure the required humidity a small plastic box containing saturated KCl salt solution was placed inside the box. The Petri-dishes were checked everyday for the emergence of first instar nymphs and the number of nymphs emerged was recorded to calculate the mean incubation period. Each nymph was considered as a replication. Five Petri-dishes were maintained for each temperature.

The lower threshold temperature (t_l) and the thermal constant (K) for the development of egg were determined using the linear regression curves fitted to the interaction of temperature with the rate of development. tl and K could be calculated from the regression equation $\mathbf{y = a+bT}$, where y is the rate of development (reciprocal of development time) at temperature T and a and b are constants, $K = 1/b$, $t_l = -a/b$

(Campbell *et al.*, 1974). Development at 27°C was outside the linear part of the growth curve and therefore, not included in the linear regression calculating the lower threshold and thermal constant.

3.3.2 Survival and development of *H. maroccana*

Development and survival of *H. maroccana* were studied at four different temperatures using five host plants. Perspex sandwich cages (75 x 38 x 8 mm) were used to study the development of different nymphal instars (Plate 2.2). Twenty mm discs cut from fully expanded leaves of the host plant away from the bulky mid rib were placed on agar media (8g/litre) the lower surface facing uppermost. Individual first instar nymphs were placed into the chamber on the leaf disc on the day of emergence. The leaf discs were placed inside a plastic box and kept in the incubator at the desired temperature under constant light conditions. The leaf discs were changed regularly where necessary to avoid deterioration of food quality and to prevent fungal and bacterial growth. The leaf cages were checked everyday for exuviae and the time spent at each instar was recorded until the nymphs became adults. The death of nymphs at different instars was also recorded to calculate nymphal survival. Twenty eight replications were maintained in the experiment at each temperature tested.

The same procedure was followed to calculate the lower threshold and thermal constant for different nymphal instars and for total nymphal duration.

3.3.3 Relative growth rate during nymphal development of *H. maroccana*

The relative growth rate (RGR) during nymphal development was calculated following van Emden (1972). He defined RGR as the weight increment per unit time per initial weight of the insect over the period between weighing and calculated as:

$$\text{Relative Growth Rate} = \frac{\text{[Log final weight-Log initial weight]}}{\text{No. of days over which weight increase is measured}}$$

About twenty of the first instar nymphs that developed on different host plants at 21°C were weighed using a micro balance to get the mean initial weight. The mean final

weight was calculated by weighing the adults emerging from different host plants at different constant temperatures. The mean relative growth rate for the nymphal period was then calculated.

3.3.4 Adult longevity of *H. maroccana* at different constant temperatures

Adult longevity of both sexes of *H. maroccana* at different constant temperatures was studied on *Primula vulgaris*. Fifth instar nymphs were collected from the mass culture and reared in the incubator in Petri-dish cages at 21°C. The cages were checked everyday for adult emergence. On emergence, each individual adult was introduced into 2 ml Eppendorf microcentrifuge tube with a piece of wet filter paper and a piece of *P. vulgaris* leaf and placed in the incubator at different constant temperatures. Leaf pieces were changed every three days to provide sufficient food. Daily observation was made for the death of adult male and females. Any adults that adhered to honeydew or to the condensed vapour were excluded from the observation.

3.3.5 Fecundity and life table parameters of *H. maroccana*

Fecundity and life table parameters of *H. maroccana* were studied in the laboratory at 21°C and 85% r.h. with *P. vulgaris* as the host plant. Fifth instar nymphs were collected from the mass culture and reared in the incubator in Petri-dish cages at 21°C. The cages were checked everyday for adult emergence.

Fifty mm leaf discs were made from the flat and fully expanded leaves of *P. vulgaris* and placed on agar media (8g/litre) in a Petri-dish, lower surface facing uppermost. *Hauptidia maroccana* has a pre-oviposition period of 7-14 days at 21°C (MacGill, 1932). Therefore, a pair of 10-day-old male and female leafhopper was introduced into each Petri-dish cage with the help of a paintbrush, avoiding any injury. Generally, the leafhoppers are found to lay eggs on the underside of the leaf remaining in an upside down position. Therefore, the Petri-dish cages were placed in plastic boxes at an angle of 45° to allow the leafhoppers to lay eggs in a natural situation. The humidity inside the boxes was maintained at 85% r.h. using a saturated KCl salt solution. The plastic boxes were kept inside the incubator at 21°C in continuous light. The pair was transferred from one leaf disc to another after every 5 days. The Petri-dishes were checked everyday.

Whenever any male died it was replaced with a live one and any observed mortality of females was also recorded. Eggs laid per female in these 5 day periods were counted and the mean daily fecundity was calculated. The experiment was started with 10 pairs. But three of them were not included in analysis due to unnatural early death of the females caused by experimental techniques.

Data on survival, age specific fecundity, and sex ratio were used to estimate different life table parameters viz., gross reproductive rate (*GRR*), net reproductive rate (R_o), capacity for increase (r_c), cohort generation time (*Tc*), generation time (*T*), doubling time (*DT*) and finite capacity for increase (λ) with the help of a QBASIC computer program (Jervis and Copland, 1996).

3.4 Statistical Analysis

The rate of development was calculated in days as 1/day (reciprocal of developmental time). Development at 27° C was outside the linear range of the growth curve and therefore not included in the linear regression analysis. Temperature from 17°C to 25°C was selected to fit a linear regression. The equation of linear regression was then used to predict the lower threshold (t_l) and the thermal constant (K). The theoretical lower threshold was calculated as t_l = -a/b, and the thermal constant K= 1/b (Campbell *et al.*, 1974). The analyses to find out the effect of temperature and host plants on incubation period, developmental period and relative growth rate were done by Analysis of Variance using SAS procedure and the means were separated by Least Significance Difference (LSD) test. The study on different host plants at different temperature was not done at the same time. Therefore, LSD was done separately for each temperature and host plant. Adult longevity at different temperatures was also subjected to Analysis of Variance using SAS procedure and the means were separated by LSD. Due to unequal replicates, the harmonic mean of the replicates was used to calculate the standard error of difference for comparing the means To compare the survival of immature stages χ^2 analysis was used. The life table was constructed with the help of a QBASIC computer program (Jervis and Copland, 1996).

3.5 Results

3.5.1 Effect of temperature and host plants on incubation period of *H. maroccana.*

The incubation period refers to the time between egg laying and the emergence of the first instar nymphs. The incubation period of *H. maroccana* was significantly influenced by both temperature and host plants (Table 3.1, Figure 3.1). Shortest incubation period (9.39 to 9.68 days) was observed at 25°C in almost all host plants except in Cucumber although the incubation in Cucumber at 25°C (9.39 days) and 27°C (9.27 days) was not significantly different. The longest incubation was observed at 17°C irrespective of the host plants, ranging from 15 to 17.09 days. Although a significant difference due to host plants has been observed on incubation, there was no specific trend among the host plants at different temperatures.

Incubation period decreased with increasing temperature from 17° to 25°C and then increased in almost all host plants, except in Cucumber. A decline in the developmental rate was observed at 27°C in all host plants, which resulted in a departure from a linear trend (Figure 3.2). The theoretical development threshold (i.e. the point where the development rate equals zero) of the egg was estimated to be in the range of 2.58°C to 7.09°C in different host plants using the linear range of temperature. The thermal constant for the development of eggs was observed to be the lowest (171.13 DD) in Primula and the highest (218.34 DD) in Sweet pepper (Table 3.3). The cubic polynomial regression analysis of data for incubation in different host plants showed a better fit than the linear regression (Table 3.2).

Table 3.1 Effect of different constant temperatures and host plants on the egg incubation of *Hauptidia maroccana*.

Host plants	Incubation period (days) Mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	16.3±0.09 b A (27)	13.5±0.09 a B (64)	9.4±0.09 b C (33)	9.3±0.09 d C (26)	df=146 *P*<0.001 LSD=0.288
Tomato	15.2±0.14 c A (45)	11.3±0.10 d B (24)	9.6±0.08 ab D (35)	10.3±0.09 b C (31)	df=131 *P*<0.001 LSD=0.334
French Bean	16.2±0.12 b A (93)	12.6±0.08 b B (63)	9.5±0.09 ab C (72)	9.6±0.13 c C (24)	df=248 *P*<0.001 LSD=0.362
Primula	17.1±0.07 a A (91)	12.5±0.07 b B (55)	9.5±0.05 ab D (88)	10.4±0.06 b C (104)	df=334 *P*<0.001 LSD=0.184
Sweet pepper	15.0±0.14 c A (23)	12.0±0.10 c B (27)	9.7±0.09 a D (28)	10.7±0.11 a C (42)	df=116 *P*<0.001 LSD=0.320
One-way ANOVA	df=274 *P*<0.001 LSD=0.3986	df=228 *P*<0.001 LSD=0.266	df=251 *P*=0.3819 NS LSD=0.2558	df=222 *P* <0.001 LSD=0.2843	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

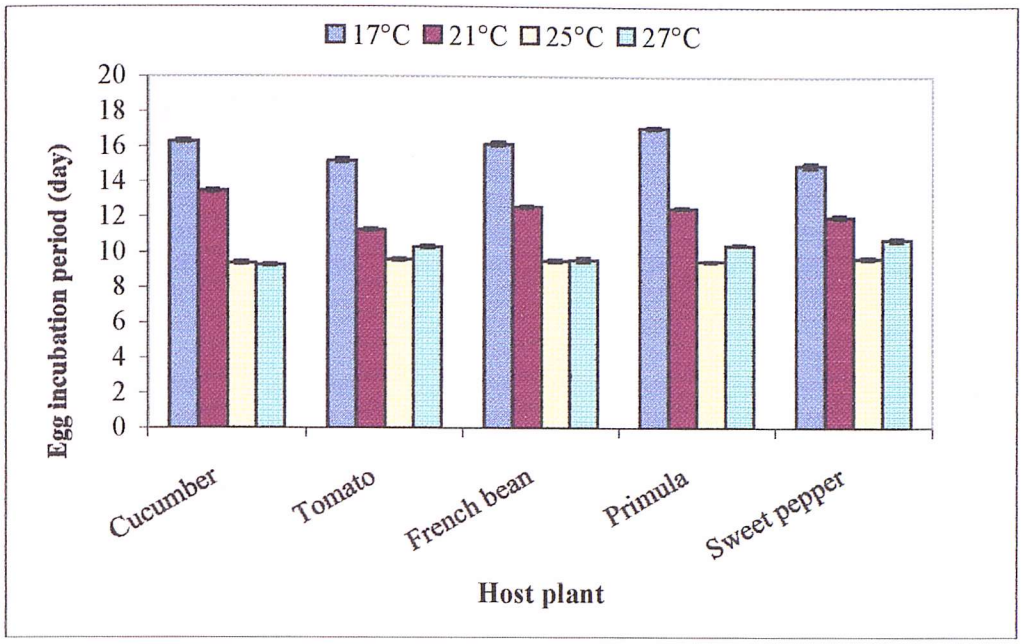


Figure 3.1 Effect of different constant temperatures and host plants on egg incubation of *Hauptidia maroccana.* Vertical bars represent the standard errors of mean.

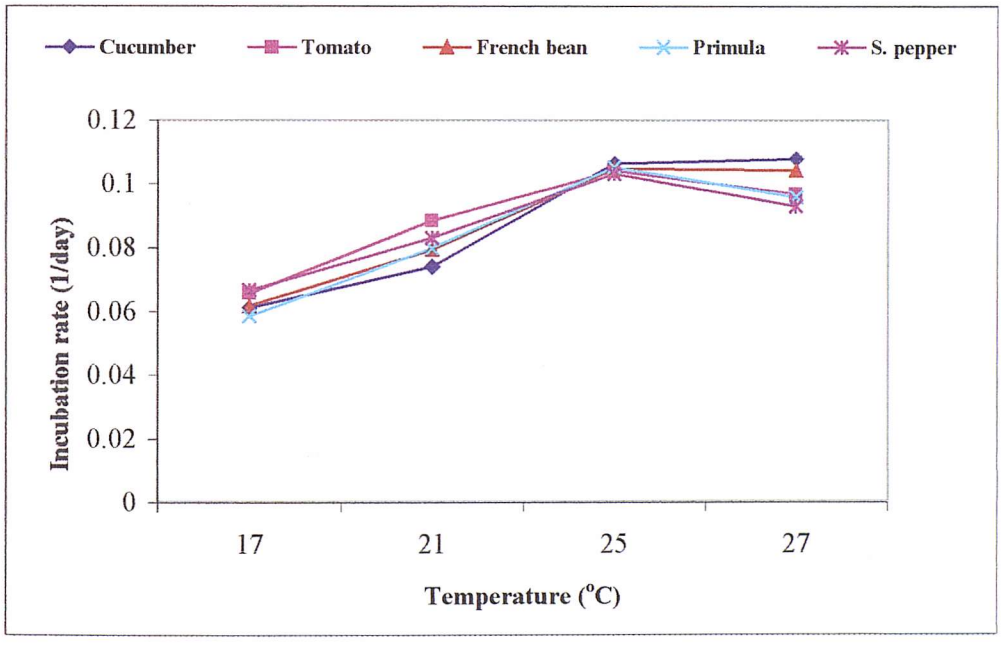


Figure 3.2 Rate of egg incubation of *Hauptidia maroccana* at four constant temperatures on five host plants.

Table 3.2 Regression analyses for the egg incubation rate of *Hauptidia maroccana* versus temperature on different host plants.

Host plants	Linear *	Quadratic (2nd order Polynomial) **	Cubic (3rd order Polynomial) **
Cucumber	$Y=0.0057x-0.0383$ $r^2= 0.9418$	$Y=8E-05x^2+0.0016x-0.0096$ $r^2= 0.9523$	$Y= -0.0084x^3+0.0602x^2-0.1089x+0.1183$ $r^2=1$
Tomato	$Y=0.0048x-0.0149$ $r^2= 0.9882$	$Y= -0.0005x^2+0.0264x-0.2329$ $r^2= 0.9706$	$Y= -0.0026x^3+0.0119x^2-0.0053x+0.0511$ $r^2=1$
French bean	$Y= 0.0054x-0.0311$ $r^2= 0.9889$	$Y= -0.0001x^2+0.0108x-0.082$ $r^2= 0.9678$	$Y= -0.0056x^3+0.0378x^2-0.0564x+0.086$ $r^2=1$
Primula	$Y= 0.0059x-0.0416$ $r^2= 0.9976$	$Y= -0.0004x^2+0.0235x-0.2156$ $r^2= 0.9296$	$Y= -0.0064x^3+0.0407x^2-0.0555x+0.0798$ $r^2=1$
Sweet pepper	$Y= 0.0046x-0.0117$ $r^2= 0.9964$	$Y= -0.0004x^2+0.0207x-0.1712$ $r^2= 0.8959$	$Y= -0.0057x^3+0.0361x^2-0.052x+0.0883$ $r^2=1$

* Regression was done based on the values at 17, 21 and 25°C

** Regression was done based on the values at 17, 21, 25 and 27°C

Table 3.3 Lower threshold (t_l) and thermal constant (K) for the development of eggs of *Hauptidia maroccana* on different host plants.

Host plants	Lower threshold (t_l) (°C)	Thermal constant (K) (DD)
Cucumber	6.75	176.76
Tomato	3.08	207.99
French bean	5.79	185.33
Primula	7.098	171.13
Sweet pepper	2.584	218.34

3.5.2 Effect of temperature and host plants on nymphal development of *H. maroccana*

Development of different nymphal instars on different host plants at different constant temperatures is presented in Table 3.4-3.8. Temperature had a significant effect on the development of each of the five nymphal instars. Development duration was always the longest at 17°C. In first instar nymphs, development duration decreased with increasing temperature up to 25°C. However, there was no significant difference in development duration of second to fifth instar nymphs at 25 and 27°C in almost all host plants. Host plants also had a significant effect on development of different nymphal instars but did not show any specific pattern at different temperatures.

Table 3.4 Effect of different constant temperatures and host plants on the development duration of 1st instar nymphs of *Hauptidia maroccana.*

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	4.2±0.09 c A (22)	2.8±0.11 c C (22)	2.6±0.11 a C (22)	3.5±0.10 a B (28)	df=90 P<0.01 LSD=0.283
Tomato	4.4±0.11 c A (22)	3.2±0.16 b B (20)	2.1±0.07 b D (20)	2.5±0.12 b C (24)	df=82, P <0.01 LSD=0.382
French Bean	4.2±0.08 c A (24)	2.6±0.12 c B (25)	2.7±0.10 a B (23)	2.3±0.09 bc C (27)	df=95 P <0.001 LSD=0.271
Primula	5.0±0.15 b A (22)	3.6±0.14 a B (20)	2.4±0.10 b C (20)	2.4±0.10 c C (22)	df=80 P <0.001 LSD=0.344
Sweet pepper	5.5±0.25 a A (21)	2.6±0.11 c BC (23)	2.8±0.08 a B (24)	2.4±0.12 bc C (24)	df=88 P<0.001 LSD=0.406
One-way ANOVA	df=106 P <0.001 LSD= 0.436	df= 105 P <0.001 LSD=0.348	df=104 P <0.001 LSD=0.264	df=120 P <0.001 LSD=0.284	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

Table 3.5 Effect of different constant temperatures and host plants on the development duration of 2nd instar nymphs of *Hauptidia maroccana.*

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	4.4±0.12 b A (22)	2.7±0.10 abB (22)	2.2±0.09 b C (22)	2.3±0.09 ab C (27)	df=89 P <0.01 LSD=0.286
Tomato	4.1±0.11 bc A (22)	2.9±0.17 a B (16)	2.1±0.07 b C (20)	2.3±0.12 ab C (23)	df=77 P <0.01 LSD=0.333
French Bean	3.9±0.17 c A (23)	2.5±0.12 b B (23)	2.6±0.13 a B (22)	2.1±0.06 b C (27)	df=91 P <0.001 LSD=0.323
Primula	4.8±0.15 a A (22)	3.1±0.17 a B (20)	2.2±0.08 b C (20)	2.2±0.09 b C (22)	df= 80 P <0.001 LSD=0.358
Sweet pepper	4.1±0.17 bc A (21)	2.7±0.09 ab B (23)	2.4±0.12 ab C (24)	2.6±0.11 a BC (22)	df=86 P <0.001 LSD=0.347
One-way ANOVA	df=105 P <0.001 LSD=0.392	df=99 P <0.05 LSD=0.366	df=103 P <0.05 LSD=0.288	df=116 P <0.05 LSD=0.265	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

Table 3.6 Effect of different constant temperatures and host plants on the development duration of 3rd instar nymphs of *Hauptidia maroccana.*

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	4.1±0.12 bc A (22)	2.7±0.11 b B (21)	2.3±0.11 ab C (20)	2.4±0.10 b BC (24)	df=83 P <0.01 LSD=0.307
Tomato	4.3±0.11 b A (21)	3.1±0.15 a B (15)	2.4±0.11 a C (20)	2.3±0.11 b C (20)	df=72 P <0.001 LSD= 0.332
French Bean	3.8±0.08 c A (23)	2.7±0.13 b B (21)	2.3±0.16 ab C (18)	2.2±0.08 b C (25)	df=83 P <0.001 LSD=0.310
Primula	5.3±0.15 a A (22)	3.2±0.10 a B (18)	2.1±0. 05 b C (20)	2.1±0.05 b C (20)	df= 76 P <0.01 LSD= 0.279
Sweet pepper	4.1±0.13 bc A (21)	3.0±0.12 ab B (23)	2.5±0.11 a C (22)	2.7±0.10 a BC (22)	P <0.001 df=84 LSD=0.317
One-way ANOVA	df=104 P <0.001 LSD=0.330	df=93 P <0.01 LSD=0.341	df=95 P <0.05 LSD=0.310	df=106 P <0.01 LSD=0.275	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

Table 3.7 Effect of different constant temperatures and host plants on the development duration of 4th instar nymphs of *Hauptidia maroccana*.

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	5.5±0.14 ab A (20)	3.3±0.156 b B (21)	2.8±0.17 ab C (16)	3.2±0.16 a BC (16)	df=69 P <0.01 LSD=0.444
Tomato	4.8±0.15 c A (21)	4.0±0.195 a B (15)	2.7±0.13 ab C (20)	2.9±0.17 a C (17)	df=69 P <0.01 LSD=0.448
French Bean	5.2±0.14 bc A (21)	3.8±0.206 a B (19)	2.3±0.11 b C (17)	2.5±0.12 b C (19)	P <0.01 df= 72 LSD=0.423
Primula	6.0±0.12 a A (21)	3.2±0.173 b B (18)	2.8±0.10 ab C (20)	2.9±0.08 a C (18)	df=73 P <0.01 LSD=0.342
Sweet pepper	5.0±0.19 bc A (21)	3.7±0.135 ab B (23)	3.0±0.16 a C (21)	3.1±0.13 a C (21)	P <0.01 df=82 LSD=0.433
One-way ANOVA	df=99 P <0.001 LSD=0.586	df=91 P <0.01 LSD=0.486	df=86 P =0.089 LSD=0.401	df=86 P <0.001 LSD=0.373	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

Table 3.8 Effect of different constant temperatures and host plants on the development duration of 5th instar nymphs of *Hauptidia maroccana*.

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	8.1±0.16 bc A (17)	5.3±0.18 b B (15)	4.1±0.18 a C (14)	4.4±0.20 ab C (7)	df=49 P <0.001 LSD= 0.546
Tomato	8.9±0.22 a A (21)	6.3±0.34 a B (14)	4.1±0.16 a C (20)	4.4±0.19 ab C (15)	df=66 P<0.01 LSD=0.647
French Bean	8.5±0.20 ab A (18)	6.3±0.24 a B (13)	4.3±0.21 a C (6)	4.1±0.15 b C (15)	df=48 P <0.001 LSD= 0.651
Primula	8.7±0.28 a A (20)	5.8±0.20 ab B (17)	4.2±0.12 a C (20)	4.5±0.12 ab B (18)	df =72 P <0.001 LSD=0.549
Sweet pepper	7.8±0.19 c A (20)	5.8±0.20 ab B (23)	4.4±0.24 a C (17)	4.9±0.19 a C (20)	df=76 P<0.001 LSD= 0.577
One-way ANOVA	df=92 P <0.001 LSD= 0.613	df=78 P <0.05 LSD= 0.546	df=72 P =0.729 NS LSD= 0.546	df=70 P <0.05 LSD= 0.546	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

Development of *H. maroccana* was significantly affected by temperature regardless of sex. Developmental period was found to decrease with a rise in temperature up to a certain limit (17-25 °C) and then increased. On average, the longest developmental time (25.9-29.5 days) was observed at 17°C and was shortest (13.4-14.7 days) at 25°C (Table 3.9). Developmental period was also affected significantly by different host plants. *Hauptidia maroccana* took longest to develop on Primula (13.5-29.5 days) over the linear range of temperature while it was shortest on Cucumber (13.9-26.1 days) (Table 3.9).

Development of male and female *H. maroccana* on different host plants under various constant temperatures is presented in Table 3.10 and 3.11. Temperature had a profound effect on male developmental period. Significant variation was observed over the linear range of temperature in all five host plants. Developmental period was always the longest (25.4-27.9 days) at 17°C and shortest (12.5-14.3 days) at 25°C (Table 3.10). Female developmental period also varied significantly over different constant temperatures. Longest developmental period (26.1-31.3 days) was observed at 17°C while it was the shortest (13.5-15.3 days) at 25°C (Table 3.11). Developmental period of both male and female were significantly affected by different host plants. Males always developed faster than females on any of the host plants at different constant temperatures.

Table 3.9 Effect of different constant temperatures and host plants on the nymphal development of *Hauptidia maroccana* (pooled sexes).

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	26.1±0. 18 b A (17)	16.8± 0.21 c B (22)	13.9± 0.15 b D (33)	15.5± 0.37 a C (10)	df=78 *P*<0.001 LSD=0.625
Tomato	26.5± 0.37 b A (20)	19.3± 0.50 a B (16)	13.4± 0.20 b C (20)	14.0± 0.30 b C (12)	df=64 *P*<0.001 LSD=1.023
French Bean	25.8±0.19 b A (18)	17.8± 0.28 b B (18)	13.6± 0.26 b C (17)	14.1± 0.32 b C (15)	df=64 *P*<0.001 LSD=0.739
Primula	29.5± 0.59 a A (19)	18.8± 0.27 a B (17)	13.5± 0.18 b C (21)	14.1± 0.25 b C (18)	df=71 *P*<0.001 LSD=1.017
Sweet pepper	26.5± 0.32 c A (21)	17.7± 0.27 b B (23)	14.7± 0.40 a C (13)	15.4± 0.22 a C (19)	df=72 *P*<0.001 LSD=0.866
One-way ANOVA	df=90 *P* <0.001 LSD=1.049	df=91 *P* <0.001 LSD=0.852	df=99 *P* <0.001 LSD=0.631	df=69 *P* <0.001 LSD=0.829	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

Table 3.10 Effect of different constant temperatures and host plants on the nymphal development of male *Hauptidia maroccana*.

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	25.6± 0.30 b A (7)	16.0± 0.00 d B (5)	13.1± 0.20 c C (9)	15.0± 0.41 aD (4)	df=21 *P*<0.001 LSD=0.790
Tomato	25.6± 0.44 b A (9)	17.8± 0.49 ab B (5)	13.3± 0.21 bc C (10)	13.6± 0.26 b C (8)	df=28 *P*<0.001 LSD=1.041
French Bean	25.4± 0.24 b A (9)	17.1± 0.23 bc B (8)	12.5± 0.29 c C (4)	13.7± 0.33 b D (6)	df=23 *P*<0.001 LSD=0.833
Primula	27.9± 0.38 a A (9)	184± 0.26 a B (8)	14.3± 0.33 a C (8)	13.6± 0.30 b C (7)	df=28 *P*<0.001 LSD=0.974
Sweet pepper	26.2± 0.42 b A (11)	16.7± 0.26 cd B (12)	14.0± 0.33 ab C (8)	15.1± 0.28 a C (10)	df=37 *P*<0.001 LSD=0.955
One-way ANOVA	df=40 *P* <0.001 LSD=1.074	df=33 *P* <0.001 LSD=0.363	df=34 *P* <0.001 LSD=0.869	df=30 *P* <0.001 LSD=0.930	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

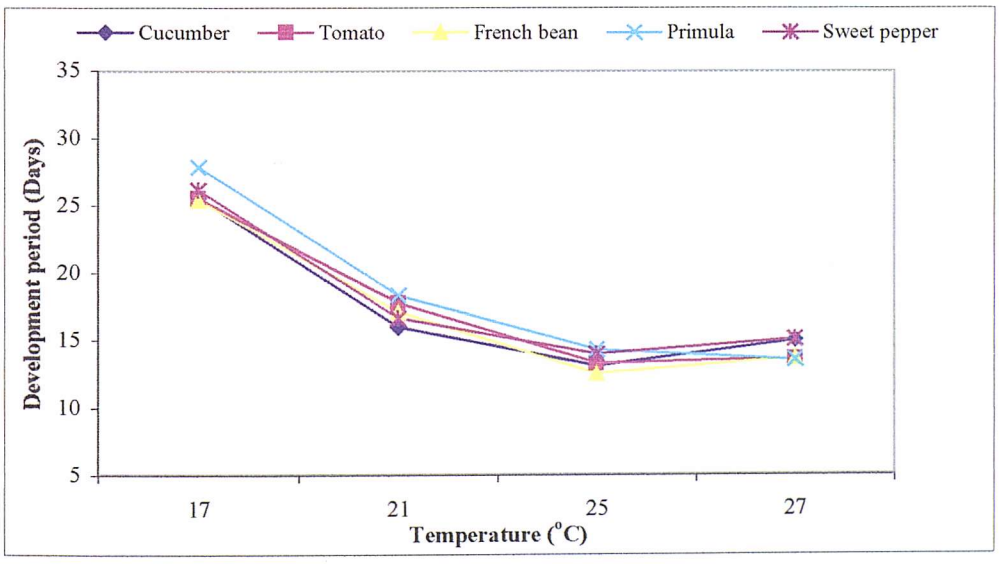


Figure 3.3 Effect of different constant temperatures and host plants on the nymphal development of male *Hauptidia maroccana*.

Table 3.11 Effect of different constant temperatures and host plants on the nymphal development of female *Hauptidia maroccana*.

Host plants	Development Period (days) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	26.4± 0.18 b A (9)	17.2± 0.35 c B (11)	14.8± 0.31 abC (6)	15.8± 0.54 a C (6)	df=28 *P*<0.001 LSD=1.051
Tomato	27.5± 0.53 b A (12)	20.0± 0.85 a B (7)	13.5± 0.31 b C (10)	14.4± 0.51 b C (5)	df=30 *P*<0.001 LSD=1.718
French Bean	26.1± 0.26 b A (9)	18.2± 0.42 bc B (10)	14.0± 0.42 b C (8)	14.3± 0.45 b C (8)	df=31 *P*<0.001 LSD=1.139
Primula	31.3± 0.85 a A (9)	19.2± 0.40 ab B (9)	14.4± 0.40 abC (9)	14.4± 0.51 b C (5)	df=28 *P*<0.001 LSD=1.792
Sweet pepper	27.1± 0.91 b A (8)	18.7± 0.34 ab B (10)	15.3± 0.72 a C (6)	15.9± 0.40 a C (7)	df=27 *P*<0.001 LSD=1.489
One-way ANOVA	df=42 *P* <0.001 LSD=1.568	df=42 *P* <0.001 LSD=1.308	df=34 *P* <0.001 LSD=1.235	df=26 *P* <0.001 LSD=1.4287	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

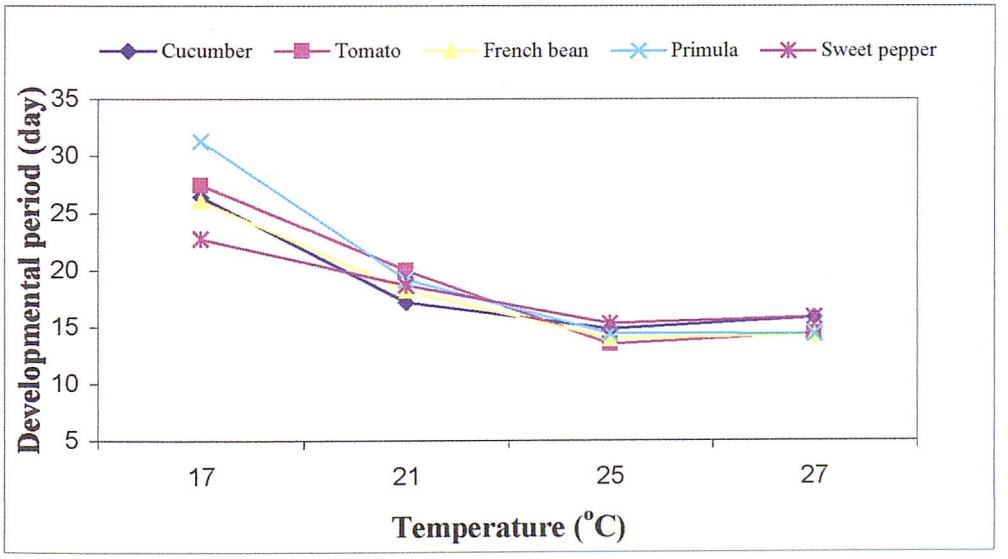


Figure 3.4 Effect of different constant temperatures and host plants on the nymphal development of female *Hauptidia maroccana*.

3.5.3 Rate of development, threshold temperature and thermal constant

Figures 3.5-3.9 show the relationship between temperature and the rate of nymphal development of *H. maroccana* on different host plants. The linear regression analyses for the rate of development show a good fit for either sex in all of the five host plants (r^2 = 0.98-1.0) (Table 3.10). The thermal constant for male and female did not show any consistent pattern among different host plants tested and ranged 196-238 °Days and 212-270 °Days for males and females, respectively (Table 3.12). The highest thermal constant was observed while developing on Sweet pepper and was lowest on Primula. The lower threshold for development estimated from linear regression was found to vary from 7.4-9.3 and 6.3-10.0 °C for male and females, respectively on different host plants. The lower threshold, regardless of sex was observed to be the highest (10°C) on Primula while it was the lowest (6.8°C) on Sweet pepper.

Table 3.12 Linear regression equations, lower threshold (tl) and thermal constant (K) for the nymphal development of *Hauptidia maroccana* on different host plants based on the data at 17, 21 and 25°C.

Host plant	Sex	Linear regression equation	r^2	t_l (°C)	K(DD)
Cucumber	Male	y = -0.0384 + 0.0047x	0.9783	8.17	212.8
	Female	y = -0.0232 + 0.0037x	0.9545	6.27	270.3
	Pooled	y = -0.0320 + 0.0042x	0.9789	7.62	238.1
Tomato	Male	y = -0.0379 + 0.0045x	0.999	8.42	222.2
	Female	y = -0.0455 + 0.0047x	0.9748	9.68	212.8
	Pooled	y = -0.0425 + 0.0046x	0.9812	9.24	217.4
French bean	Male	y = -0.0476+ 0.0051x	0.9987	9.33	196.1
	Female	y = -0.0320 + 0.0041x	1	7.81	243.9
	Pooled	y = -0.0351 + 0.0043x	1	8.16	232.6
Primula	Male	y = -0.0359 + 0.0042x	0.9971	8.55	238.1
	Female	y = -0.0471 + 0.0047x	0.9981	10.02	212.8
	Pooled	y = -0.0520 + 0.0050x	0.9995	10.40	200.0
Sweet pepper	Male	y = -0.0312 + 0.0042x	0.9687	7.43	238.1
	Female	y = 0.0227 + 0.0036x	0.9898	6.30	227.8
	Pooled	y = -0.0257 + 0.0038x	0.9826	6.76	263.2

Table 3.13 Linear regression equations, thermal constant (K) and the lower threshold (tl) for different nymphal instars of *Hauptidia maroccana* on different host plants based on the data at 17, 21 and 25°C.

	Regression equation	r^2	t_l (°C)	K(DD)
1st instar				
Cucumber	Y= 0.0884x+0.135	0.8504	-1.53	11.31
Tomato	Y= 0.1247x+0.089	0.9685	-0.71	8.02
French bean	Y= 0.0688x+0.1984	0.6783	-2.88	14.53
Primula	Y= 0.1118x+0.0796	0.9733	-0.71	8.94
Sweet pepper	Y= 0.0877x+0.1325	0.6348	-0.66	7.55
2nd instar				
Cucumber	Y= 0.1095x+0.129	0.9796	-1.18	9.13
Tomato	Y= 0.1158x+0.1219	0.99	-1.05	8.64
French bean	Y= 0.0665x+0.2184	0.688	-3.28	15.04
Primula	Y= 0.1277x+0.0787	0.9982	-0.62	7.83
Sweet pepper	Y= 0.0864x+0.1709	0.933	-0.51	5.85
3rd instar				
Cucumber	Y= 0.0939x+0.1642	0.9589	-1.75	16.65
Tomato	Y= 0.0929x+0.1387	0.9999	-1.49	10.76
French bean	Y= 0.0887x+0.1806	0.9749	-2.04	11.27
Primula	Y= 0.1490x+0.0313	0.9882	-0.21	6.71
Sweet pepper	Y= 0.0781x+0.1696	0.993	-0.46	5.90
4th instar				
Cucumber	Y= 0.0901x+0.1035	0.9634	-1.15	11.09
Tomato	Y= 0.0812x+0.1136	0.9282	-1.40	12.32
French bean	Y= 0.1229x+0.0501	0.9409	-0.41	8.14
Primula	Y= 0.0985x+0.0833	0.9338	-0.85	10.15
Sweet pepper	Y= 00666x+0.1346	0.999	-0.49	7.43
5th instar				
Cucumber	Y= 0.0587x+0.0676	0.9954	-1.15	17.04
Tomato	Y= 0.0655x+0.0409	0.9716	-0.62	15.28
French bean	Y= 0.0567x+0.0557	0.9747	-0.97	17.64
Primula	Y= 0.0616x+0.0518	0.9981	-0.84	16.23
Sweet pepper	Y= 0.0496x+0.0769	0.9961	-0.64	13.0

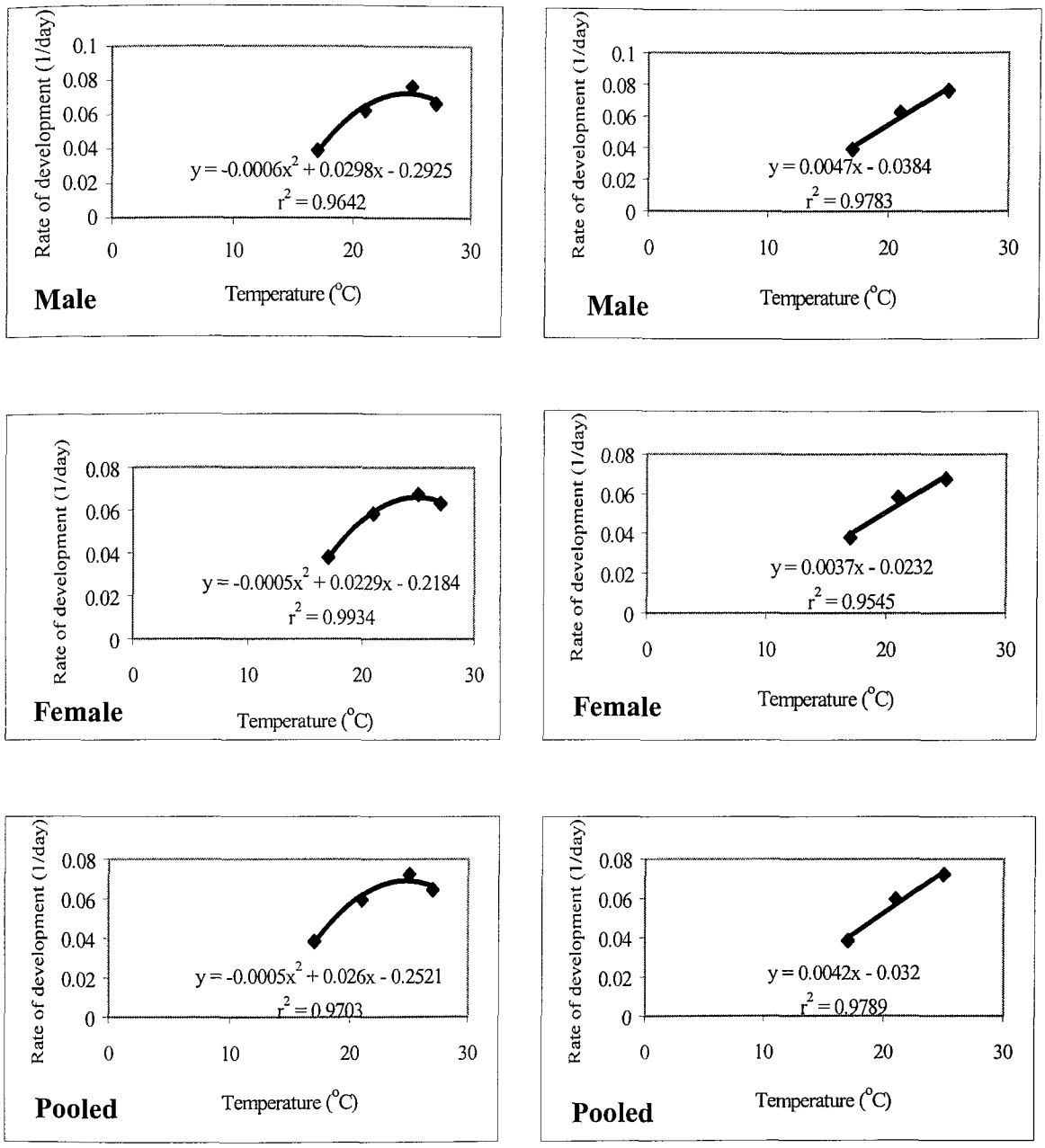


Figure 3.5 **Polynomial and linear relationship between temperature and nymphal developmental rate of *Hauptidia maroccana* on Cucumber.**

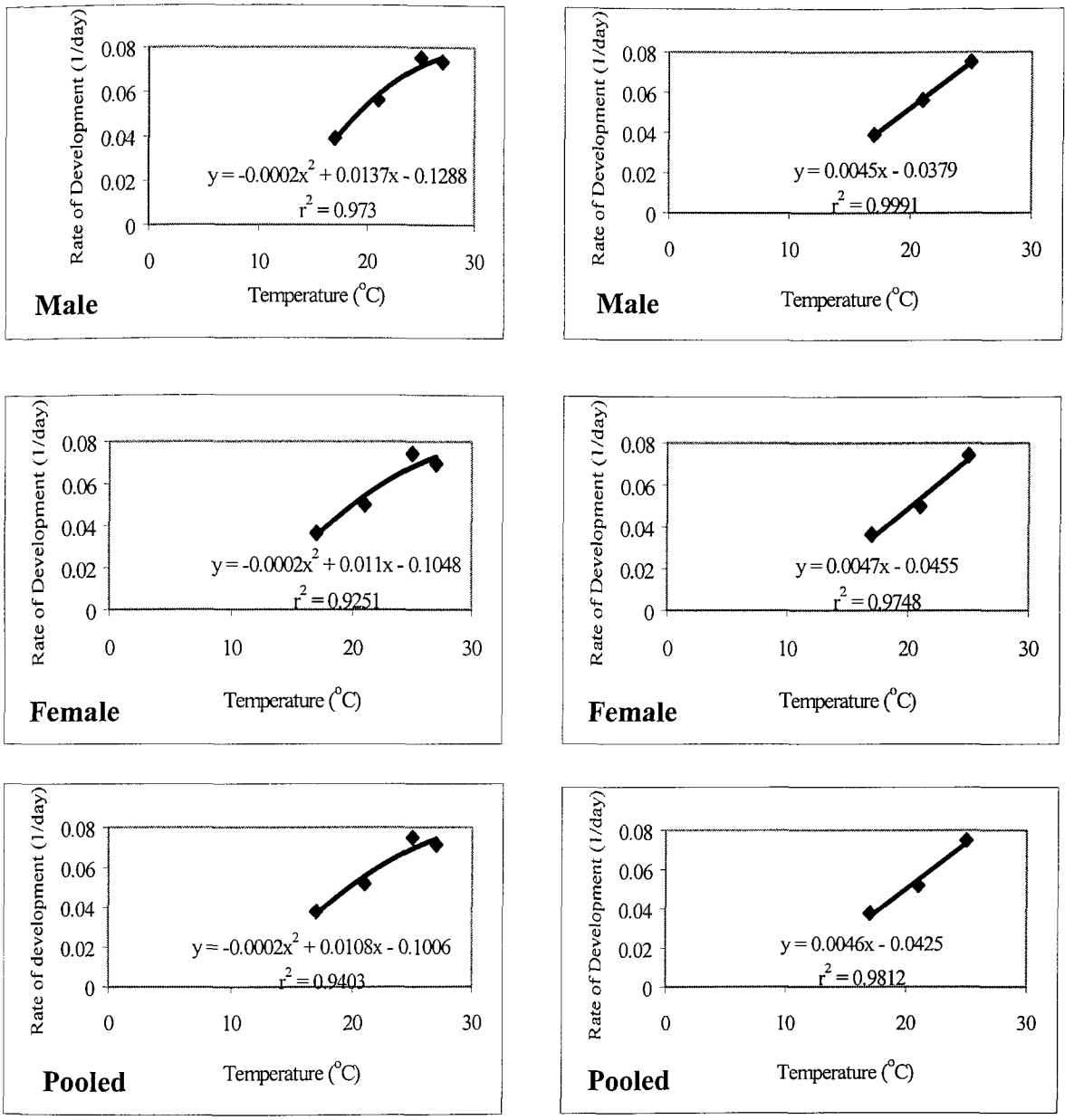
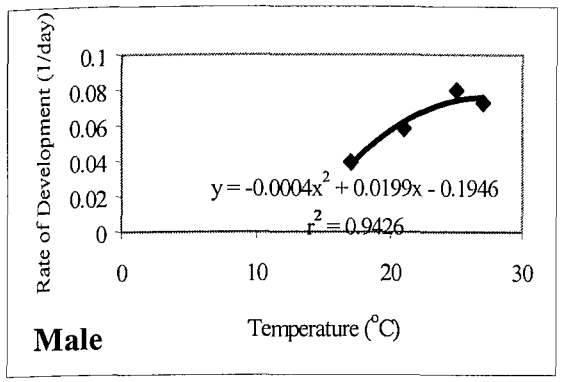
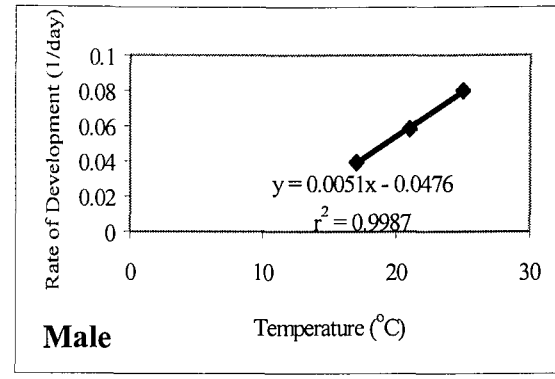
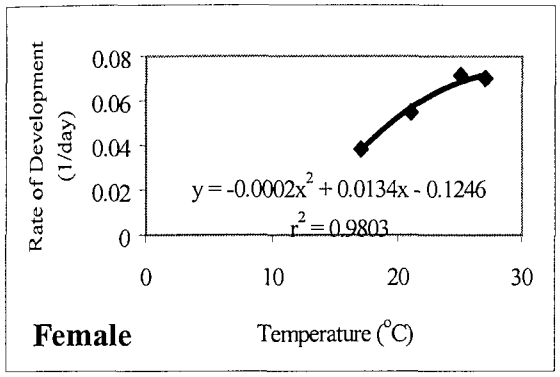
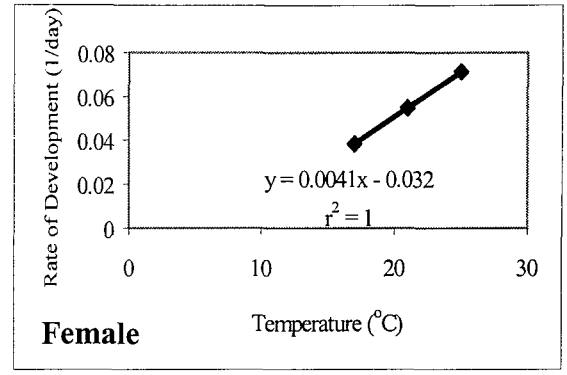


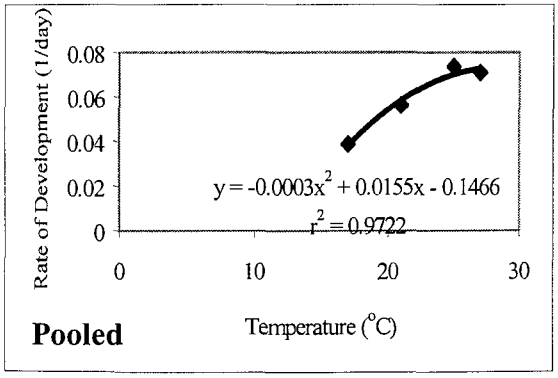
Figure 3.6 **Polynomial and linear relationship between temperature and nymphal developmental rate of *Hauptidia maroccana* on Tomato.**











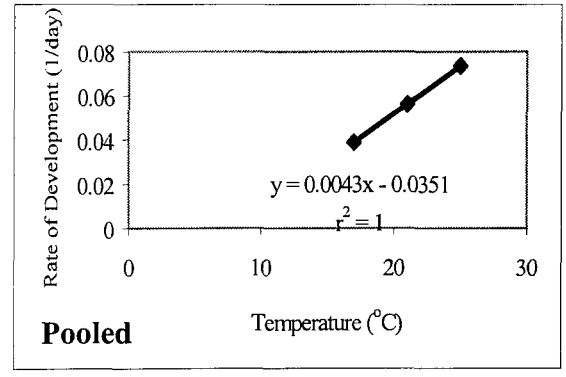
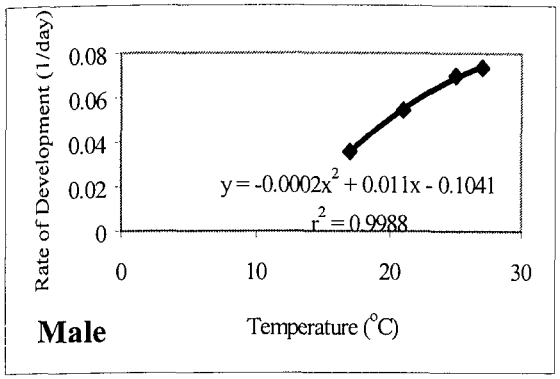
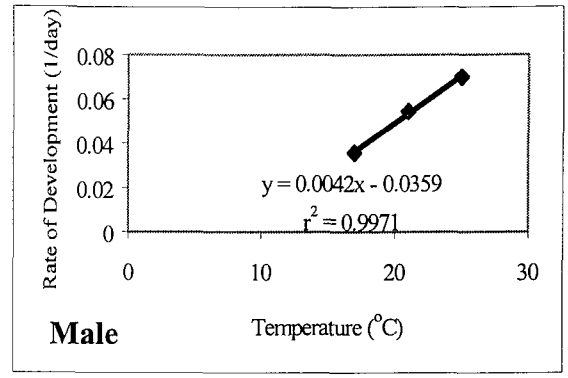
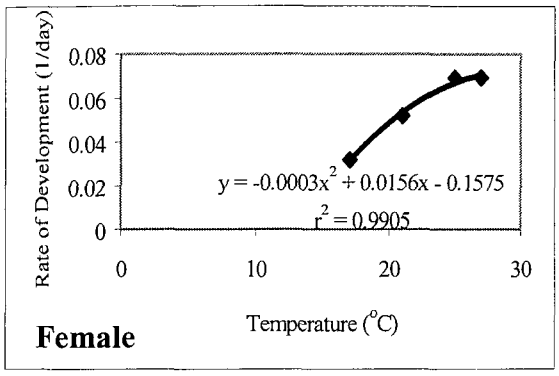
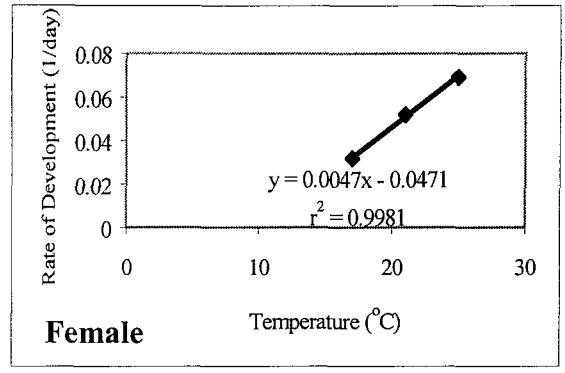


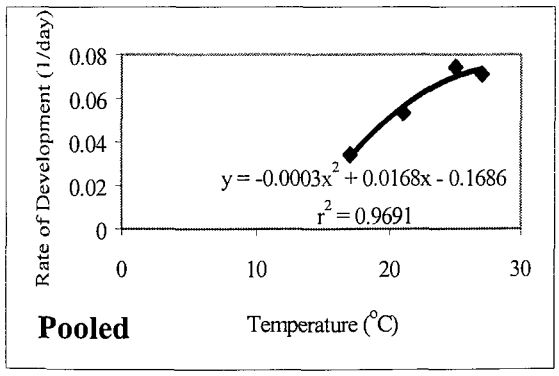
Figure 3.7 **Polynomial and linear relationship between temperature and nymphal developmental rate of *Hauptidia maroccana* on French bean.**











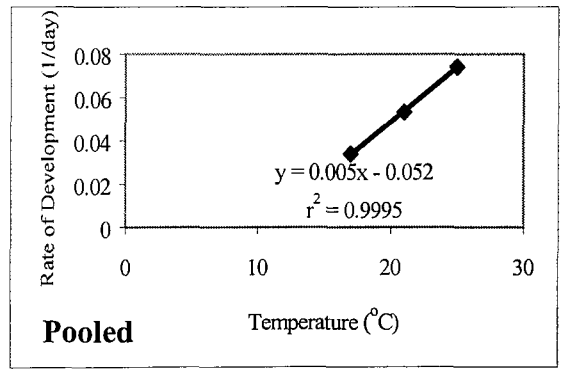
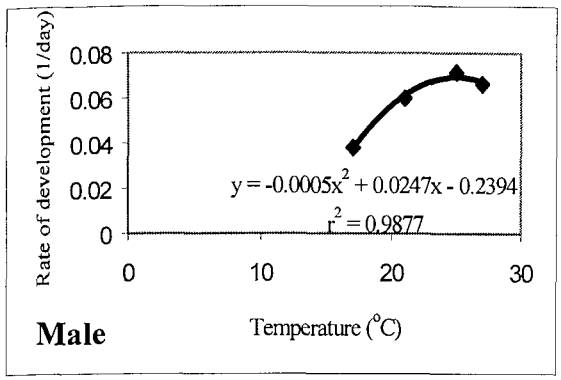
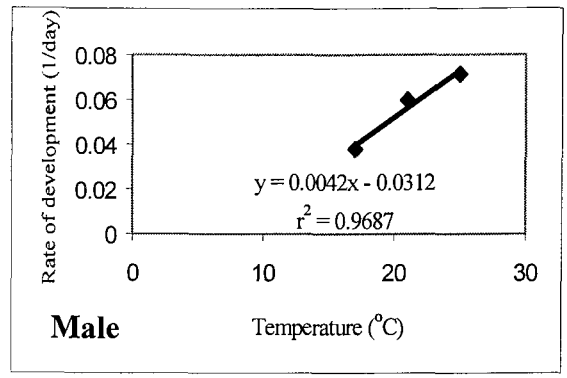
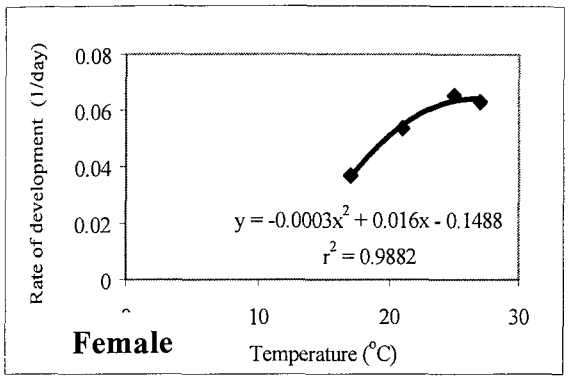
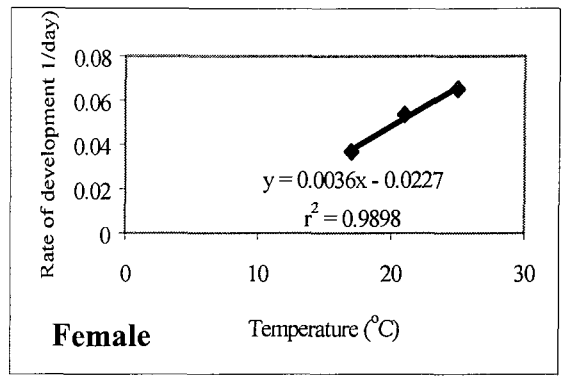


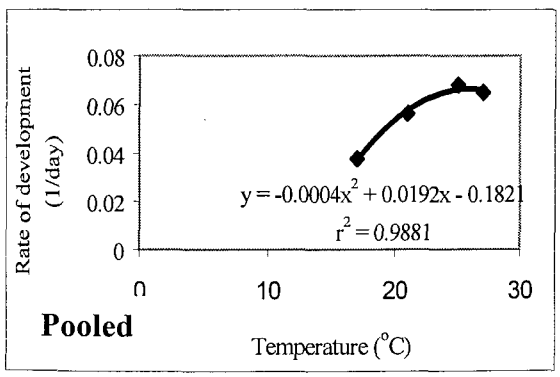
Figure 3.8 **Polynomial and linear relationship between temperature and nymphal developmental rate of *Hauptidia maroccana* on Primula.**











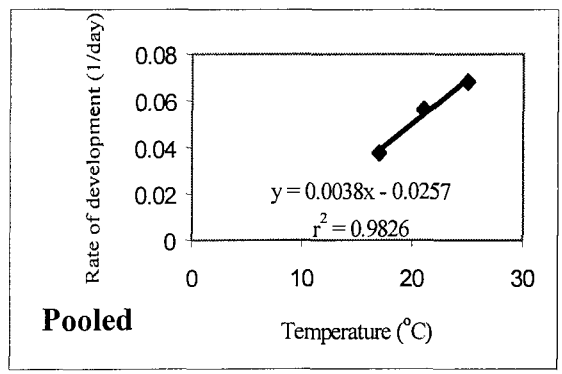


Figure 3.9 Polynomial and linear relationship between temperature and nymphal developmental rate of *Hauptidia maroccana* on Sweet pepper.

3.5.4 Survival of immature stages

Both temperature and host plant had a significant effect on overall survival of nymphal stages of *H. maroccana*. Highest survival was observed at 17°C. Rate of survival was found to decrease with a rise in temperature. After 25°C survival rate declines sharply on almost all host plants (Table 3.14). On average, 75-100% nymphs survived at 17°C while at 27°C, only 25-83% survived on different host plants. Different host plants also had a significant effect on nymphal survival. On French bean, survival was always the lowest (26-75%) followed by Cucumber (25-77%). On Primula, there was no significant variation in survival at different temperatures. Survival of *H. maroccana* nymphs on different host plants at different constant temperatures is presented in Table 3.15-3.19.

Table 3.14 Effects of different constant temperatures and host plants on the overall survival of *Hauptidia maroccana* during nymphal development.

Host plant	% Survival from first instar nymph to adult mean±s.e.				χ^2 test
	17°C	21°C	25°C	27°C	
Cucumber	77.27 ± 9.93 (17/22)	72.73 ± 9.50 (16/22)	63.64 ± 10.26 (14/22)	25.00± 8.18 (7/28)	df=3 P<0.001 χ^2=18.04
Primula	90.90 ± 6.13 (20/22)	85.00 ± 7.98 (17/20)	100.00 ± 0 (20/20)	81.82± 8.22 (18/22)	df=3 P=0.248 χ^2=4.127
French Bean	75.00 ± 8.84 (18/24)	52.00 ± 9.99 (13/25)	26.09 ± 9.16 (6/23)	55.56± 9.56 (15/27)	df=3 P=0.010 χ^2=11.41
Tomato	95.45 ± 4.44 (21/22)	70.00 ± 0.25 (14/20)	100.00 ± 0 (20/20)	62.50± 9.88 (15/24)	df=3 P=0.002 χ^2=14.817
Sweet Pepper	100.00±0 (24/24)	100.00 ± 0 (23/23)	70.83 ± 9.28 (17/24)	83.33± 5.38 (20/24)	df=3 P=0.003 χ^2=13.23
χ^2 test	df=4 P=0.042 χ^2=9.96	df=4 P=0.003 χ^2=16.30	df=4 P<0.001 χ^2=39.16	df=4 P<0.001 χ^2=24.38	

Figures in parentheses indicate number of nymphs reached adulthood/initial no. of nymphs used.
SE calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion survived, q =1-p. P value calculated from χ^2 analysis.

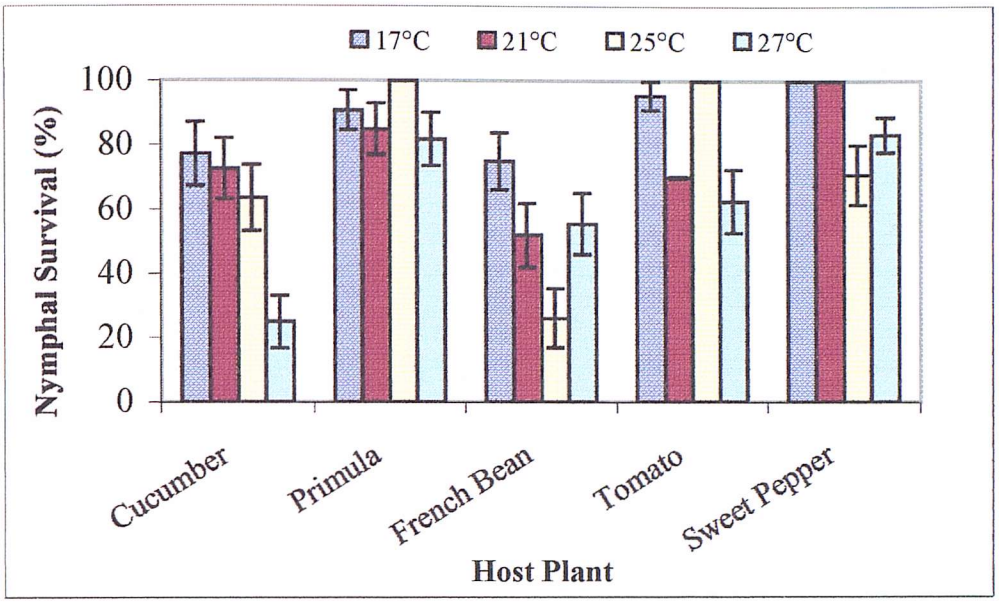


Figure 3.10 **Effect of different constant temperatures and host plants on the overall survival of *Hauptidia maroccana* during nymphal development. Vertical bars represent the standard errors of mean.**

Table 3.15 Effects of different constant temperatures on the survival of different nymphal instars of *Hauptidia maroccana* during development on Cucumber.

Nymphal instar	% survival to next instar mean ± s.e.* (n)**				χ^2-test
	17°C	21°C	25°C	27°C	
1st	100±0 (22/22)	100±0 (22/22)	100±0 (22/22)	100±0 (28/28)	-
2nd	100±0 (22/22)	100±0 (22/22)	100± 0 (22/22)	96.43±3.51 (27/28)	-
3rd	100±0 (22/22)	95.45±4.44 (21/22)	90.9±6.13 (20/22)	85.71±6.61 (24/28)	χ^2 =4.03 df =3 *P* >0.05
4th	90.9±4.33 (20/22)	95.45±4.44 (21/22)	72.73±9.5 (16/22)	57.14± 9.35 (16/28)	χ^2 =13.34 df =3 *P* <0.05
5th	77.27±9.93 (17/22)	72.73±9.5 (16/22)	63.64± 10.26 (14/22)	17.86±7.24 (5/28)	χ^2 =23.50 df =3 *P* <0.001
χ^2-test	χ^2=14.65 df= 4 *P* <0.01	χ^2 =16.99 df= 4 *P* <0.01	χ^2 =19.31 df= 4 *P* <0.001	χ^2 =64.88 df= 4 *P* <0.001	

* : s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where *p*= proportion survived, *q* =1-p

'-' χ^2 analysis could not be possible due to lack of data.

** : n = no reached next instar /initial no. of nymphs used.

P value calculated from χ^2 analysis.

Table 3.16 Effects of different constant temperatures on the survival of different nymphal instars of *Hauptidia maroccana* during development on Tomato.

Nymphal instar	% survival to next instar mean ± s.e.* (n)**				χ^2-test
	17°C	21°C	25°C	27°C	
1st	100±0 (22/22)	100±0 (20/20)	100±0 (20/20)	100±0 (24/24)	-
2nd	100±0 (22/22)	80±8.94 (16/20)	100± 0 (20/20)	95.83±4.07 (23/24)	χ^2 =10.06 df= 3 P <0.05
3rd	95.45±4.44 (21/22)	75±9.68 (15/20)	100±0 (20/20)	83.33±7.61 (20/24)	χ^2 =7.78 df=3 P <0.05
4th	95.45±4.44 (21/22)	75±9.68 (15/20)	100± 0 (20/20)	70.83±9.28 (17/24)	χ^2 =10.69 df =3 P <0.05
5th	95.45±4.44 (21/22)	70±10.25 (14/20)	100± 0 (20/20)	65.23±9.88 (15/24)	χ^2 =14.82 df =3 P <0.01
χ^2-test	-	χ^2 =6.88 df= 4 P >0.05, NS	-	χ^2 =16.97 df= 4 P <0.01	

* : s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion survived, q =1-p

'-' χ^2 analysis could not be possible due to lack of data.

(n) ** = no reached next instar /initial no. of nymphs used.

P value calculated from χ^2 analysis.

Table 3.17 Effects of different constant temperatures on the survival of different nymphal instars of *Hauptidia maroccana* during development on French bean.

Nymphal instar	% survival to next instar mean ± s.e.* (n)**				χ^2-test
	17°C	21°C	25°C	27°C	
1st	100±0 (24/24)	100±0 (25/25)	100±0 (23/23)	100±0 (27/27)	-
2nd	95.83±4.08 (23/24)	92±5.43 (23/25)	95.65±4.25 (22/23)	100±0 (27/27)	-
3rd	95.83±4.08 (23/24)	84±7.33 (21/25)	78.26±8.6 (18/23)	92.59±3.56 (25/27)	χ^2 =4.34 df=3 *P*>0.05 NS
4th	87.5±6.75 (21/24)	76±8.54 (19/25)	73.91±9.15 (17/23)	70.37±8.79 (19/27)	χ^2 =2.28 df=3 *P* >0.05 NS
5th	75.0±8.84 (20/24)	52±9.99 (13/25)	26.09±9.16 (6/23)	55.55±9.56 (15/27)	χ^2 =11.41 df=3 *P* <0.01
χ^2-test	χ^2 =11.41 df=4 *P* <0.05	χ^2=21.87 df=4 *P* <0.001	χ^2=42.15 df=4 *P* <0.001	χ^2 =31.28 df=4 *P* <0.001	

* : s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion survived, q =1-p

'-' χ^2 analysis could not be possible due to lack of data.

(n) ** = no reached next instar /initial no. of nymphs used.

P value calculated from χ^2 analysis.

Table 3.18 Effects of different constant temperatures on the survival of different nymphal instars of *Hauptidia maroccana* during development on Primula.

Nymphal instar	% survival to next instar mean ± s.e.* (n)**				χ^2 -test
	17°C	21°C	25°C	27°C	
1st	100±0 (22/22)	100±0 (20/20)	100±0 (20/20)	95.45±4.44 (21/22)	-
2nd	100±0 (22/22)	100±0 (20/20)	100± 0 (20/20)	95.45±4.44 (21/22)	-
3rd	100±0 (22/22)	95.00±4.87 (19/20)	100±0 (20/20)	95.45±4.44 (15/22)	χ^2 =7.6 df=3 *P* =<0.01
4th	100±0 (22/22)	95.00±4.87 (19/20)	100± 0 (20/20)	81.82± 7.12 (18/22)	χ^2 =8.25 df =3 *P* =<0.05
5th	90.90±6.13 (20/22)	85.00± 7.89 (17/20)	100± 0 (20/20)	81.82±8.22 (18/22)	χ^2 =4.13 df=3 *P* =0.248
χ^2 –test	-	-	-	χ^2 =8.78 df= 4 *P* =0.067	

* : s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where *p*= proportion survived, *q* =1-p

'-' χ^2 analysis could not be possible due to lack of data.

(n) ** = no reached next instar /initial no. of nymphs used.

P value calculated from χ^2 analysis.

Table 3.19 Effects of different constant temperatures on the survival of different nymphal instars of *Hauptidia maroccana* during development on Sweet pepper

Nymphal instar	% survival to next instar mean ± s.e.* (n)**				χ^2-test
	17°C	21°C	25°C	27°C	
1st	100±0 (21/21)	100±0 (23/23)	100±0 (24/24)	95.45±4.44 (21/22)	-
2nd	100±0 (21/21)	100±0 (23/23)	100± 0 (24/24)	95.45±4.44 (21/22)	-
3rd	100±0 (21/21)	100±0 (23/23)	91.67±5.64 (22/24)	95.45±4.44 (15/22)	-
4th	100±0 (21/21)	100±0 (23/23)	87.5±6.75 (21/24)	81.82± 7.12 (18/22)	χ^2 =5.9 df= 3 P >0.05
5th	100±0 (21/21)	100±0 (23/23)	70.83±9.28 (17/24)	81.82±8.22 (18/22)	χ^2 =13.2 df =3 P <0.01
χ^2-test	-	-	χ^2=11.8 df= 4 P <0.05	χ^2 =4.4 df =4 P >0.05	

* : s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion survived, q =1-p

'-' χ^2 analysis could not be possible due to lack of data.

(n) ** = no reached next instar /initial no. of nymphs used.

P value calculated from χ^2 analysis.

3.5.5 Relative growth rate (RGR) of *H. maroccana*

The effect of temperature and host plant on RGR of *H. maroccana* is presented in Table 3.21 –3.23. The weight of 1st instar nymphs used to calculate RGR is presented in Table 3.20. A significant difference was observed in RGR from nymph to adults developed at different constant temperatures. RGR for both males and females tend to be faster with increasing temperature. On average, RGR was almost always slower on Primula than on other host plants. RGR for males varied from 0.015 to 0.121 mg/mg/day on different host plants at 17° C while it was 0.172-0.224 mg/mg/day at 25° C (Table 3.21). A slower RGR was observed for females than for males, 0.09-0.115 mg/mg/day at 17° C and 0.164-0.206 mg/mg/day at 25° C (Table 3.22).

Table 3.20 Weight of 1st instar nymphs of *Hauptidia maroccana* emerging from different host plants at 21°C.

Host Plant	n	Weight (mg)*
Cucumber	16	0.038 ± 0.0014 ab
Tomato	21	0.035 ± 0.0008 b
French Bean	11	0.035 ± 0.0014 b
Primula	17	0.039 ± 0.0011 ab
Sweet pepper	20	0.041 ± 0.0006 a

Means in columns, followed by the same letter are not significantly different from each other at $P \leq 0.05$
One-way ANOVA followed by Least Significant Difference test (LSD=0.003, df= 80, $P<0.001$)
n = no. of insects as replicates.
* Weight used to calculate RGR.

Table 3.21 **Effect of different constant temperatures and host plants on the relative growth rate of male *Hauptidia maroccana* during nymphal development.**

Host plants	Relative growth rate (mg/mg/day) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	0.105± 0.0014 a C (7)	0.166± 0.0018 a B (4)	0.184± 0.0060 c A (6)	0.172± 0.0051 a B (4)	df=17 P<0.001 LSD=0.0129
Tomato	0.108± 0.0021 a D (9)	0.153± 0.0034 a C (5)	0.203± 0.0035 b A (10)	0.189± 0.0042 a B (7)	df=27 P<0.001 LSD=0.0103
French Bean	0.109± 0.0012 a C (9)	0.158± 0.0019 a B (5)	0.224± 0.0072 a A (4)	0.221± 0.0080 a A (7)	df=21 P<0.001 LSD=0.0119
Primula	0.095± 0.0018 b D (8)	0.144± 0.0023 a C (8)	0.175± 0.0059 c A (8)	0.157± 0.0029 a B (10)	df=30 P<0.001 LSD=0.0101
Sweet pepper	0.121± 0.0020 b B (10)	0.149± 0.0061 a AB (9)	2± 0.0051 c AB (4)	0.201± 0.0477 a A (10)	df=29 P=0.2255 LSD=0.0930
One-way ANOVA	df=38 P<0.001 LSD=0.0048	df=26 P=0.3612 LSD=0.0226	df=27 P<0.001 LSD=0.0168	df=33 P=0.7554 LSD=0.0879	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates

Table 3.22 Effect of different constant temperatures and host plants on the relative growth rate of female *Hauptidia maroccana* during nymphal development.

Host plants	Relative growth rate (mg/mg/day) mean±s.e. (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	0.107± 0.0009 b C (10)	0.159± 0.0039 ab B (10)	0.187± 0.0029 b A (9)	0.168± 0.0063 b B (6)	df=31 *P*<0.001 LSD=0.0102
Tomato	0.109±0 .0021 d D (11)	0.163± 0.0064 a C (7)	0.206± 0.0043 a A (10)	0.190± 0.0078 a B (5)	df=29 *P*<0.001 LSD=0.014
French Bean	0.115± 0.0015 a C (9)	0.152± 0.0052 abc B (7)	0.201± 0.0043 ab A (7)	0.169± 0.0144 ab B (7)	df=26 *P*<0.001 LSD=0.0217
Primula	0.090± 0.0023 d D (9)	0.142± 0.0031 c C (9)	0.187± 0.0079 b A (8)	0.162±.0028 b B (10)	df=32 *P*<0.001 LSD=0.0124
Sweet pepper	0.113± 0.0015 c C (8)	0.149± 0.0039 bc B (10)	0.164± 0.0076 c A (6)	0.162± 0.0027 b A (10)	df=30 *P*<0.001 LSD=0.0129
One-way ANOVA	df=41 *P*<0.001 LSD=0.0069	df=36 *P*<0.05 LSD=0.0127	df=35 *P*<0.001 LSD=0.0159	df=33 *P*=0.0156 LSD=0.0210	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

3.5.6 Effect of temperature on adult longevity of *H. maroccamna*.

The adult longevity of male and female *H. maroccana* is presented in Table 3.23. Longevity of both male and female significantly decreased with increasing temperature. Adult longevity was longest (49.21 and 58.85 days at 17°C) and shortest (15.04 and 23.93 days at 27°C) for males and females, respectively. Females lived significantly longer than the males at 27°C. Whereas, the longevity of male and female at 21°C was not significantly different ($P>0.05$) from that observed at 25°C (Table 3.23).

The distribution of survival pattern presented in Figure 3.11 shows the 50% survival point for male as 47.5, 40 and 12 days after emergence at 21, 25 and 27°C, respectively, whereas, for female the 50% survival point was observed as 58, 43 and 23 days after emergence at 17, 25 and 27°C, respectively.

Table 3.23 Effect of different constant temperatures on the adult longevity of male and female *Hauptidia maroccana*.

Temperature (°C)	Longevity (days) mean±s.e.				Two-Sample t test
	n	Male	n	Female	
21	14	49.21±3.85 a A	13	58.85±3.37 a A	P=0.072 t=-1.88, df=24
25	14	41.21±1.22 b A	14	41.50±2.22 b A	P=0.91 t=-0.11, df=20
27	23	15.04±0.96 c B	14	23.93±1.29 c A	P<0.001 t=-5.53, df=26
One-way ANOVA		LSD=6.0024 df=48 P<0.001		LSD=6.8461 df=38 P<0.001	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P\leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

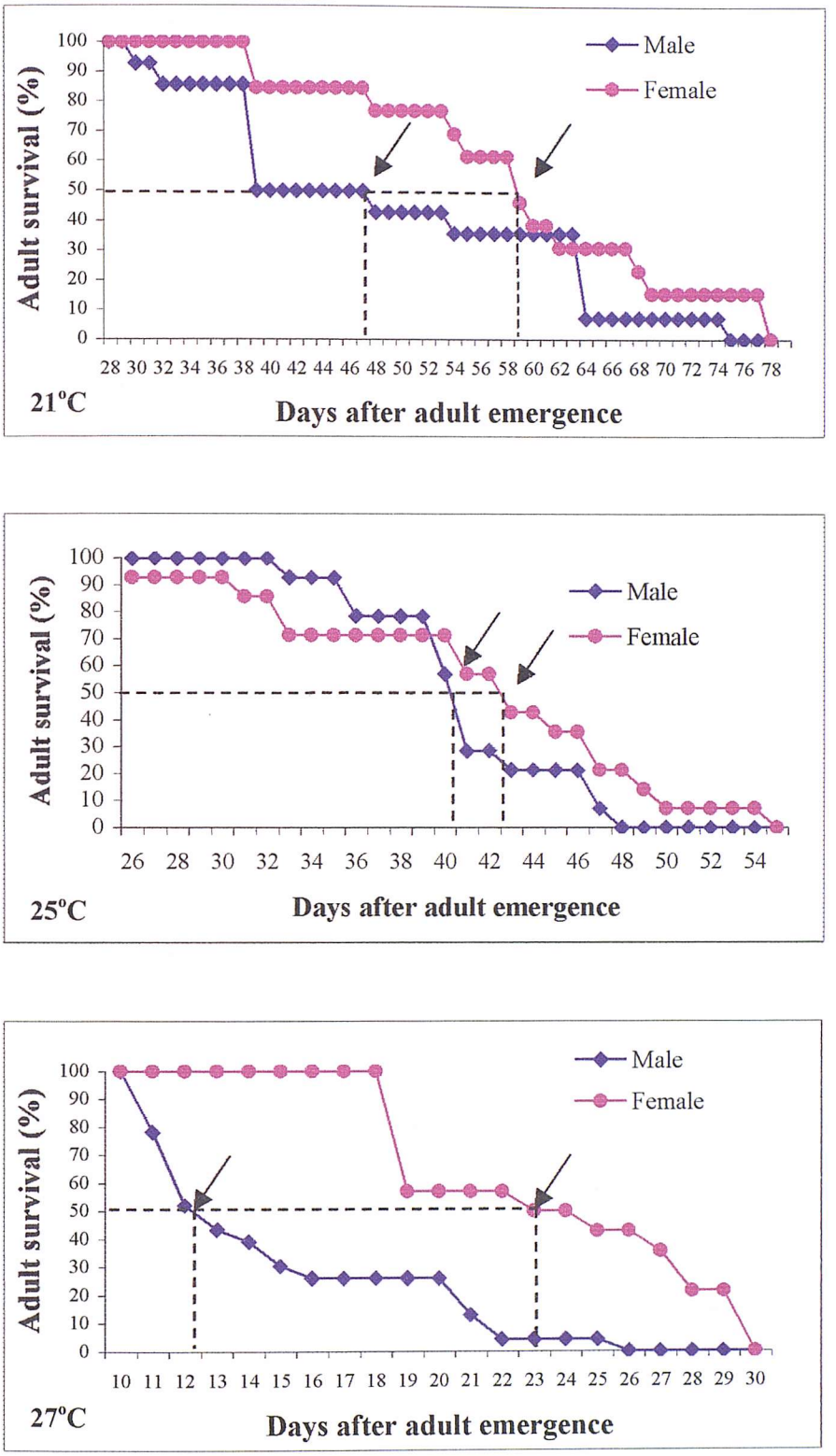


Figure 3.11 Survival pattern of male and female *Hauptidia maroccana* at different constant temperatures. Arrows indicate the 50% survival point.

3.5.7 Fecundity and life table parameters of *H. maroccana*

The mean number of eggs laid by *H. maroccana* throughout its lifetime is presented in Figure 3.12. The maximum number of eggs were laid from day 11-15 after adult emergence. In general, oviposition rate declined with the age of the adults. The pattern of age-specific fecundity (m_x) and the survival rate (l_x) are demonstrated in Figure 3.13. The intrinsic rate of natural increase and other life table parameters are presented in Table 3.24. The net reproductive rate of *H. maroccana* was 5.5 females/female/generation and the intrinsic rate of increase was 0.033 female eggs/female/day. The population doubling time was observed as 20.79 days.

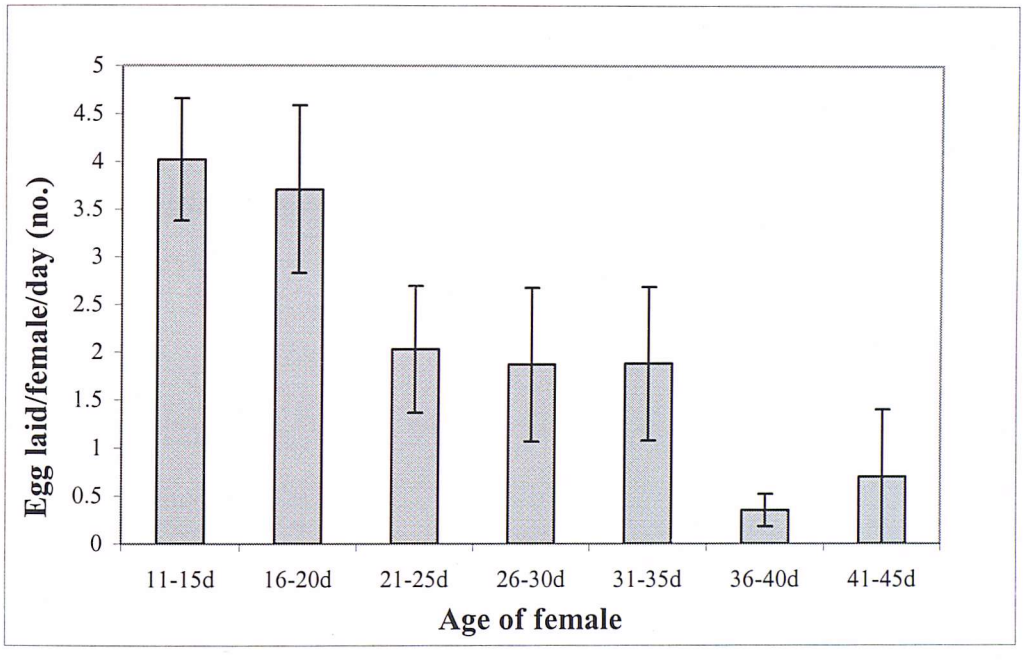


Figure 3.12 Age specific fecundity of *Hauptidia maroccana* females on the host plant Primula at 21°C. Vertical bars represent the standard errors of mean (7 replications).

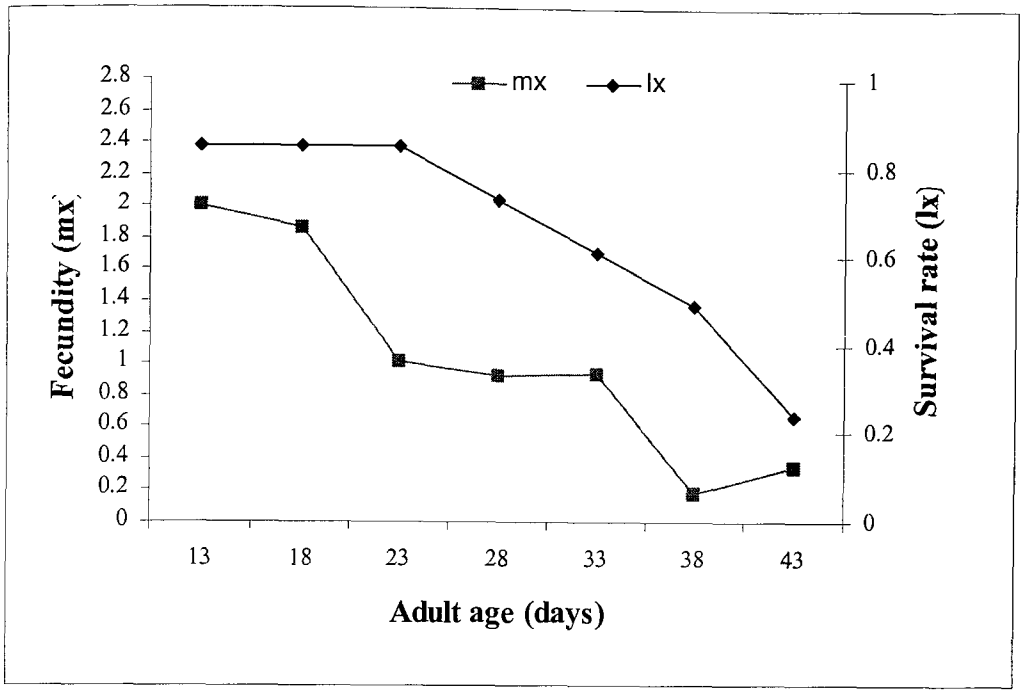


Figure 3.13 **Fecundity and survival rate of *Hauptidia maroccana* on Primula at 21°C. Age specific fecundity (m_x) is calculated considering 50% female proportions (Copland and Soeprapto, 1985).**

Table 3.24 **Life table parameters of the glasshouse leafhopper *Hauptidia maroccana* at 21°C on the host plant Primula.**

Life table parameters	
Gross reproductive rate (*GRR*)	7.2899 **Female eggs/female/generation**
Net reproductive rate (R_o)	5.581 **Females/female/generation**
Capacity of increase (r_c)	0.03279 **Female offspring/female/day**
Intrinsic rate of increase (r_m)	0.03341 **Female eggs/female/day**
Cohort generation time (*Tc*)	52.4260 **Days**
Generation time (*T*)	51.5680 **Days**
Finite capacity for increase (λ)	1.0339 **Female offspring/female/day**
Doubling time (*DT*)	20.7892 **Days**

3.6 Discussion

3.6.1 Egg incubation and nymphal development of *H. maroccana* at different constant temperatures on different host plants

The result showed that as in many other insects, temperature influenced each developmental stage (egg to adult) of *H. maroccana*. The shortest and the longest egg incubation were observed at 25 and 17°C, respectively, irrespective of host plant. The lower threshold and the thermal constant for the development of eggs ranged 2.58 to 7.09°C and 171.1 to 218.3°Days, respectively in different host plants.

Temperature and host plants had a significant effect on the development of each of the five nymphal instars (Table 3.4-3.8). Developmental period of each instar tended to shorten with increasing temperature within the temperature range of 17 to 25°C on different host plants. Total development duration of nymphal stages of *H maroccana* decreased with increasing temperature on different host plants (Table 3.9-3.11). The increase in developmental rate with increasing temperature was consistent with the description of temperature effect on insect development by many authors (Wagner *et al.*, 1984; Teulon and Penman, 1991; Shishehbor and Brennan, 1996; Wang and Tsai, 1996; Soultani, 1997). The developmental rate of individual nymphal instars and total nymphal period of *H. maroccana* is linearly related to temperature over a certain range of temperatures in different host plants (r^2= 0.97-1.0) (Table 3.12). The slope of the curves relating developmental rate to temperature was similar to that observed for many other insect species (Campbell *et al.*, 1974; Lamb *et al.*, 1987; McDonald *et al.*, 1998). Liu and Tsai (1998) also described a linear trend in the development of spider mites *Tetranychus tumidus* Banks at the range of 15-30°C. Development rate decreased sharply at 35°C. The decline in developmental rate at 27°C indicated that the optimum temperature for development could be 17-25°C. This linear model estimates a threshold temperature of 6.76 to 10.40°C and a requirement of 200 to 238 degree days for nymphal development (Table 3.12). The use of non-linear models to estimate developmental rate was considered unnecessary, because although at low and high sections of the developmental rate versus temperature curve the relationship is often non-linear, this was not apparent in this study, over the total range of temperature used.

In Primula, *H. maroccana* took a longer time to develop and the lower development threshold was the highest for both males and females among all the host plants. The high lower threshold in Primula indicated that they tolerate a narrow range of temperature on Primula but a minimum day degree was needed for development. On the other hand, the lowest lower development threshold and the highest day degree requirement was observed in Sweet pepper for development. It can be concluded that the variation in developmental threshold in different host plants can be attributed to the nutritional quality of the host plant. Many authors reported a high developmental threshold in the host plant with a high nutritional quality (Lamb and Loschiavo, 1981; Dixon *et al.*, 1982)

. The proportion of the developmental period allocated to each immature stage was found to be almost similar at different temperatures and sometimes the periods were compensated by one another among different instars (Figure 3.14). The nymphs spent the maximum time of their total developmental period during 5th instar at all temperatures.

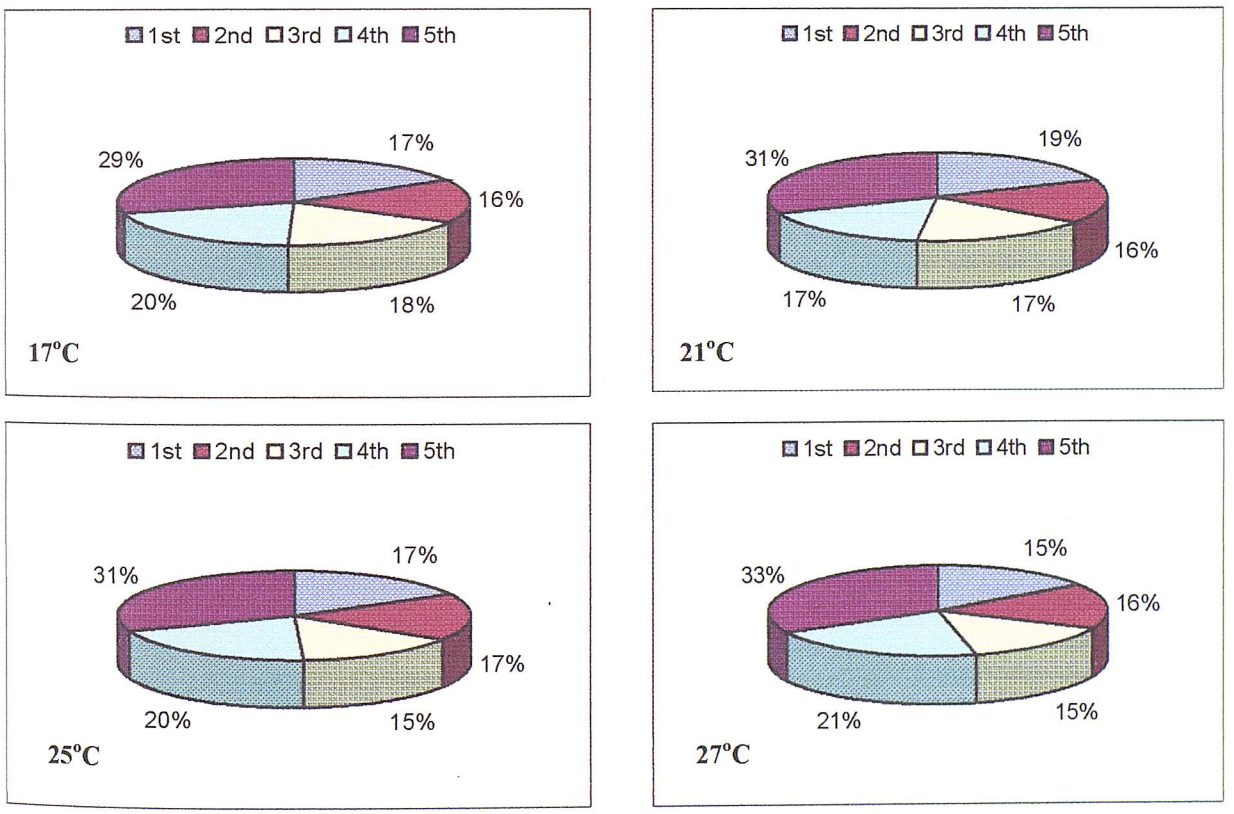


Figure 3.14: Time spent (% of total nymphal period) during nymphal development of *Hauptidia maroccana* at different constant temperatures.

3.6.2 Survival of immature stages

There were significant differences in the survival of nymphal stages at different temperatures. In a preliminary trial, 99% of the nymphs died just after emergence at 31°C. Therefore development was not studied at 31°C. The lowest survival at 27°C indicated that the upper development threshold of *H. maroccana* lies between 25-27°C for nymphal stages. High mortality was observed in the later nymphal instar than in the earlier stages. Among different host plants, lowest survival was observed in French bean resulting from the high mortality of the nymphs. Most of the mortality was due to catching of the nymphs on the hooked shaped trichomes. On the other hand, highest nymphal survival was observed in Primula. This differential mortality at the nymphal stages is due to the leaf topography and the presence of different trichomes. Elden and McCaslin (1997) also reported high mortality in adults (13-96%) and nymphs (0-33%) of potato leafhopper due to the presence of glandular hairs in alfalfa (*Medicago* species).

3.6.3 Growth rate of *H. maroccana*

The response of adult weight to rearing temperature has not been studied for as many insects as that of developmental rate, and is less closely related to rearing temperature than developmental period (Lamb *et al.*, 1987). Weight of adults usually decreases with increasing temperature (Lamb *et al.*, 1987). Results of the present study showed a significant decrease in adult weight of *H. maroccana* with increasing temperature (Appendix 1) which is attributed to faster development and high RGR in different host plants (Table 3.21-3.23). Females were found to have a lower RGR than that of males at 17 and 25°C (Table 3.23).

The main factor in reducing the rate of population growth at 17° C was the very long developmental time although the weight of adults was slightly higher at low temperatures. There was not any consistent relationship between host plants and RGR. However RGR of *H. maroccana* was almost always lower in Primula among the different host plants. In general the results obtained from this investigation among other things, shows the considerable shortening of growth duration with increasing temperature, contributing a faster intrinsic rate of population increase (Price, 1984).

3.6.4 Adult longevity of *H. maroccana* at different constant temperatures

Temperature significantly affected the adult longevity of both male and female *H. maroccana*. Longevity decreased with increasing temperature for both sexes. Adult longevity was considerably longer for female than for male, which is in agreement with the observation of Copland and Soeprapto (1985). Asante *et al.* (1991) and Wang *et al.* (1997) observed a similar phenomena in *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae) and *Aphis nasturii* Kaltenbach, (Homoptera: Aphididae), with the life span of the female being extended at low temperatures.

3.6.5 Fecundity and life table parameters of *H. maroccana*

On average female *H. maroccana* laid 65.6±13.7 eggs, ranging from 21-139 eggs per female in their 40±2.86 days average lifetime. Females were found to lay most of their eggs during the first 20 days of their emergence. Oviposition rate decreased with the age of adult female and only a few eggs were laid in the later part of their life (Figure 3.11).

The intrinsic rate of increase, mean generation time and population doubling time are useful indices of population growth under a given set of growing conditions (Tsai and Liu, 2000). Birch (1948) described intrinsic rate of natural increase (r_m) as a useful summary parameter to characterize the influence of abiotic factors, in particular temperature, on the growth potential of an insect.

In the present study, the life table parameters analysis clearly indicated that the age specific fecundity (m_x) decreased sharply after 18 days of their emergence. As most of the eggs has been laid in the first few weeks of their emergence, oviposition in the latter part did not contribute much to the r_m values. A slight increase of m_x after 38 days might be due to very low survival, which gave a relatively high m_x. Birch (1948) reported that the first two and three weeks of the oviposition by *Sitophilus oryzae* (L.) contributed 85% and 94%, respectively towards the final r_m value. Similarly in *H. marocccana*, the r_m is influenced to a greater extent by the fecundity of the earlier part of their oviposition period.

In general, temperature and host plants had a significant influence on *H. maroccana* survivorship, growth and development and longevity and fecundity. This study of temperature and host plant dependent development and survivorship may contribute to improving rearing methods of the glasshouse leafhopper and this first approach needs to be complemented by evaluating the different life history parameters in different host plants at fluctuating as well as constant temperatures.

Chapter 4

Effect of host egg age on some components of parasitoid fitness

4.1 Introduction

In parasitoid wasps, host quality is associated with the successful development and emergence of wasps in the process of parasitism (Mackauer and Sequeira, 1993) and is related directly to successful parasitism, development duration and the size of parasitoid offspring (Karamaouna and Copland, 2000). Host size frequently influences the parasitoid's development time, the size of the developing parasitoid and its fitness as an adult (Murdoch *et al.*, 1992; Godfray, 1994; Muller *et al.*, 1996). Host size is also known to affect some life history characteristics, including fecundity, development time, and progeny sex ratio in some parasitoids (Smith, 1993; Karamaouna and Copland, 2000). Larger hosts contain more resources and consequently yield larger and more female parasitoid offspring (Godfray, 1994; Karamaouna and Copland, 2000). Larger male and females usually live longer than smaller individuals, larger females have a higher daily and life time fecundity than smaller females, while larger males copulate with more females and sire more daughters than smaller males (de Jong and van Alphen, 1989; Godfray, 1994; Ode *et al.*, 1996).

Numerous studies have shown that certain host stages (small host) are more likely to be used for host feeding than oviposition (Kidd and Jervis, 1991; Rosenheim and Rosen, 1992; Heimpel and Rosenheim, 1995). Host quality may therefore influence the fitness of a parasitoid.

4.2. Objectives of the study

The aim of the present work was to determine the influence of different ages of *H. maroccana* eggs on various aspects of male and female *A. atomus*:

a) developmental time of the parasitoid *A. atomus*
b) offspring size of the parasitoid
c) parasitoid survival (successful adult emergence)
d) potential fecundity of the female wasps
e) the offspring sex ratio (percentage of females out of total offspring emerged).

The null hypothesis:

The age of host egg does not have any affect on the development time, offspring size, survival, potential fecundity and the sex ratio of *A. atomus*.

4.3 Materials and Methods

A laboratory study was carried out to determine whether the developmental time, size, survival, fecundity and sex ratio of the parasitoid are related to host age. Three different ages of *H maroccana* eggs (i.e. 1-3, 4-6, 7-9 days-old) were used in this experiment. To obtain different ages of egg, healthy potted plants of *Primula vulgaris* with fully expanded leaves were placed in the mass culture of *H. maroccana* for three days. A large number of host insects were maintained inside the Perspex cage to ensure plenty of eggs. After seventy two hours of infestation the plants were removed from the Perspex cages containing 1-3 day-old leafhopper eggs. Leaf discs of 20 mm diameter containing leafhopper eggs were placed on agar medium in Petri-dishes with lower surface uppermost. The Petri-dishes containing leafhopper eggs were marked individually and kept in the incubator at 21°C. Thus, 1-3, 4-6 and 7-9 day old eggs were obtained 0, 3 and 6 days, respectively after placing the leaf discs on the agar medium.

One freshly emerged mated adult female of *A. atomus* along with a male was introduced into each Petri-dish containing leaf discs with a specific age of leafhopper eggs and placed in the incubator at a constant temperature (21°C). The parasitoid wasps were allowed to lay eggs for 24 hours. Each pair was removed 24 hours after infestation. Each agar plate was marked individually and kept in the incubator at 21°C for the development of parasitoid eggs. A total of 10 replications were maintained.

Ten days after laying eggs by *A. atomus*, leaves were cut into small pieces containing an individual parasitized egg (those which turned red) (Plate 4.1) and placed into 0.5ml clear plastic Eppendorf safe-locking microcentrifuge tubes with a piece of wet filter paper. These tubes were placed in the incubator in a plastic sandwich box. The humidity inside the boxes was maintained at 85% r.h. using saturated KCl salt solution. The tubes were checked everyday for the emergence of adult *A. atomus*. The data on emergence of individual parasitoids was recorded and sex was determined by the shape of the antennae (Plate 4.2). The duration of development of male and female wasps and the secondary sex ratio were calculated from these data. Another ten replications were done in the same way to calculate the survival of the parasitoid.

As a pro-ovigenic parasitoid, *A. atomus* adults emerge with all of its lifetime egg complements. Therefore, the females were dissected under a binocular microscope and the total number of eggs in the ovaries was counted (Plate 4.3 and 4.4). The parasitoid survival was calculated on the basis of the total emerging adults out of the total parasitized eggs (red eggs). Wasp hind tibia length (HTL) was used as an index of wasp size. Measurements of HTL and head width were made using a calibrated ocular micrometer mounted in the eyepiece of a binocular microscope.

The same procedure was followed for 4-6 and 7-9 day-old eggs. Ten Petri-dishes were used for each of the three age classes. In addition, ten *H. maroccana* eggs from each age class were taken out of the leaf vein carefully. Measurements of length and width of the eggs were done under a binocular microscope fitted with a calibrated ocular micrometer in the eyepiece. The approximate volume of the eggs were calculated as $\pi r^2 h$ where, π=3.14, r= half of the egg width and h= the length of the egg.

4.4 Statistical Analysis

In determining the development duration, each individual emerging parasitoid was considered as one replicate and subjected to one way analysis of variance (ANOVA) in SAS. When ANOVA showed significance, means for different ages of eggs were separated by Least Significant Difference (LSD) test. Due to unequal replication, Standard Error of Difference (SED) values for the separation of means were used, based

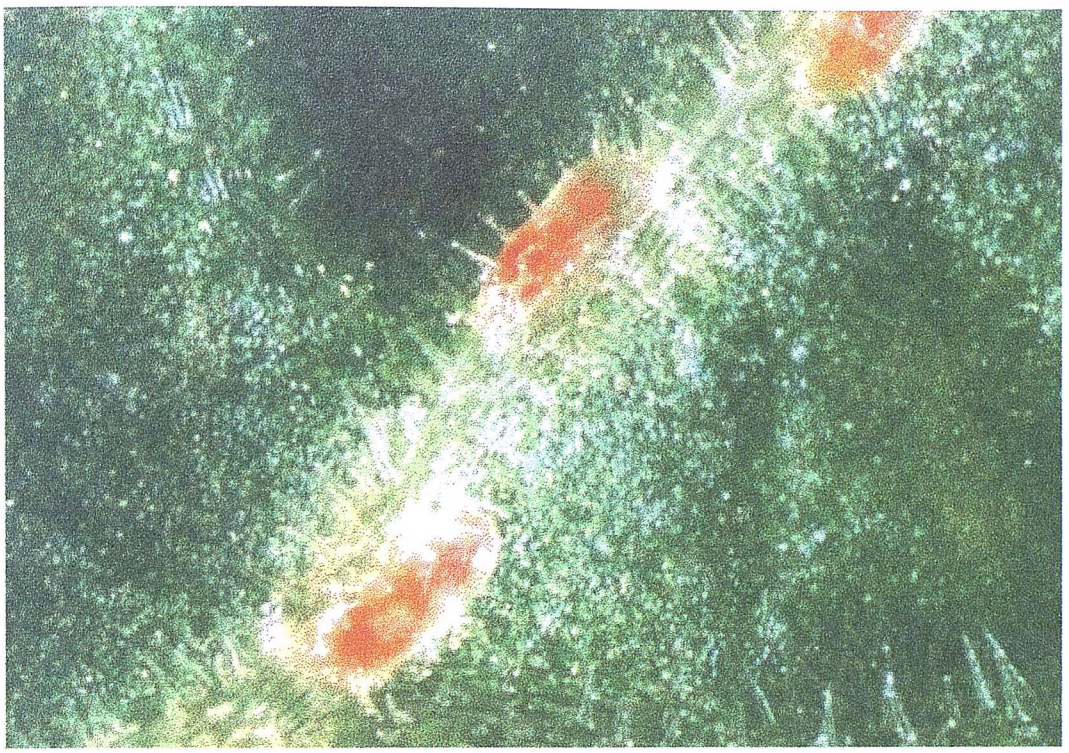


Plate 4.1: Parasitized eggs of *Hauptidia maroccana* by *Anagrus atomus* (x 50).

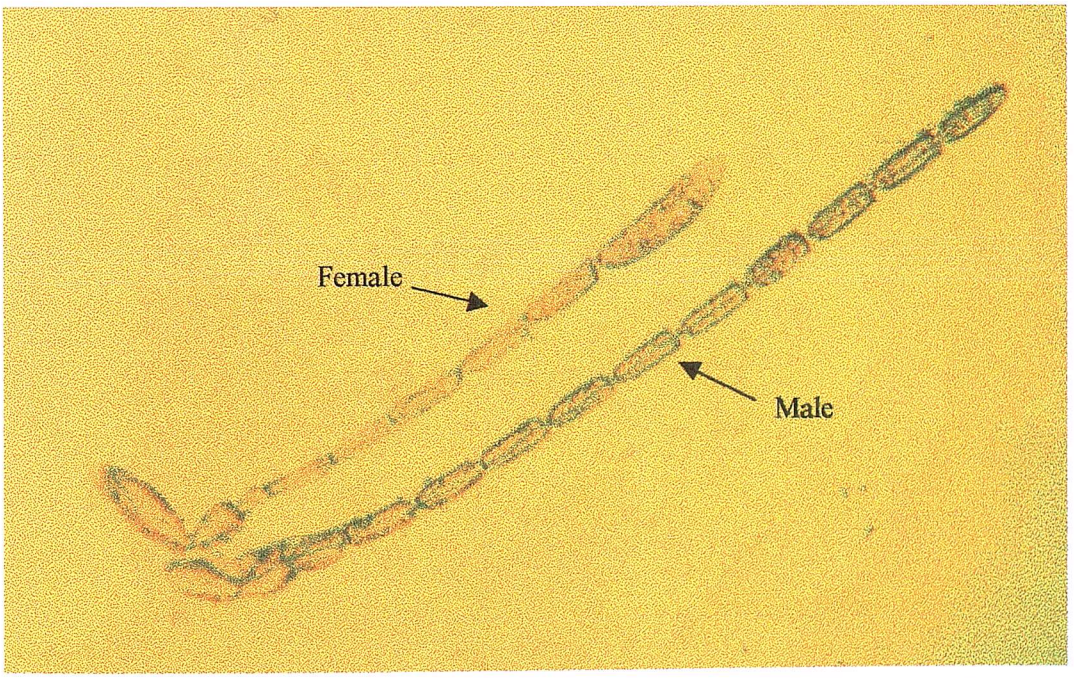


Plate 4.2: Male and female antennae of *Anagrus atomus* adults (x 150).



Plate 4.3 : Dissected abdomen of *Anagrus atomus* showing the matures eggs in the ovary (x 100).



Plate 4.4: Eggs of *Anagrus atomus* after dissecting the adult parasitoid wasp (x 150).

on the harmonic mean of the number of replicates (SAS Institute Inc., 1988). Head width, hind tibia length and potential fecundity of the adult females were also analysed using one-way ANOVA in SAS. Development duration of male and female was compared using Two-sample t test in MINITAB.

The effect of different ages of eggs on adult emergence and parasitoid sex ratio was determined by using χ^2 analysis provided that the expected value was more than five. In addition regression analysis was conducted in order to investigate any possible influence of the host size on parasitoid size and potential fecundity in MINITAB.

4.5 Results

4.5.1 Relationship between age and size of *H. maroccana* eggs

The volume of *Hauptidia maroccana* eggs in relation to their age is presented in Table 4.1. No significant difference was observed in egg volume with increasing age.

Table 4.1 Relationship between age and size of *H. maroccana* eggs.

Age of the eggs (days)	n	Volume (mm^3) Mean±s.e.
1-3	10	0.018±0.008 a
4-6	10	0.020±0.002 a
7-9	10	0.031±0.002 a
One-way ANOVA		P= 0.1388, df=27, LSD= 0.0138

Means in columns, followed by the same letter are not significantly different from each other at $P \leq 0.05$
One-way ANOVA followed by Least Significant Difference test.
n = no. of eggs measured.

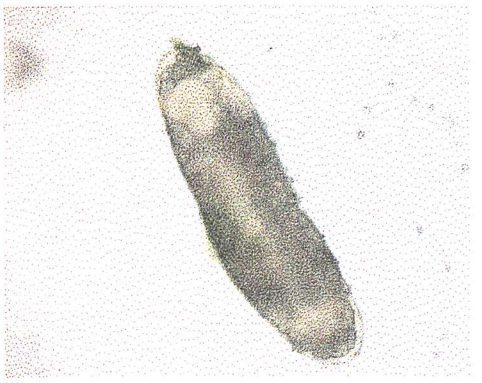
4.5.2 Developmental duration of *A. atomus*

Mean developmental duration from egg to adult of both male and female *A. atomus* was significantly affected by the age of host eggs (female: $P<0.001$, df=354, LSD= 0.3818; male: $P<0.001$, df= 170, LSD= 0.4289) (Table 4.2). Faster development has been observed in both males and females in older eggs (7-9 day-old). The longest duration was observed on 4-6 day-old eggs for both males and females. Males were always found to develop faster than the females regardless of host age ($P<0.001$). Some of the eggs were dissected at 1, 2, 3, 5, 7 and 9 days after parasitization and the morphological differences are presented in Plate 4.5.

Table 4.2 Pre-imaginal development time (days) for male and female wasps of *Anagrus atomus* in three different age classes of the host *Hauptidia maroccana* eggs.

Host age (days)	Development time (days) mean± s.e.				Two-sample t test
	n	Female parasitoid	n	Male parasitoid	
1-3	124	17.80±0.008 a A	77	16.75±0.099 b B	t=-6.34 df= 174 P <0.001
4-6	84	18.07±0.120 a A	41	17.32±0.133 a B	t=-4.22 df= 99 P <0.001
7-9	149	17.17±0.155 b A	55	15.84±0.209 c B	t=-5.11 df= 117 P <0.001
One-way ANOVA		P<0.001 df= 354 LSD= 0.3818		P<0.001 df= 170 LSD= 0.4289	

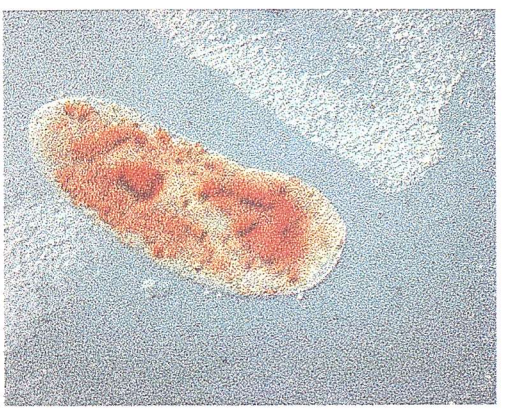
Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P\leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.



One day



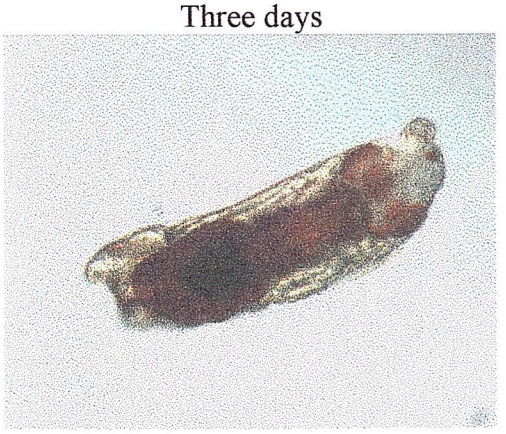
Two days



Three days



Five days



Seven days



Nine days

Plate 4.5: Immature stages of *Anagrus atomus* at increasing days after oviposition (x 75).

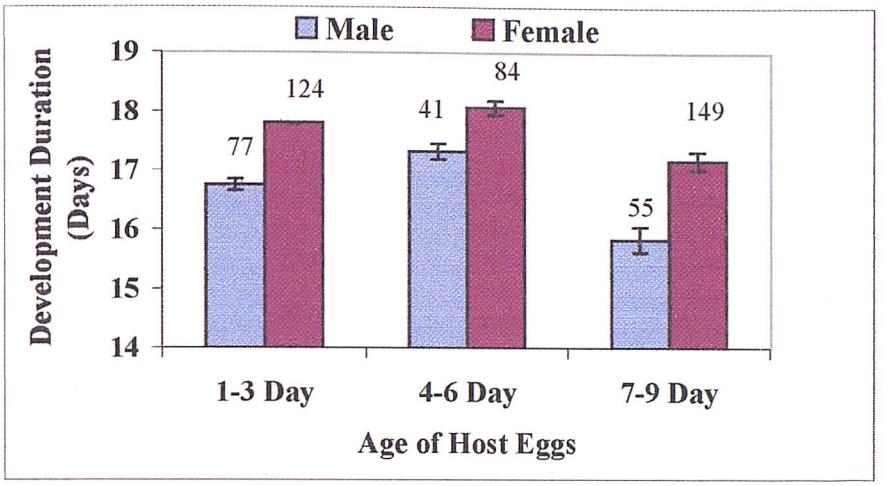


Figure 4.1 Pre-imaginal development time (days) for male and female of *Anagrus atomus* in three different age classes of the host *Hauptidia maroccana* eggs. Vertical bars represent the standard error of mean. Values above the bars represent number of observations (n).

4.5.3 Parasitoid survival in relation to host age

Survival of parasitoid progeny was measured as the percentage of the total number of host eggs parasitized. The χ^2 analysis showed a significant effect (P= 0.001, df=2, χ^2=13.86) of host age on parasitoid survival rate (Table 4.3). *Anagrus atomus* was able to develop successfully from all of the three host classes. However the age-related host size differences affected the survival, with the parasitoids being more successful when parasitizing the intermediate group. The highest percentage (80.59) of successful parasitoid emergence occurred in 4-6 day-old host eggs and declined in older eggs (7-9 day-old).

Table 4.3 Survival of pre imaginal stages of *Anagrus atomus* in different age classes of the host *Hauptidia maroccana* eggs.

Host age (days)	No. of parasitized (red) eggs	Number of adults emerged	Adult emergence (%)
1-3	217	150	69.12±3.14
4-6	134	108	80.59±3.42
7-9	228	141	61.84±3.22
χ^2 analysis			χ^2=13.86 df=2 , P< 0.01

s.e. was calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion female, q =1-p P value calculated from χ^2 analysis.

4.5.3 Parasitiod size in relation to host age

Measurements of head width and hind tibia length shows that size of the emerging *A. atomus* was affected significantly by different sizes of the host. Larger females emerged from the youngest class of the host (1-3 day-old. Size of the emerging female wasps did not vary significantly among 4-6 and 7-9 day-old eggs (Table 4.4).

Table 4.4 Mean head width and hind tibia length of female *Anagrus atomus* emerged from different age classes of the host *Hauptidia maroccana* eggs.

Host age (days)	Female parasitoid offspring			
	Head width		Hind tibia length	
	n	mean± s.e.	n	mean± s.e.
1-3	44	0.181±0.0010 a	44	0.201±0.0014 a
4-6	25	0.173±0.0011 b	25	0.191±0.0015 b
7-9	20	0.169±0.0017 b	20	0.187±0.0025 b
One-way ANOVA	P<0.001 df= 74 LSD= 0.004		P<0.001 df= 86 LSD= 0.0051	

Means in columns, followed by the same letter are not significantly different from each other at $P \leq 0.05$
One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates

4.5.4 Potential fecundity of the parasitoid

The number of mature oocytes contained in pro-ovigenic parasitoids ovaries at the time of their eclosion, represents the potential life time fecundity (Jervis and Copland, 1996). Results presented in Table 4.5 show that the potential fecundity of the females was not significantly influenced by different ages of *H. maroccana* eggs (P>0.05, df=84, LSD= 3.43).

Table 4.5 Potential fecundity (number of eggs in the ovaries on emergence) of *Anagrus atomus* emerged from different age classes of the host *Hauptidia maroccana* eggs reared at 21°C in Primula.

Host age (days)	Mean number of eggs	
	n	mean± s.e.
1-3	34	28.94±1.36 a
4-6	32	27.13±0.98 a
7-9	21	28.76±1.06 a
One-way ANOVA		P>0.05, NS df= 84 LSD= 3.43

Means in column, followed by the same letter are not significantly different from each other at $P \leq 0.05$
One-way ANOVA followed by Least Significant Difference test.
n = no. of insects dissected.

4.5.5 Host effect on sex ratio of the parasitoid

Host age played a significant role in determining the sex ratio of *A. atomus* with a tendency to emerge more female (fertilized) eggs from older hosts. The χ^2 analysis showed a significant difference in sex ratio of the parasitoids emerging from different aged host eggs (χ^2 = 5.93, df=2, P=0.05) (Table 4.6). A female biased sex ratio was observed in the offspring from all of the three classes of the host.

Table 4.6 Influence of different age classes of *Hauptidia maroccana* eggs on the secondary sex ratio of the parasitoid *Anagrus atomus*.

Host age (days)	Adult emergence (no.)			Emerged Female % ± s.e.
	Male	Female	Total	
1-3	77	124	201	61.69±3.42
4-6	41	84	125	67.20±4.20
7-9	55	149	204	73.04±3.11
				$\chi^2 = 5.93$, df =2 P =0.052

s.e. was calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion female, q =1-p P value calculated from χ^2 analysis.

4.6 Discussion

4.6.1 Host effect on development and survival of parasitoid

In the present study faster development was observed in the older eggs (7-9 day-old), which in turn reduces the adult size of the emerging parasitoids. The earlier stages of hosts may have less nutrient supply for the parasitoid at the time of parasitization and slowing down of the host development after parasitization may delay the development of the parasitoid in early instar hosts. A delayed development in younger hosts is also reported in many insect parasitoids (Lohr *et al.*, 1988; Islam and Copland, 1997). The faster development of daughters in large hosts decreases generation time, which is inversely related to the intrinsic growth rate of the parasitoid population. Thus if the fecundity is constant, any decrease in generation time will increase the value of its growth rate and thus enhance parasitoid fitness (Pandey and Singh, 1999). The early emergence of males could be an added advantage for the parasitoid in preventing oviposition by virgin females.

Pak *et al.* (1986) suggested the nutritional resources and the energy that an egg contains remain constant throughout embryonic development. However this is probably not the case. Roberson *et al.* (1987) challenged this assumption and showed not only that *Edovum putleri*, an eulophid, oviposited less often in older eggs, but also progeny mortality was greater in older eggs. In the present study, parasitoid survival was found to

reduce in older eggs. Survival ranged from 62-81% in different ages of host. Highest survival was observed in 4-6 day-old eggs. A number of authors have shown that successful parasitism of eggs by various egg parasitoids declines as the host ages (Powell and Shepard, 1982; Strand and Vinson, 1983; Reznik and Umarova, 1990).

4.6.2 Host effect on fecundity and size of emerging parasitoid

The potential fecundity of *A. atomus* was not influenced by the host age. This result is not in agreement with other authors working on different groups where they mentioned significantly more eggs at emergence in the females that developed in larger hosts (King and King, 1994; Harvey and Thompson, 1995; Karamaouna and Copland, 2000).

Parasitoid size is often used as an index of parasitoid quality, especially for female parasitoids. Thus, an attempt was made to investigate the relationship between host age and parasitoid size and its influence on parasitoid quality, i.e., egg load. In the present study, size of the emerging parasitoids was significantly affected by host age. Larger females emerged from the youngest host, which contradicts the findings of Miura and Kobayashi (1998) and King and Lee (1994). They mentioned a decrease in hind tibia length of the emerging female parasitoid of *Trichogramma chilonis* Ishii as the host eggs become older and of larger volume

4.6.3 Host effect on parasitoid sex ratio

In the current study it was not possible to demonstrate a change in host size with age. However, under host quality-dependent sex allocation (i.e., host age-dependent in this case), parasitoids are commonly observed to lay female eggs in large hosts and male eggs in small hosts. In this case adult parasitoid size depends strongly on host size and it seems likely that females gain greater marginal benefits than males from increased adult size (Mayhew and Godfray, 1997). Many models have been developed which predict adaptive sex allocation in response to host quality. In many solitary parasitoid species, adult size is correlated with host size and adult size is in turn correlated with fitness. The

greater fitness of bigger females may, for example, be due to a greater fecundity and longevity (Hardy, 1994).

In the current study, a female-biased sex ratio has been observed in the offspring of *A. atomus* developing from different ages of host eggs and significantly affected by host age (P =0.052, df=2, χ^2 = 5.93). More female offspring were found to emerge from the older hosts. Similar observations have been found by many authors on different insect parasitoids (Bernal *et al.*, 1999; Karamaouna and Copland, 2000). The results are based on the sex ratio at emergence, which may not correspond to the sex allocation at oviposition. Differential mortality during larval development will influence sex at emergence, giving a misleading impression of preferences and sex allocation strategies. Female-biased sex ratio in parasitic Hymenoptera have been reported by many authors (Croft and Copland, 1995; Heinz, 1998; Bertschy *et al.*, 2000).

Arrhenotokous species of parasitoid Hymenoptera, by controlling fertilization, may also dictate the sex ratio of their offspring and adjust their sex ratio in response to host size. Several studies have shown that female (fertilized) eggs are oviposited more often on large hosts, and male (unfertilized) eggs are oviposited more often on small hosts by the idiobionts (Croft and Copland, 1995; Lampson *et al.*, 1996; Fidgen *et al.*, 2000). However, even in koinobionts, Harvey et al. (1999) found that newly eclosed adults of the solitary parasitoid *Cotesia rubecula* were actually larger when developing in earlier instar than in later instars of the host. Similarly in *Cotesia* glomerata, emerging adult parasitoid size was correlated negatively with the host instar. These result have profound implications for clutch size models in which host quality is predicted to be correlated positively with host instar, and greater in large host species than in small host species. In idiobiotic parasitoids, where eggs are deposited in a non-growing host stage, host size is considered to be a good predictor of host resources at the time of oviposition (Nechols and Kikuchi, 1985). Presumably, females are laid on larger hosts more often than male if they require more food than males to complete their development and/or if they gain more in fitness than males with increasing size. Sex ratio directly contributes to the intrinsic rate of increase and could indirectly contribute to several other attributes of effective natural enemies such as density-dependent performance, competitive ability and interference among parasitoids (Hall, 1993). In the present experiment, older hosts

produced more female offspring, which may be an advantage in augmentative biological control.

However, later on in these studies *A. atomus* from the same source revealed a proportion of thelytokous individuals. It is not known whether thelytoky played a role in sex determination in this study.

Chapter 5

Effects of four levels of temperature and three different host plants on the developmental duration, longevity, fecundity and adult size of the parasitoid *Anagrus atomus* developing in the egg of glasshouse leafhopper *Hauptidia maroccana*.

5.1 Introduction

Extrinsic factors such as temperature, humidity, photoperiod, and nutrition are examples of key environmental elements that may affect fecundity. As a poikilotherm animal, the developmental rate of insects depends on the environmental temperature to which they are exposed (Andrewartha and Birch, 1954; Kitching, 1977), therefore, a lower developmental threshold and associated thermal constant may be useful indicators of an insect's abundance and distribution (Messenger, 1959). Moreover, the potential of a parasitoid as a natural enemy is dependent to a large extent on the physical environment (Tillman and Powell, 1991). The development duration of many parasitoids is reported to be prolonged at low temperatures (Cave and Gaylor, 1988; James and Warren, 1991; Hentz *et al.*, 1998).

The lower threshold of insects in warm climates was found to be higher than that in a cooler climate and reductions in thresholds are compensated by a corresponding increase in development time (Campbell *et al.*, 1974). Therefore, temperature, like other environmental factors, may increase heterogeneity of a natural enemy complex by causing a varied response and lead to improved pest population stability over a range of conditions (James, 1993). Temperature may also exert a considerable influence on some biological characteristics of insects such as developmental rate, longevity, fecundity and sex ratio (Jervis and Copland, 1996). No detailed study on the development of *A. atomus* in relation to different constant temperatures and host plants has been done so far. Therefore, the present study was undertaken with the following objectives.

5.2 Objectives of the Study

The purpose of this study is to investigate the influence of different constant temperatures and host plants on the overall development of *A. atomus* by observing the following parameters:

1. development duration of the parasitoid *A. atomus.*
2. the developmental rate and estimation of the lower threshold and thermal constant
3. the potential fecundity.
4. size of the adult parasitoids and the relationship between fecundity and the size of female parasitoid.

These data would be useful in predicting the generation phenology of the parasitoid and evaluate the potential of *A. atomus* for a successful biological control of *H. maroccana.*

The null hypotheisis:

Temperature and host plant do not have any affect on development duration, potential fecundity and adult size of the parasitoid *A. atomus.*

5.3 Materials and Methods

5.3.1 Development duration, rate of development, thermal constant, lower threshold, fecundity and size of *A. atomus*

The effect of temperature and host plants on biological characteristics of *A. atomus* such as development duration, rate of development, lower threshold, potential fecundity and parasitoid size were investigated in laboratory experiments at four constant temperatures. Mixed colour cultivar of Primula was used as host plants.

Four to six-day-old leafhopper eggs were used in the experiment. Healthy potted plants of Primula with 4-5 fully expanded leaves were placed in the mass culture of leafhopper inside a Perspex cage. A large number of leafhoppers were maintained inside the Perspex cage to ensure adequate eggs were laid. The plants were taken out of the cage after 72 hours of infestation. After removal of the plants, 50 mm leaf discs were made from the leaves containing leafhoppers egg and placed on agar (8g/litre) in 50 mm Petri-dish cages, lower surface facing uppermost. The Petri-dish cages were placed in a plastic

box and put inside the incubator at 21°C in continuous light with 85% r.h.. Thus, 4-6 day old eggs were obtained three days after placing the leaf discs.

Two freshly emerged mated females of *A. atomus* were introduced into each Petri-dish containing the leaf disc. Using a camel hair brush, 50% honey solution was smeared over the leaf disc which served as a food for *A. atomus*. The adult females were allowed to lay eggs for 24 h and then removed from the arena. The Petri-dishes were placed in temperature controlled illuminated incubators at 17, 21, 25 and 27°C in continuous light conditions to allow the eggs to develop. Five Petri-dishes were used at each temperature.

Ten days after laying eggs by *A. atomus*, leaves were cut into small pieces containing individual parasitized eggs (those which turned red) and placed into 0.5ml Eppendorf safe-locked microcentrifuge tubes with a piece of wet filter paper. These tubes were placed in a plastic sandwich box in the incubator at the respective temperature until adult emergence. The humidity inside the boxes was maintained at 85% r.h. using a saturated KCl salt solution. The tubes were checked everyday for the emergence of adult *A. atomus*. The data on emergence of individual parasitoids was recorded and adult sex was determined by the shape of the antennae (clubbed in female). The following information was obtainfed:

1) total developmental time from oviposition to emergence of the adult male and female *A. atomus* was recorded.
2) the development duration of male and females was determined from the emerged adults.
3) the females, which are pro-ovigenic were dissected under a binocular microscope and the total eggs in the ovaries were counted.
4) the length of the hind tibia and head width was measured to determine the size of the emerging male and female wasps.

5.3.2 Adult longevity of *A. atomus* at different constant temperature

Newly emerged individual adults of *A. atomus* were placed inside 0.5 ml Eppendorf safe-locked microcentrifuge tubes and placed in a plastic box. Relative humidity inside the box was maintained at 85% r.h. using a saturated KCl salt solution. Fifty percent honey solution was smeared on the lid as a source of food for the parasitoid. The small holes on the top of the lid allowed honey solution to leak inside which the parasitoid could feed upon. The plastic boxes were placed inside the incubator at 17, 21, 25 and 27°C. The adults were transferred to fresh tubes every other day and fresh honey solution was provided. The tubes were checked everyday for survival and mortality of the parasitoid adults was recorded. Any unnatural mortality caused to the parasitoid by adhering to the honey solution or to the condensed vapour was excluded from the analysis. Forty five parasitoids were used in each temperature and each parasitoid was considered as one replication.

5.3.3 Effect of Different host plants on the development of *A. atomus*.

The same method was followed as in 5.3.1 except the following three host plants were used in this experiment:

1. Primula, *Primula vulgaris* Huds. (var. Mixed colour)
2. French bean, *Phaseolus vulgaris* L. (var. Prince) and
3. Cucumber, *Cucumis sativus* L. (var. Bush champion)

5.4 Experimental design and Statistical Analysis

The rate of development was calculated in days as 1/day (reciprocal of developmental time). Development at 27°C was not included in the linear regression and therefore, polynomial regression was done over the range of 17-27°C. Temperature from 17°C to 25°C was selected to fit a linear regression. The equation of linear regression was then used to predict the lower threshold (tl) and the thermal constant (K). The theoretical lower threshold was calculated as tl= -a/b, and the thermal constant K= 1/b (Campbell *et al.*, 1974). The analyses to find out the effect of temperature and host plants on developmental period, potential fecundity, parasitoid size and adult longevity were done by Analysis of Variance using SAS procedure and the means were separated by LSD test

(P=0.05). Due to unequal replicates, the harmonic mean of the replicates was used to calculate the standard error of difference for comparing the means. Two-sample t tests were used in comparing male and female. Regression analysis was carried out to find any relationship between temperature and fecundity and parasitoid size and fecundity.

5.5 Results

5.5.1 Effect of temperature on the development of *A. atomus*

5.5.1.1 Development duration, developmental rate, lower threshold and thermal constant and parasitoid emergence

Development data of male and female *A. atomus* at various constant temperatures are presented in Table 5.1 and Figure 5.1. The parasitoid *A. atomus* developed successfully to adults over a temperature range of 17 to 27°C. Development of both male and female parasitoid wasps was significantly affected by temperature (male: P<0.001, df=366, LSD=0.364; female: P<0.001, df= 342, LSD= 0.386). The developmental duration of both sexes of the parasitoid decreased significantly with increasing temperature. The longest developmental duration for both sexes was found at 17°C. But the shortest development duration for the male was observed at 27°C. However, development duration of the female was not significantly different at 25 and 27°C. At 17°C, development from egg to adult took 25.19 and 26.06 days while at 27°C it was 12.9 and 12 days for females and males, respectively.

Table 5.1 Duration for development of *Anagrus atomus* males and females at different constant temperatures.

Temperature (°C)	Duration of development (days) mean±s.e.				Two-sample t test
	n	Male	N	Female	
17	97	25.19±0.141 a A	78	26.06±0.197 a B	t=-3.63, df= 145 P <0.001
21	113	17.58±0.132 b A	118	18.44±0.156 b B	t=-4.18, df= 224 P<0.001
25	108	12.56±0.079 c A	185	12.77±0.064 c B	t=-2.08, df =232 P <0.05
27	52	12.00± 0.135 d A	65	12.86±0.158 c B	t=-4.15, df =114 P <0.001
One-way ANOVA		P <0.001 df=366 LSD=0.364		P <0.001 df= 342 LSD=0.386	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

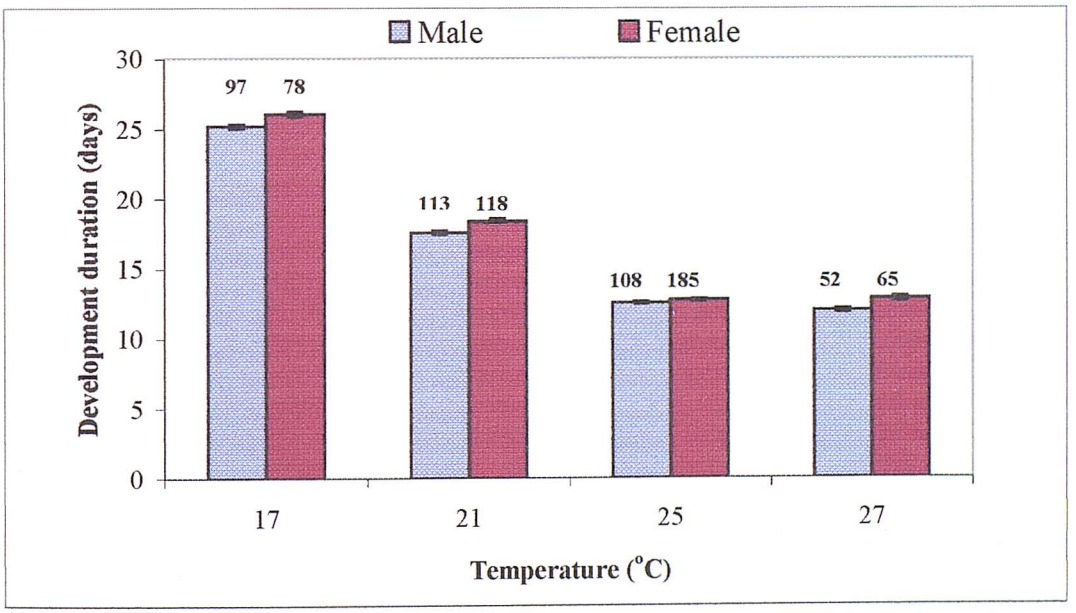


Figure 5.1 Development duration of male and female *Anagrus atomus* on the eggs of *Hauptidia maroccana* at four constant temperatures. Vertical bars represent the standard errors of mean. Values at the top of the bars represent the number of observation.

The linear relationship of the parasitoid development time with temperature has a good fit within the linear range of temperature (17 to 25°C) (Devt $_{male}$= -1.5788x+51.597, r^2=0.9862) (Devt $_{female}$= -1.6613x+53.976, r^2=0.9929). But the linear regression analysis for the rate of development for male and female showed a good fit which is described by the regression line, Y= 0.005x-0.046 (r^2= 0.9937), and Y= 0.005x-0.478 (r^2= 0.9858), respectively (Figure 5.2 and 5.3).

The lower threshold for male and female parasitoids was similar, 9.2 and 9.56°C, respectively while the thermal constant was identical (200°Days) for both sexes (Table 5.2).

Table 5.2 Lower development threshold (Tl) and thermal constant (K) for the development of *Anagrus atomus*.

	Male	Female
Lower threshold (°C)	9.2	9.56
Thermal constant (DD)	200	200
r^2 value	0.9937	0.9858
Regression equation	Y= 0.005x-0.046	Y= 0.005x-0.478

Polynomial regression for the rate of development at all temperature shows a good fit for both sexes. Y= -6E-05x^2+0.0072x-0.0662, r^2=0.9879 and Y=-0.0001x^2+0.0093x-0.088, r^2=0.9652 for the male and female, respectively.

Figure 5.4 shows the total number of adults emerged per day at different constant temperatures. Emergence peaked the same day for male and female at almost all temperatures except at 17°C where the male emergence peaked one day earlier than that of the female. Emergence was completed within six days from first emergence.

The distribution of the cumulative percentage of emergence is presented in Figure 5.5. Fifty percent of the adult emergence occurred after approximately 24, 17, 12 and 11.5 days at 17, 21, 25 and 27°C, respectively.

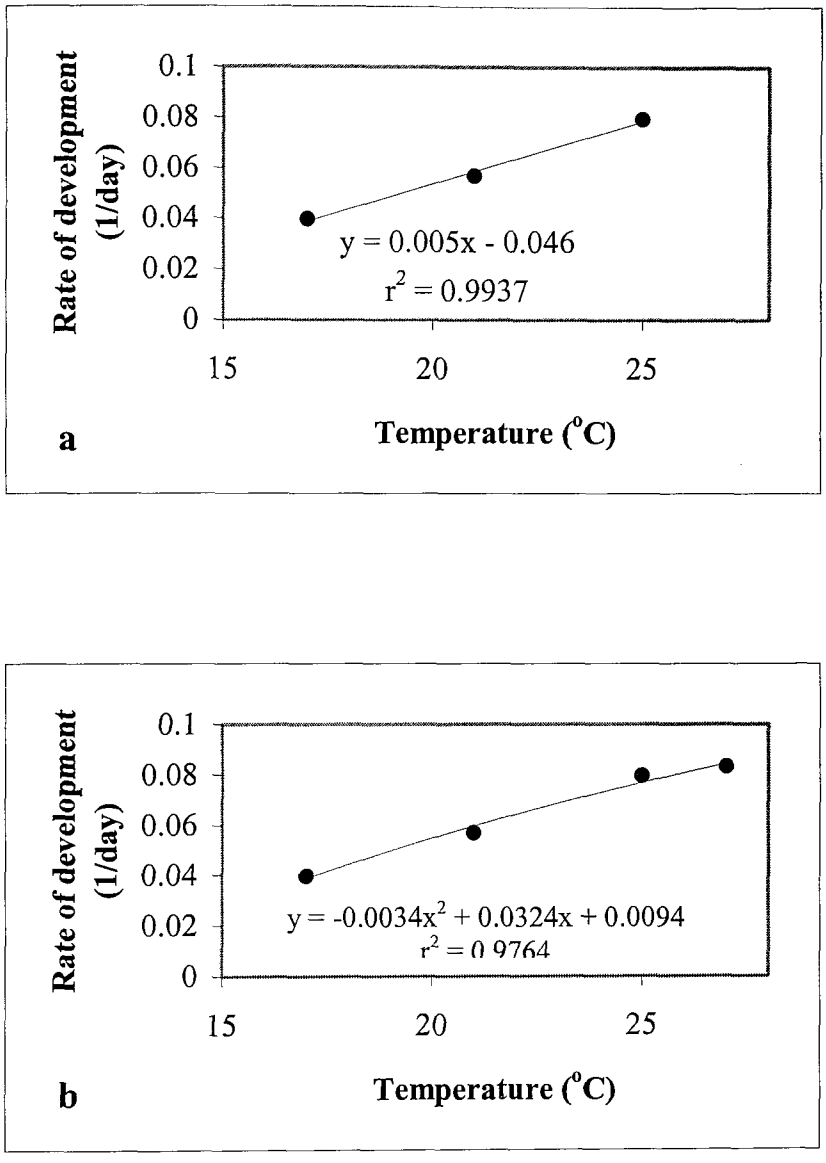


Figure 5.2 **Relationship between temperature and pre-imaginal developmental rate of the egg parasitoid *Anagrus atomus* males a) linear and b) polynomial on eggs of glasshouse leafhopper *Hauptidia maroccana.***

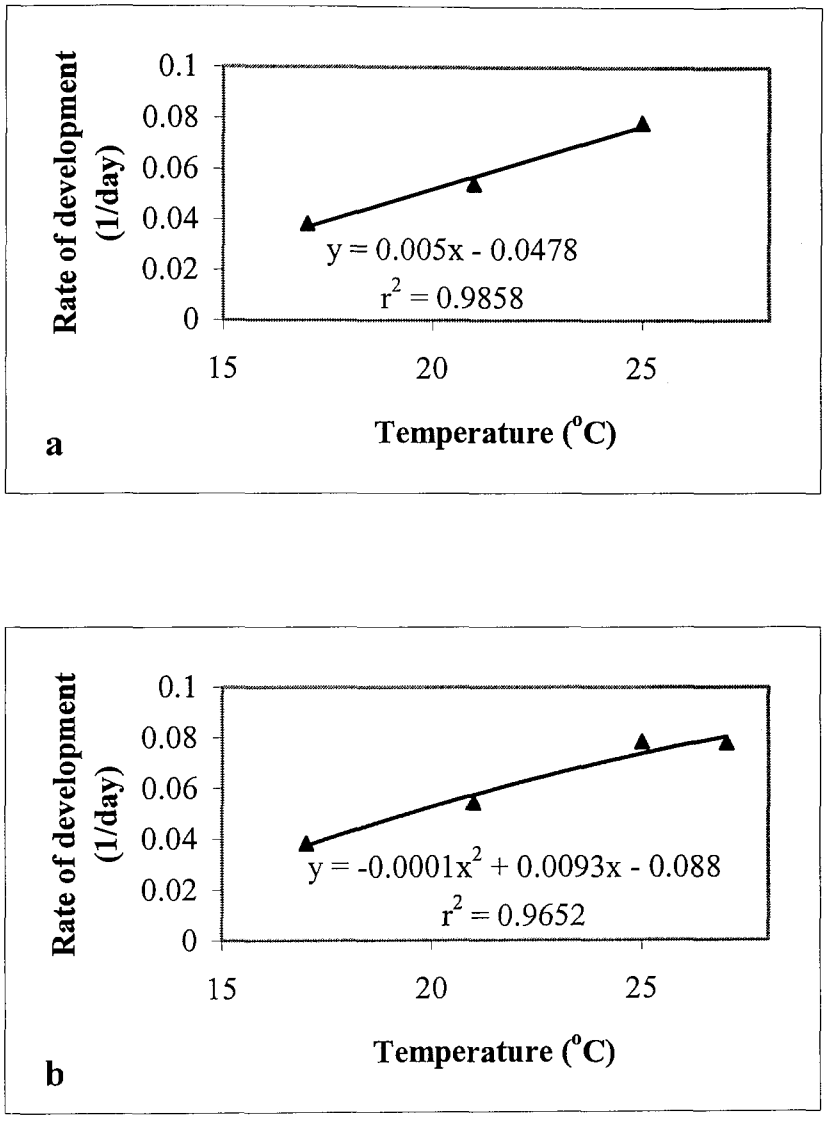


Figure 5.3 **Relationship between temperature and pre-imaginal developmental rate of the egg parasitoid *Anagrus atomus* female a) linear and b) polynomial on eggs of glasshouse leafhopper *Hauptidia maroccana.***

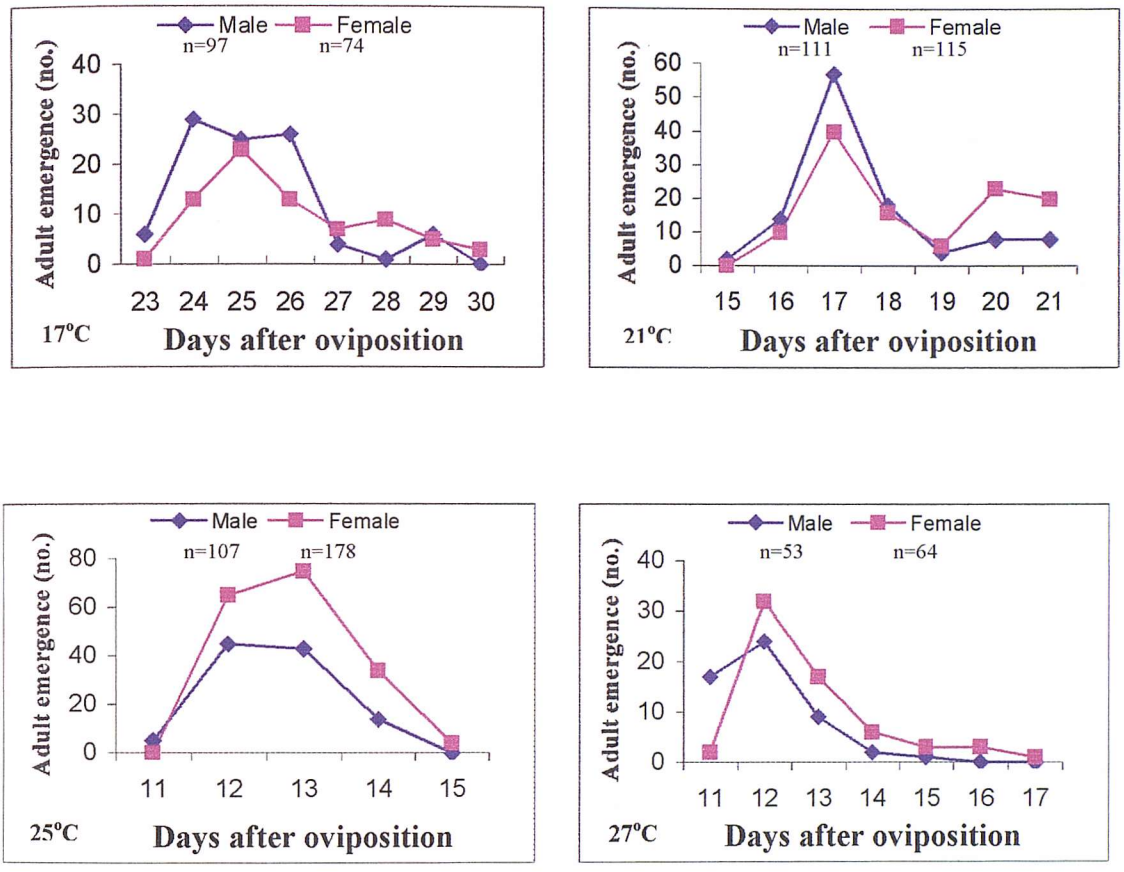


Figure 5.4 **Daily emergence of *Anagrus atomus* developed on the eggs of *Hauptidia maroccana* at four constant temperatures.**

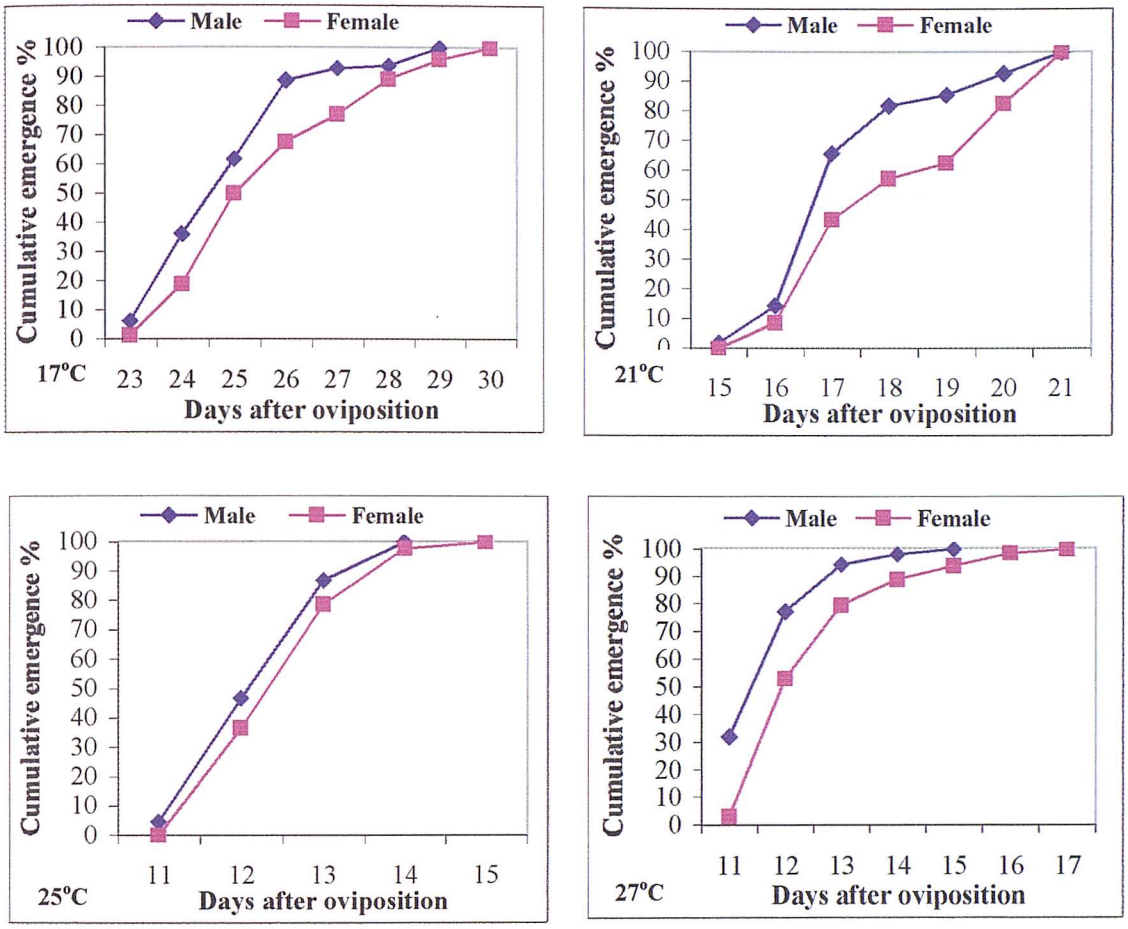


Figure 5.5 **Distribution of the cumulative percent of emergence of *Anagrus atomus* adults developed on the eggs of *Hauptidia maroccana* at four constant temperatures.**

5.5.1.2 Effect of temperature on the potential fecundity of *A. atomus*

The potential fecundity or the total number of eggs counted upon the emergence of the female parasitoid wasps at four different temperatures is presented in Table 5.3. Results showed that temperature had a highly significant effect on the potential fecundity of *A. atomus* ($P \leq 0.001$, ANOVA). Potential fecundity decreased with increasing temperature. The maximum number of eggs (37) was found in wasps developed at lower temperature (17°C) and the minimum (22) at the highest temperature (27°C). In general, there was a linear decreasing trend in the numbers of egg found in the ovary with increasing temperature (Figure 5.6).

Table 5.3 Potential fecundity of *Anagrus atomus* emerging at different constant temperatures.

Temperature (°C)	n	Eggs per female on emergence (no.) mean±s.e.
17	28	37.29±0.886 a
21	34	31.56±1.229 b
25	27	26.44±1.665 c
27	23	22.52±1.683 d
One-way ANOVA		$P<0.001$, df= 108 LSD=3.867

Means in a column, followed by the same letter are not significantly different from each other at $P \leq 0.05$
One-way ANOVA followed by Least Significant Difference test.
n = no. of insects dissected.

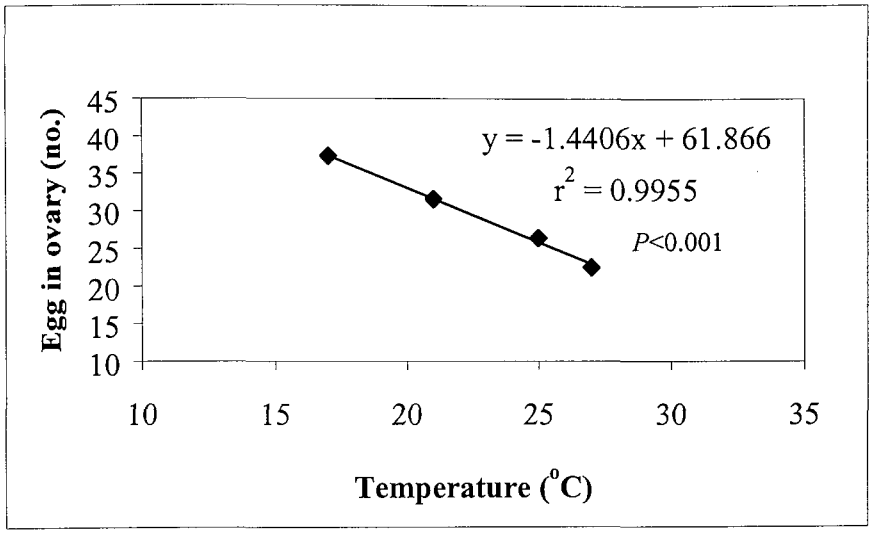


Figure 5.6 Linearity of relationship between temperatures and the potential fecundity of *Anagrus atomus*.

5.5.1.3 Effect of temperature on the size of the adult *A. atomus*

The effect of temperature on the hind tibial length and head width of the male and female *A. atomus* is presented in Table 5.4, Figure 5.7 and 5.8. Hind tibia length of both male and female was significantly affected by temperature (male: $P< 0.01$, df=83, LSD=0.0071; female: $P< 0.001$, df=119, LSD=0.0057). The increase in temperature from 17 to 27°C had a negative effect on the size of the parasitoid (represented by hind tibia length). Hind tibia length of both male and female was the longest at 17°C and the shortest at 27°C. Male head width was significantly affected by temperature ($P< 0.05$, df=86, LSD=0.0048) while that of the female remained unaffected by temperature.

Hind tibia of the male wasp was longer than that of the female at 25 and 27°C while the head width of the male was always greater than that of the female developed at the same temperature.

Additionally, an attempt was made to find out whether there was a relationship between potential fecundity versus parasitoid size. Regression analysis between parasitoid size and potential fecundity indicated a significant linear relationship (y= 235.3x-12.687, $r^2= 0.1935$, $P=0.02$) (Figure 5.9).

Table 5.4 Effect of different constant temperatures on head width and hind tibia length of *Anagrus atomus*.

Tempe-rature (°C)	Hind Tibia length (mm) mean± s.e. (n)		Two-Sample t test	Head width (mm) mean± s.e. (n)		Two-Sample t test
	Male	Female		Male	Female	
17	0.20±0.0019 aA (24)	0.199±0.0023 aA (28)	t=0.30 df=49 p>0.05	0.182±0.0018 abA (23)	0.175±0.0015 aB (28)	t=3.06 df=44 *P* <0.01
21	0.199±0.003 aA (14)	0.197±0.0023 aA (37)	t=0.69 df=28 p>0.05	0.186±0.0031 aA (14)	0.174±0.0014 aB (37)	t=3.29 df=18 *P* <0.01
25	0.198±0.0009 abA (19)	0.188±0.0016 bB (31)	t=5.38 df=45 P<0.001	0.180±0.0011 bA (21)	0.176±0.0016 aB (33)	t=2.17 df=51 *P* <0.05
27	0.193±0.0015 bA (30)	0.184±0.0015 bB (27)	t=3.84 df=54 p<0.001	0.179±0.0011 bA (32)	0.173±0.0017 aB (25)	t=2.92 df=41 *P* <0.01
One-way ANOVA	*P* <0.01 df=83 LSD=0.0071	*P* <0.001 df=119 LSD=0.0057		*P* <0.05 df=86 LSD=0.0048	*P* >0.05 df=119 LSD=0.0044	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
(n) = no. of insects as replicates.

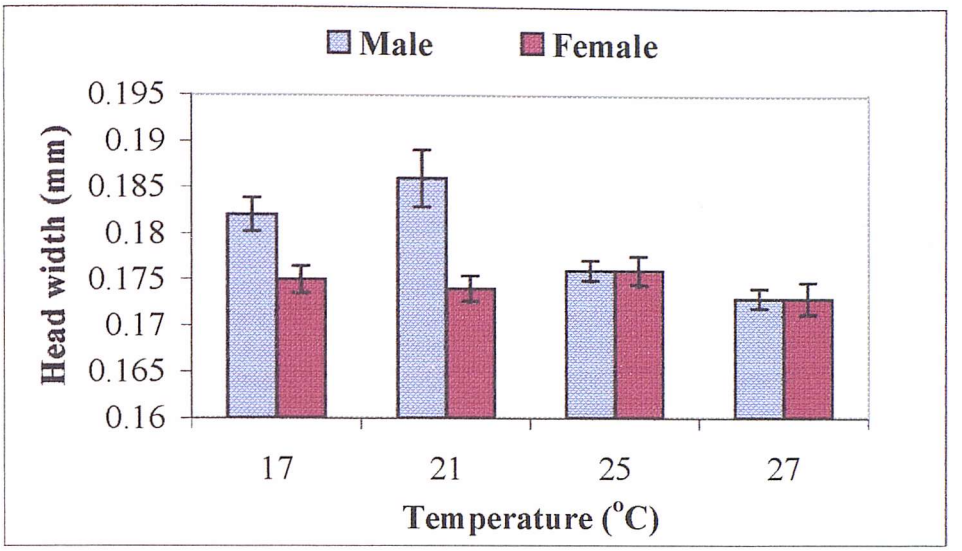


Figure 5.7 **Effect of different constant temperatures on head width of adult male and female *Anagrus atomus*. Vertical bars represent the standard errors of mean.**

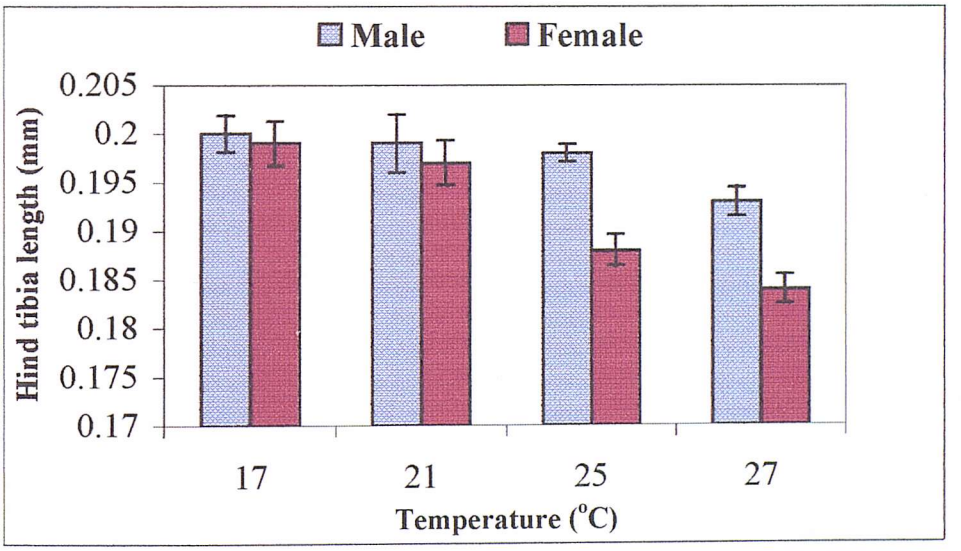


Figure 5.8 **Effect of different constant temperatures on hind tibia length of adult male and female *Anagrus atomus*. Vertical bars represent the standard errors of mean.**

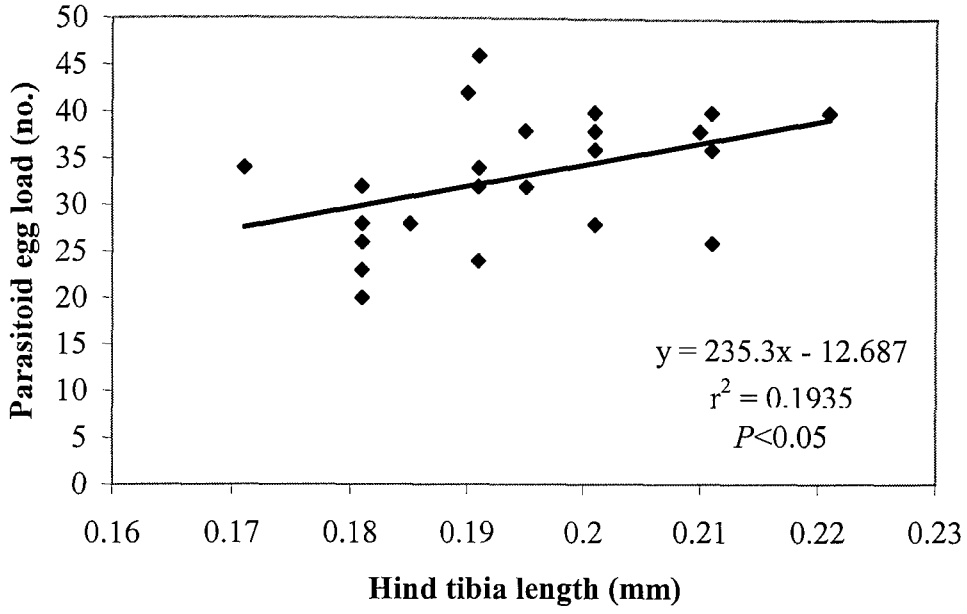


Figure 5.9 Relationship between size and potential fecundity of *Anagrus atomus* developed at 21°C.

5.5.1.4 Longevity of adult *A. atomus*

The mean adult longevity of male and female *A. atomus* feeding on honey solution is presented in Table 5.5. There was a significant effect of temperature on adult longevity of both sexes (male: $P<0.001$, df=107, LSD= 1.248; female: $P<0.001$, df= 94, LSD= 1.408). The females lived for maximum 13 days at 17°C while they lived for only 4 days at 27°C. The greatest longevity (11days) of the male was observed at 17°C while it was only 4 days at 27°C. There was no significant difference between the longevity of male and females at different temperatures except at 21°C where females lived longer than the males ($P<0.001$, df=45, t=-6.12).

Table 5.5 Adult longevity of *Anagrus atomus* male and female at different constant temperatures.

Temperature (°C)	Adult longevity (days)				Two-sample t test
	Male		Female		
	n	mean±s.e.	n	mean±s.e.	
17	16	11.25±0.854 a A	24	13.04±0.658 a A	t= -1.66, df= 30 *P*= 0.11
21	30	7.60±0.459 b A	21	11.76±0.762 a B	t= -6.12, df= 45 *P*<0.001
25	39	5.21±0.297 c A	15	5.93±0.565 b A	t= -1.14, df= 22 *P*= 0.27
27	26	4.19±0.254 c A	38	4.84±0.276 b A	t= -1.73, df= 61 *P*= 0.088
One-way ANOVA	*P*<0.001 Df=107 LSD=1.248		*P*<0.001 df=94 LSD=1.408		

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

5.5.2 Host plant effect on the development of *A. atomus*

5.5.2.1 Duration of development in *A. atomus*

Effect of different host plants on the development duration of the *parasitoid A. atomus* is presented in Table 5.6. Development duration from egg to adult varied significantly according to the host plants (male: *P*<0.001, df=195, LSD=0.4601; female: *P*<0.001, df=262, LSD= 0.5085) and differed between the sexes in Primula (*P*<0.001, df=145, t=-3.63) and French bean (P<0.001, df= 103, t= -6.05). Shortest development duration (14.97 days and 15.35 days) was observed in Cucumber while it was the longest (17.58 days and 18.44 days) in Primula for the male and female, respectively. Males always developed faster than female in Primula and French bean while in Cucumber, development duration of male and female were not significantly different.

Table 5.6 Duration for development of *Anagrus atomus* males and females on leafhopper eggs in different host plants.

Host plant	Duration of development (days) Mean±s.e.				Two-sample t test
	n	Male	n	Female	
Primula	113	17.58±0.132 a A	118	18.44±0.156 a B	t=-3.63, df= 145 P <0.001
French Bean	47	16.08±0.128 b A	59	17.25±0.144 b B	t=-6.05, df= 103 P<0.001
Cucumber	38	14.97±0.133 c A	88	15.35±0.199 c A	t=-1.58, df =123 P >0.05 ,NS
One-way ANOVA		P <0.001 df= 195 LSD=0.4601		P <0.001 df= 262 LSD=0.5085	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P\leq$ 0.05. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

5.5.2.2 Potential fecundity of *A. atomus* emerging from leafhopper eggs in different host plants

Different host plants significantly affected potential fecundity of *A. atomus*. The highest number of eggs was found in the adults developed in French bean and the lowest in those developed in Cucumber (Table 5.7).

Table 5.7 Potential fecundity of *Anagrus atomus* emerging from leafhopper eggs in different host plants at 21°C.

Host Plant	n	Eggs (no.) per female on emergence mean±s.e.
Primula	34	31.56±1.229 ab
French Bean	35	33.17±0.971 a
Cucumber	24	28.67±1.360 b
One-way ANOVA		P=0.038 df= 90 LSD=3.3433

Means in a column, followed by the same letter are not significantly different from each other at $P\leq$ 0.05
One-way ANOVA followed by Least Significant Difference test.
n = no. of insects dissected.

5.5.2.3 Size of adult *A. atomus* emerging from leafhopper eggs in different host plants

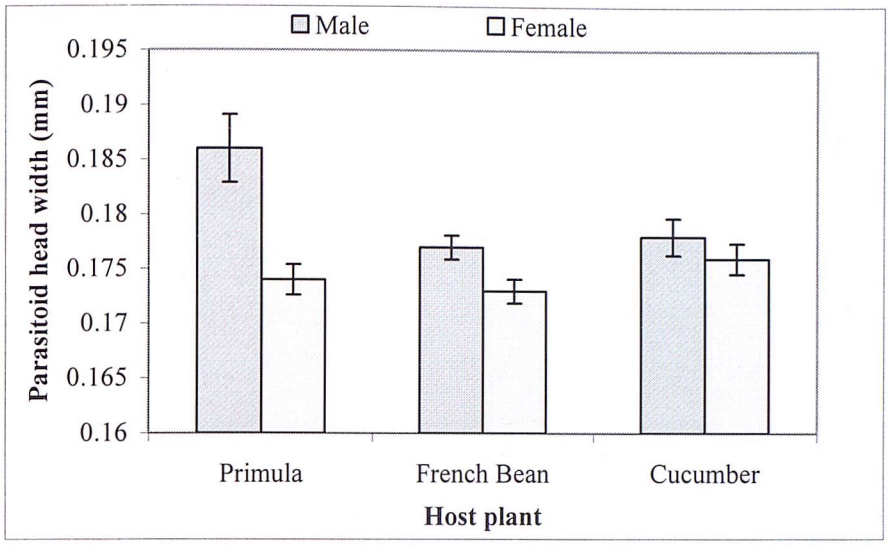
Host plants did not significantly affect hind tibia length of the male. But significant differences were observed in the hind tibia length of the females ($P<0.05$, df=90, LSD=0.0063) developed in different host plants (Table 5.8). Head width of males was affected significantly by different host plants ($P<0.01$, df=46, LSD= 0.0056) while that of females was unaffected. Interestingly, unlike other hymenopteran parasitoids, *A. atomus* males were always larger in size than the females.

Table 5.8 Effect of different host plants on head width and hind tibia length of *Anagrus atomus* at 21°C.

Host Plant	Hind Tibia length (mm) mean±s.e. (n)		Two-Sample t test	Head width (mm) mean±s.e. (n)		Two-Sample t test
	Male	Female		Male	Female	
Primula	0.199±0.0031 a A (14)	0.197±0.0023 a A (37)	t=0.69 df=28 P>0.05	0.186±0.0031 a A (14)	0.174±0.0014 a B (37)	t=3.06 df=44 *P* <0.01
French Bean	0.194±0.0023 a A (20)	0.189±0.0019 b A (36)	t=1.56 df=43 P>0.05	0.177±0.001 b A (20)	0.173±0.0011 a B (37)	t=2.54 df=50 *P* <0.05
Cucumber	0.195±0.0033 a A (15)	0.195±0.0019 ab A (20)	t=-0.01 Df=22 P>0.05	0.178±0.0017 b A (15)	0.176±0.0014 a A (20)	t=0.92 df=30 *P* >0.05
One-way ANOVA	NS, *P* =0.31 df=46 LSD=0.0082	*P* <0.05 df=90 LSD=0.0063		*P* <0.01 df=46 LSD=0.0056	NS, *P* =0.28 df=91 LSD=0.0044	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P\leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

Head width



Hind tibia length

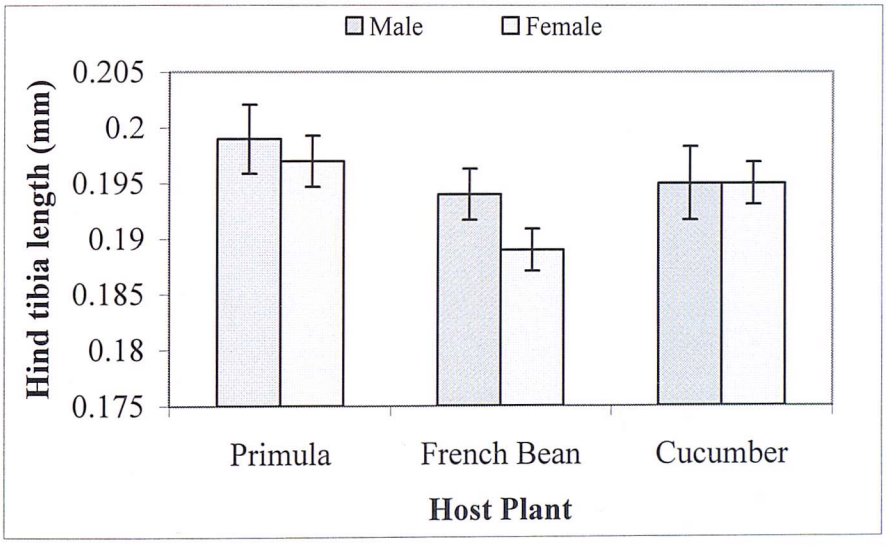


Figure 5.10 **Effect of different host plants on the adult size of *Anagrus atomus* at 21°C. Vertical bars represent the standard errors of mean.**

5.6 Discussion

5.6.1 Effect of temperature on the development of *A. atomus*

Most studies of the effect of temperature on insects assume that there is a close relationship between development time, fecundity and longevity, across temperature. In the present study, the egg parasitoid *A. atomus* developed successfully over a range of temperature (17-27°C). Emergence of the adults peaked on the second to third day although it continued on to the sixth or seventh day. A similar pattern of emergence was observed for both sexes.

As expected, the development of *A. atomus* was found to be dependent on temperature. An increase in temperature showed a faster development of both male and females. The total development of *A. atomus* from egg to adult ranged from 12 to 25 days and 12.9 to 26.1 days at 17-27°C for the male and females, respectively. Development duration was reduced to just half in both male and female when the temperature was increased from 17°C to 25°C. The developmental duration from egg to adult was observed to be 17.6 and 18.4 days at 21°C for male and female, respectively. This result is in agreement with the observation of MacGill (1934). He reported the average developmental duration of *A. atomus* as 14-18 days at 26-27°C. Cooper (1934) described the developmental duration of 16-21 days at 18-24°C. No significant difference was observed in the development of female wasp between 25 and 27°C. Total developmental times are variable among different species within the genus *Anagrus* (Appendix 2). No report on temperature dependent development of *A. atomus* has been found so far. The faster development with increasing temperature is well known in poikilotherms and has been documented by many authors (Miura and Kobayashi, 1993; Balsdon *et al.*, 1996; Ponsonby and Copland, 1996; Hart *et al.*, 1997; McDougall and Mills, 1997; Hentz *et al.*, 1998). Therefore, this result will provide some guidance for rearing the parasitoid at a suitable temperature.

Male *A. atomus* was always found to develop faster than females (Table 5.1). This early emergence of sons is an added advantage for the parasitoid in preventing oviposition by virgin females (Biswas and Singh, 1995). An exception has been reported in *A. takeyanus* where males emerged later than females (Balsdon *et al.*, 1996).

The simple linear or degree-day model used in this study showed a significant linearity among temperature and the rate of insect development. This linear trend is a more common phenomenon in laboratory studies at constant temperatures and most commonly used for predicting and forecasting insect development within the range of temperatures (Campbell *et al.*, 1974). This is surprising because enzyme reaction rates increase exponentially with temperature, so the relation between insect development and temperature is expected to be sigmoid, i.e. roughly exponential through the lower range of temperature but falling off at high temperatures (Gilbert and Raworth, 1996). The widely adopted measure of 'day degrees' relies on linearity. This relation between development and temperature is the result of selection, as it changes when the insects are exposed for many generations to different temperature regimes (Huey *et al.*, 1991). The estimated lower threshold temperature for male (9.2°C) and female (9.6°C) indicated that the parasitoid can develop within a wide range of temperature having the same day degree (200DD) requirements. Thus the temperature dependent relationship between host and parasitoid allows us to make prediction regarding the release of appropriate biological control agents. The temperature dependent developmental requirement is an assessment of parasitoid growth rates relative to the host growth rate (Campbell *et al.*, 1974). In general, the thresholds for parasites are higher than that of their hosts and for all practical purposes the parasite season cannot start earlier than those of the host. Similarly, in the present study the lower thresholds for both male (9.2°C) and female (9.56°C) *A. atomus* were found to be higher than that of its host egg of *H. maroccana* (7.09°C).

5.6.2 Effect of temperature on potential fecundity *of A. atomus*

The potential fecundity and the actual fecundity are two important criteria to characterise a successful biological control agent. Pro-ovigenic insects complete oogenesis either before or very soon after eclosion, having used food reserves built up during the larval period (Jervis and Copland, 1996). Thus, the number of mature oocytes in the ovarioles at emergence can be a useful convenient estimate of the potential fecundity for pro-ovigenic species. As a pro-ovigenic species, the potential fecundity of *A. atomus* was observed by dissecting the emerging adults. A significant declining trend has been observed in the potential fecundity of *A. atomus* with an increase in the development temperatures (Table 5.3). The regression analysis of the potential fecundity versus rearing temperature showed a significant linear relationship (y= -1.4406x+61.866,

r^2= 0.9955, P<0.001). Zarokosta (1999) observed the same trend while studying the potential fecundity of *Eretmocerus* sp. at different constant temperatures. On average, the potential fecundity of the females developed at 17°C was observed to be 37 while at 27°C it reduced to 22.5.

The effectiveness of a parasitoid species as a biological control agent is linked to the quality of an individual parasitoid's fitness (Ridgway and Morrison, 1985). Female size, which may reflect the initial egg load, can be used as a measure of potential reproductive output of many insects. The simple formula, 'larger body = more eggs' allows the expectation of a strong correlation between fecundity and body size, at least for those insects that mainly use resources accumulated during the larval stage for egg production (Honek, 1993). Like other physical factors temperature influences the size of the developing parasitoids (Jervis and Copland, 1996). In the present study, parasitoid size was inversely affected by increasing temperatures (Table 5.4).

A positive linear relationship (Y= 235.3x-12.687, r^2=0.1935, P<0.05) has been observed in the potential fecundity and the size of *A. atomus* (Figure 5.9). Usually large females were always found to be more fecund (Hardy *et al.*, 1981, 1992,; Croft and Copland, 1995; Lampson *et al.*, 1996). This positive relationship between the number of mature oocytes and female size can have important implications for a foraging model (Jervis and Copland, 1996).

5.6.3 Effect of temperature on longevity of *A. atomus*

The life span of a female parasitoid wasp is important in respect to its actual fecundity. A pro-ovigenic parasitoid like *A. atomus* that emerges with all the mature eggs present can lay its eggs within first few days of emergence. However, temperature plays an important role on adult survival. The mean longevity of either sex of *A. atomus* was found to decrease with increasing temperature (Table 5.5). The decrease in female longevity with increasing temperature has also been reported in some other parasitoid species (Naranjo, 1993; Balsdon *et al.*, 1996; Baitha and Ram, 1998; Hentz *et al.*, 1998).

5.6.4 Effect of host plants on the development, potential fecundity and size of *A. atomus*

The development duration, size of both male and female, and the potential fecundity of *A. atomus* was significantly affected by different host plants (Table 5.6). Faster development was observed in both sexes on Cucumber, which lead to smaller females with a lower egg load (Table 5.7 and 5.8). On the other hand, development duration was the longest on Primula and this contributed to the larger females, which were more fecund than those emerging from the other host plants. This differential effect of host plants on development of *A. atomus* might be due to some antibiosis effect of Cucumber that adversely affected parasitoid fitness (size and fecundity). This antibiosis type of resistance mechanism, which acts unfavourably on the second trophic level may also influence the third level. The life table parameters and the searching behaviour of the parasitoid on different host plants might provide an idea about the antibiosis effect of those host plants. However, the observations were made under laboratory conditions on only one generation of the parasitoid. Therefore, it is difficult to predict the long term effect of this type of resistance over several generations under natural conditions.

Thus the information in this present study on the developmental biology *of A. atomus* in the egg of *H. maroccana* may provide information on the effectiveness *of A. atomus* and contribute to the biological control programme of glasshouse leafhopper *H. maroccana*.

Chapter 6

Effect of three different host plants and three constant temperatures (17, 21 and 25°C) on the fecundity and life table parameters of *A. atomus.*

6.1 Introduction

The effectiveness of a parasitoid species as a biocontrol agent depends to a large extent on the fitness of the individual parasitoid (Ridgway and Morrison, 1985). The lifetime fecundity or reproductive potential of a parasitoid has frequently been cited in the biological control as an important characteristic of successful biological control agents (Lane *et al.*, 1999). Hence, the evaluation of a natural enemy as a biocontrol agent needs a great deal of study of the influence of these factors on potential and realised fecundity, and even fertility (Jervis and Copland, 1996).

The number of eggs present in the ovary at a time is a useful conservative estimate of the potential fecundity for many species. In pro-ovigenic parasitoids, which emerge as adults with full lifetime complement of mature eggs, the egg load is simply a decresing function of eggs laid, and there is thus no distinction between egg-load limitation and fecundity limitation (Lane *et al.*, 1999). Female size, which may reflect the number of eggs present in the oviduct, can be used as a measure of potential reproductive output of many insects. The size of the parasitoid can easily be estimated by measuring the head width (Liu, 1985; van den Assem *et al.*, 1989) or the hind tibia length (Heinz and Parrella, 1989).

A life table is an age specific summary of the mortality rates operating on a population. Deevey (1947) defined the life table as a compact summary of certain vital statistics of a population. Therefore, the construction of a life table is an important component in the understanding of the population dynamics of a species (Southwood, 1978). Population growth of a particular species may be expressed as either the intergeneration net reproductive rate of increase (R_o) or the present intrinsic rate of natural increase (r_m) (van Driesche and Bellows, 1996).

The construction of age specific life tables of parasitoids in the laboratory comprise the study of the gross reproductive rate (*GRR*), net reproductive rate (R_o), intrinsic rate of natural increase (r_m) and other associated life table parameters like mean generation time (*T*), finite capacity for increase (λ) and doubling time (*DT*). The innate capacity for increase (r_c) and the cohort generation time (*Tc*) are approximation for the intrinsic rate of natural increase and the generation time respectively. All of these life table parameters can be calculated from the data on age-specific survival (l_x) and the age-specific fertility (m_x), the proportion of female surviving in each age class (Tripathi and Singh, 1990; Asante *et al.*, 1991).

Intrinsic rate of natural increase (r_m) can be used for the selection of a candidate parasitoid species in a biological control programme and in the absence of other criteria, the species with the greatest value of the parameters would be the most favourable (Jervis and Copland, 1996).

6.2 Objectives of the study

Experiments were conducted to study the effects of different host plants on the following aspects related to life table parameters of the parasitoid *A. atomus* at different constant temperatures:

1. age-specific fecundity of the parasitoid
2. actual fecundity during life time
3. intrinsic rate of population increase (r_m) under laboratory conditions.

The null hypothesis:

The fecundity and life table parameters of the parasitoid *A. atomus* are independent of temperature and host plant.

6.3 Materials and Methods

Fecundity and life table parameters of *A. atomus* were studied on the eggs of *H. maroccana* on three host plants and at three constant temperatures viz., 17, 21 and 25°C. Following three host plants were used in the experiment:

1. Cucumber, *Cucumis sativus* L. (var. Bush champion) Cruciferae
2. French bean, *Phaseolus vulgaris* L. (var. Prince) Leguminosae and
3. Primula, *Primula vulgaris* Huds. (var. Mixed colour) Primulacae

Leaf discs of the host plant containing 4-6 day-old *H. maroccana* eggs were placed with their lower surface facing upwards on top of a layer of 8g/l agar in 6 cm diameter Petri-dishes. The desired ages of eggs were obtained using the methods described in Chapter 4.

A single freshly emerged mated female of *A. atomus* was introduced carefully into each Petri-dish with the help of a fine tipped paintbrush avoiding any damage. Fifty percent honey solution was smeared over the leaf surface as food for the parasitoid. The Petri-dish cages were then placed in the incubator at 17, 21 and 25°C. The female parasitoid wasps were transferred to a new Petri-dish cage containing leafhopper eggs every other day until the female wasp died. Each female wasp was considered as one replication. Five replications were maintained at each temperature and host plant.

The Petri-dish cages with parasitized eggs were marked individually and incubated at the respective temperatures. Ten days after laying eggs by *A. atomus*, the eggs of *H. maroccana* that turned red were counted and the total fecundity and daily fecundity were calculated. The leaves were cut into small pieces containing an individual parasitized egg and placed into 5 ml Eppendorf microcentrifuge tube with a piece of wet filter paper. These tubes were placed in the incubator in a plastic sandwich box. The humidity inside the boxes was maintained at 85% r.h. using saturated KCl salt solution. The tubes were checked everyday for the emergence of adult *A. atomus*. The data on emergence of individual parasitoid were recorded and adult sex was determined on the basis of the antennae shape.

6.4 Statistical analysis

The effect of temperature and host plants on survival and secondary sex ratio of *A. atomus* was done by χ^2 analysis. Data on survival of the female wasp, daily fecundity, total fecundity and sex ratio were used to estimate different life table parameters viz., gross reproductive rate (*GRR*), net reproductive rate (R_o), capacity for increase (r_c), cohort generation time (*Tc*), generation time (*T*), doubling time (*DT*) and finite capacity for increase (λ) with the help of QBASIC computer software (Jervis and Copland, 1996).

6.5 Results

6.5.1 Survival, sex ratio, life table parameters and fecundity of the parasitoid *A. atomus* on the eggs of *H. maroccana* laid on different host plants

6.5.1.1 Survival of *A. atomus*

Percent emergence of parasitoid adults from the eggs of *H. maroccana* on different host plants is presented in Table 6.1. Survival was calculated from the number of emerging parasitoids to the total number of eggs laid per female during her lifetime. χ^2 analysis showed a significant difference in the parasitoid survival on different host plants ($P< 0.01$, df= 2, $\chi^2= 12.94$). The highest (89.3%) and the lowest (72.5%) survival was observed on cucumber and on French bean, respectively.

Table 6.1 Survival of *Anagrus atomus* during preimaginal development on three different host plants at 21°C.

Host Plant	Parasitized eggs (no.)	Parasitoid emerged (no.)	Parasitoid Survival (%)
Primula	107	83	77.6
French Bean	167	121	72.5
Cucumber	131	117	89.3
χ^2 analysis	$\chi^2= 12.944$, df=2, $P<0.01$		

6.5.1.2 Sex ratio of *A. atomus*

The secondary sex ratio of *A. atomus* emerging from the eggs of *H. maroccana* laid on different host plants is presented in Table 6.2. A significant difference was observed among the sex ratio of the parasitoid wasp emerging from different host plants (P=0.05, df= 2, χ^2= 5.84). The proportion of females was the highest in French bean and lowest in Primula.

Table 6.2 Effect of three different host plants on the sex ratio of *Anagrus atomus* emerging at 21°C.

	Primula	French bean	Cucumber
Parasitoid emerged	83	121	117
Males	25	20	31
Females	58	101	86
Sex ratio (Γ:E)	1: 2.3	1: 5.1	1: 2.8
χ^2 analysis	χ^2=5.843, df= 2, P= 0.054		

6.5.1.3 Age specific realised fecundity and survival of *A. atomus* on different host plants

A. atomus adults start to lay eggs just after emergence. The highest daily reproduction was observed within the first few days of emergence. Maximum eggs were laid on the first day of emergence in each host plant (Figure 6.1). Only a few eggs were laid in the 3rd to 6th day of their life. The cumulative fecundity of *A. atomus* indicated that 50% of the eggs had been laid during the first 2 days of their reproductive life (Figure 6.2).

The actual fecundity of *A. atomus* was not affected by different host plants, although more eggs were laid on French bean than on Primula and Cucumber (Figure 6.3). Age specific distribution of the proportion of females of the parasitoid *A. atomus* emerging from the eggs of *H. maroccana* on different host plants is presented in Table 6.3. In French bean, females lived for a longer period and contributed to the highest total fecundity. The weighted mean of the female proportion was the highest in French bean. The age specific fecundity (m_x) and the survival (l_x) is presented in Figure 6.4.

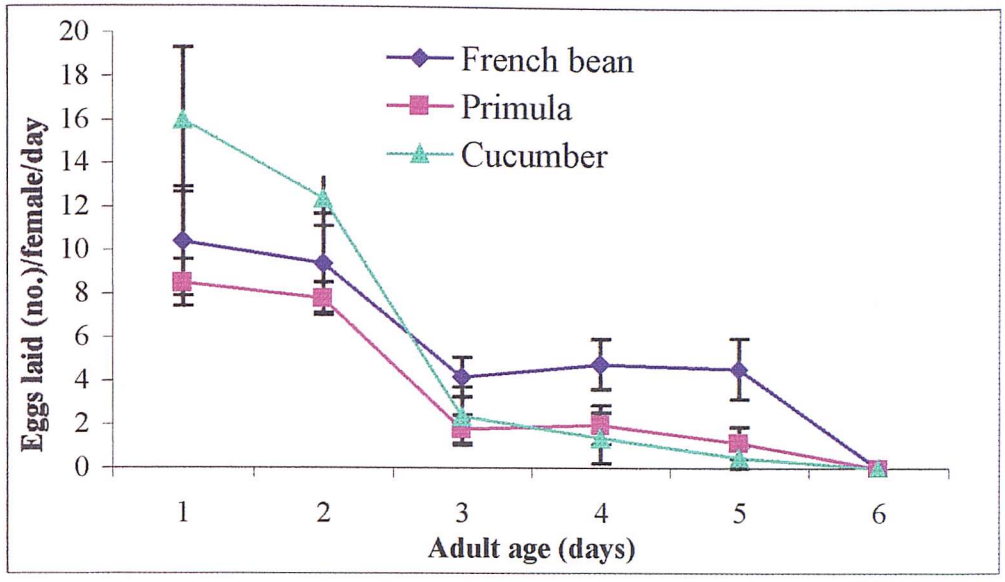


Figure 6.1 **Mean daily fecundity of the egg parasitoid *Anagrus atomus* on different host plants at 21°C. Vertical bars represent the standard errors of mean.**

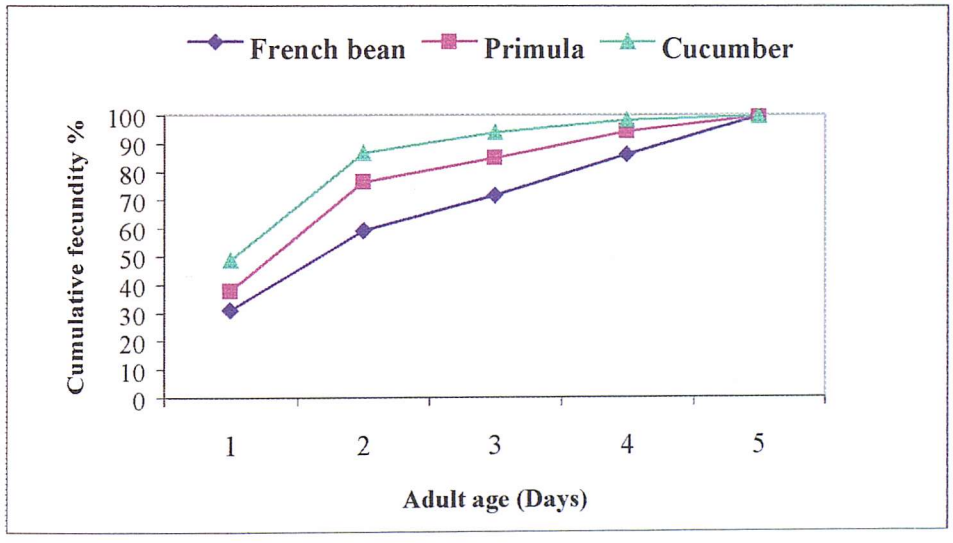


Figure 6.2 **Cumulative fecundity of the egg parasitoid *Anagrus atomus* on the egg of *Hauptidia maroccana* laid in different host plants.**

Figure 6.3 **Actual and potential fecundity of *Anagrus atomus* emerging from different host plants.**

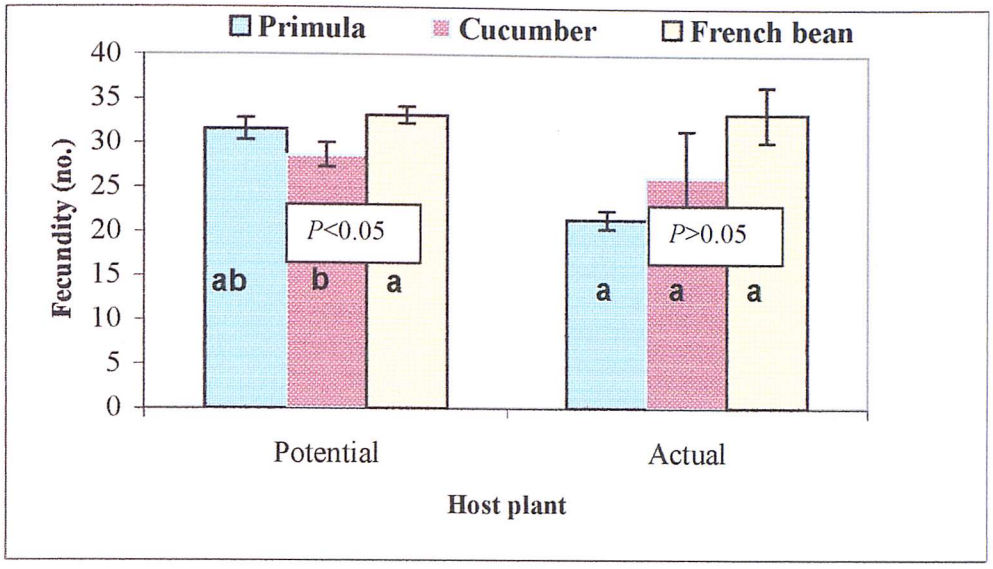


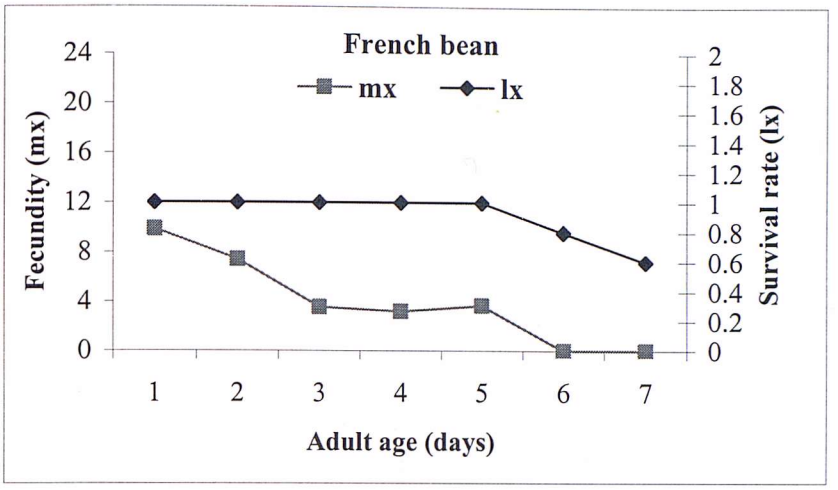
Table 6.3 **Host plant effects on the secondary sex ratio (female proportion) of *Anagrus atomus* on *Hauptidia maroccana* eggs at 21°C.**

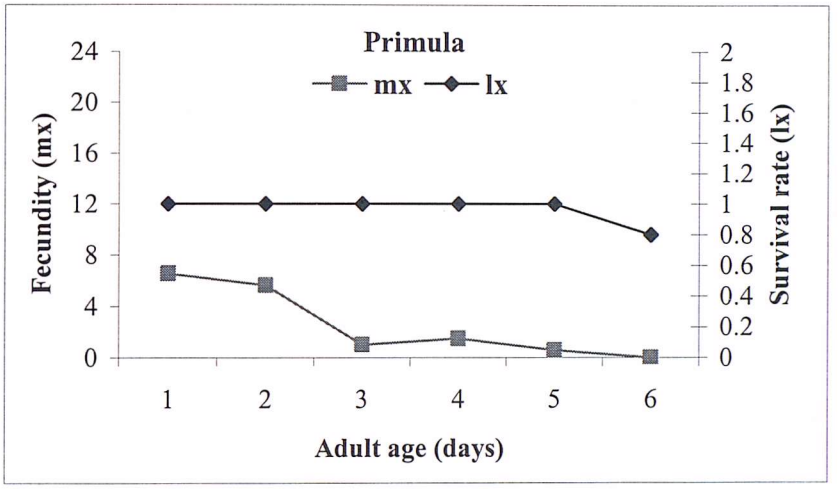
Maternal age (days)	Living females (n)	Ovipositing females (n)	Total Offspring (n)	Female offspring (n)	Female proportion
Primula*					
1	5	5	34	26	0.76
2	5	5	29	21	0.72
3	5	4	7	4	0.57
4	5	3	8	6	0.75
5	5	3	4	2	0.50
6	4	0	0	0	0
French bean**					
1	5	5	40	38	0.95
2	5	5	38	30	0.79
3	5	5	13	11	0.85
4	5	5	15	10	0.67
5	5	4	15	12	0.80
6	4	0	0	0	0
7	3	0	0	0	0
Cucumber***					
1	5	5	71	54	0.76
2	5	5	29	19	0.66
3	5	4	11	9	0.82
4	5	2	6	4	0.67
5	2	1	0	0	0

* Age of the female at emergence= 18.44 days Weighted mean of female proportion= 0.67

* * Age of the female at emergence=17.25 days; Weighted mean of female proportion= 0.81

*** Age of the female at emergence=15.35 days; Weighted mean of female proportion= 0.65





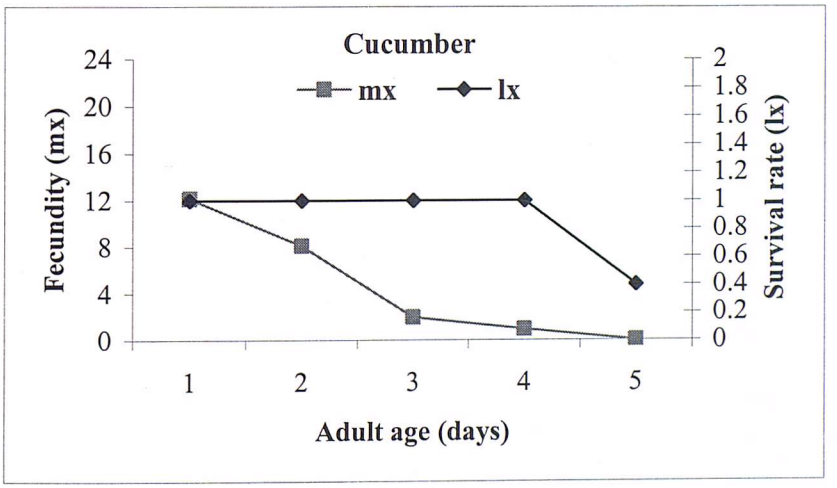


Figure 6.4 **Fecundity and survival rates of the egg parasitoid *Anagrus atomus* on the eggs of *Hauptidia maroccana* laid on different host plants at 21°C.**

6.5.1.4 Life table parameters of *A. atomus* on different host plants

The intrinsic rate of natural increase (r_m) and the associated life table parameters of *A. atomus* ovipositing on the egg of *Hauptidia maroccana* laid on different host plants are presented in Table 6.4. The lowest and the highest r_m value was observed while the host eggs were laid in Primula and Cucumber, respectively.

Table 6.4 Life table parameters of the parasitoid *Anagrus atomus* ovipositing on the egg of *Hauptidia maroccana* laid on different host plants.

Life table parameters	Primula	French bean	Cucumber
Gross reproductive rate (*GRR*)	16.36	27.73	23.18 **Female eggs/female/generation**
Net reproductive rate (R_o)	15.36	27.73	23.18 **Females/female/generation**
Capacity of increase (r_c)	0.14	0.18	0.20 **Female offspring/female/day**
Intrinsic rate of increase (r_m)	0.14	0.18	0.20 **Female eggs/female/day**
Cohort generation time (*Tc*)	19.39	18.65	15.97 **Days**
Generation time (*T*)	19.31	18.48	15.92 **Days**
Finite capacity for increase (λ)	1.15	1.20	1.22 **Female offspring/female/day**
Doubling time (*DT*)	4.89	3.86	3.48 **Days**

6.5.2 Survival, sex ratio, fecundity and life table parameters of the parasitoid *A. atomus* on the eggs of *H. maroccana* at different constant temperatures

6.5.2.1 Survival of the immature stages *A. atomus* at different constant temperatures

χ^2 analyses showed a significant effect of temperature on the survival of immature stages of *A. atomus*. The survival of the immature stages decreased from 90.1% to 56.6% with increasing temperature from 17 to 27°C (Table 6.5).

Table 6.5 Survival of *Anagrus atomus* during preimaginal development at different constant temperatures.

Temperature (°C)	Parasitized eggs (no.)	Parasitoid emerged (no.)	Parasitoid Survival (%)
17	171	154	90.1
21	107	83	77.6
25	125	87	69.6
27	145	82	56.6
χ^2 analysis	χ^2= 47.933, df= 3, P<0.001		

6.5.2.2 Sex ratio of *Anagrus atomus* emerging at different constant temperatures

Sex ratio of the emerging parasitoids *A. atomus* over the temperature regime of 17-25°C is presented in Table 6.6. The χ^2 analyses did not show any significant effect of temperature on parasitoid sex ratio (P= 0.288). Sex ratio of the emerging parasitoid (Γ:E) was always female biased at each temperature.

Table 6.6 Effect of three different constant temperatures on the sex ratio based on the total emerging adults of *Anagrus atomus*.

	17°C	21°C	25°C
Total adults emerged	154	83	87
Males	33	25	19
Females	121	58	68
Sex ratio (Γ:E)	1: 3.7	1: 2.3	1: 3.6
χ^2 analysis	χ^2=2.493, df= 2, P= 0.288		

6.5.2.3 Age specific fecundity and survival of *A. atomus* at different constant temperatures

Anagrus atomus laid most of her eggs on thef first two days of emergence. Females lived longer at 17°C and completed laying eggs within 7 days of emergence, whereas at 25°C females completed laying egg within 4 days of emergence. Cumulative fecundity of *A. atomus* indicated that the females laid 50% of their eggs during the first 2 days of their reproductive life (Figure 6.6). Temperature significantly affected both potential and actual fecundity of *A. atomus* (Figure 6.8). Both potential and actual fecundity was the highest at 17°C.

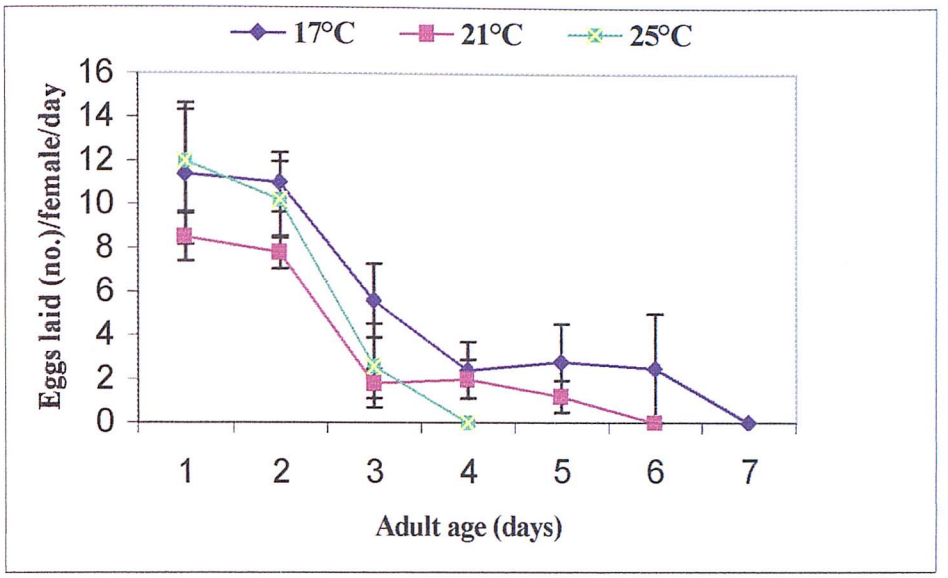


Figure 6.5 **Mean daily fecundity (actual) of the egg parasitoid *Anagrus atomus* at different constant temperatures. Vertical bars represent the standard errors of mean.**

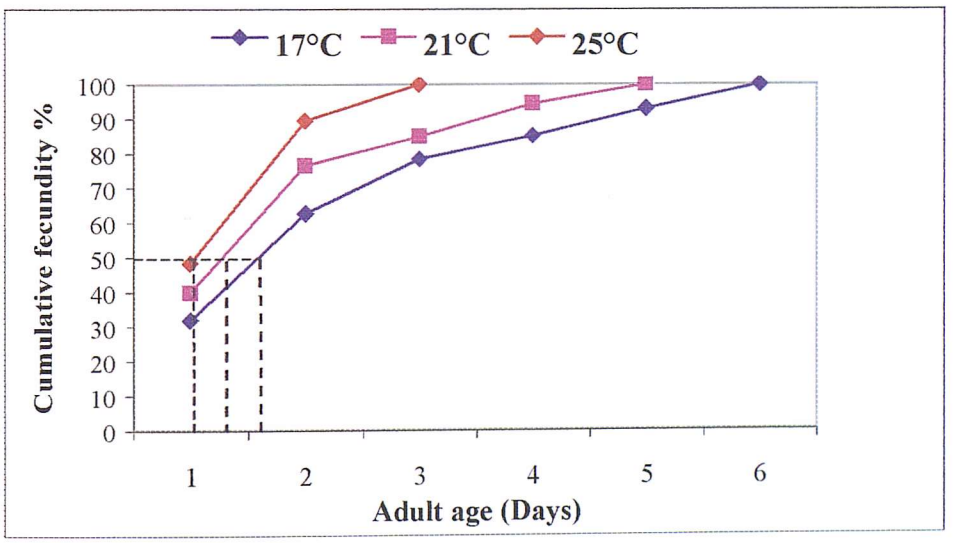


Figure 6.6 **Cumulative fecundity of *Anagrus atomus* at different constant temperatures.**

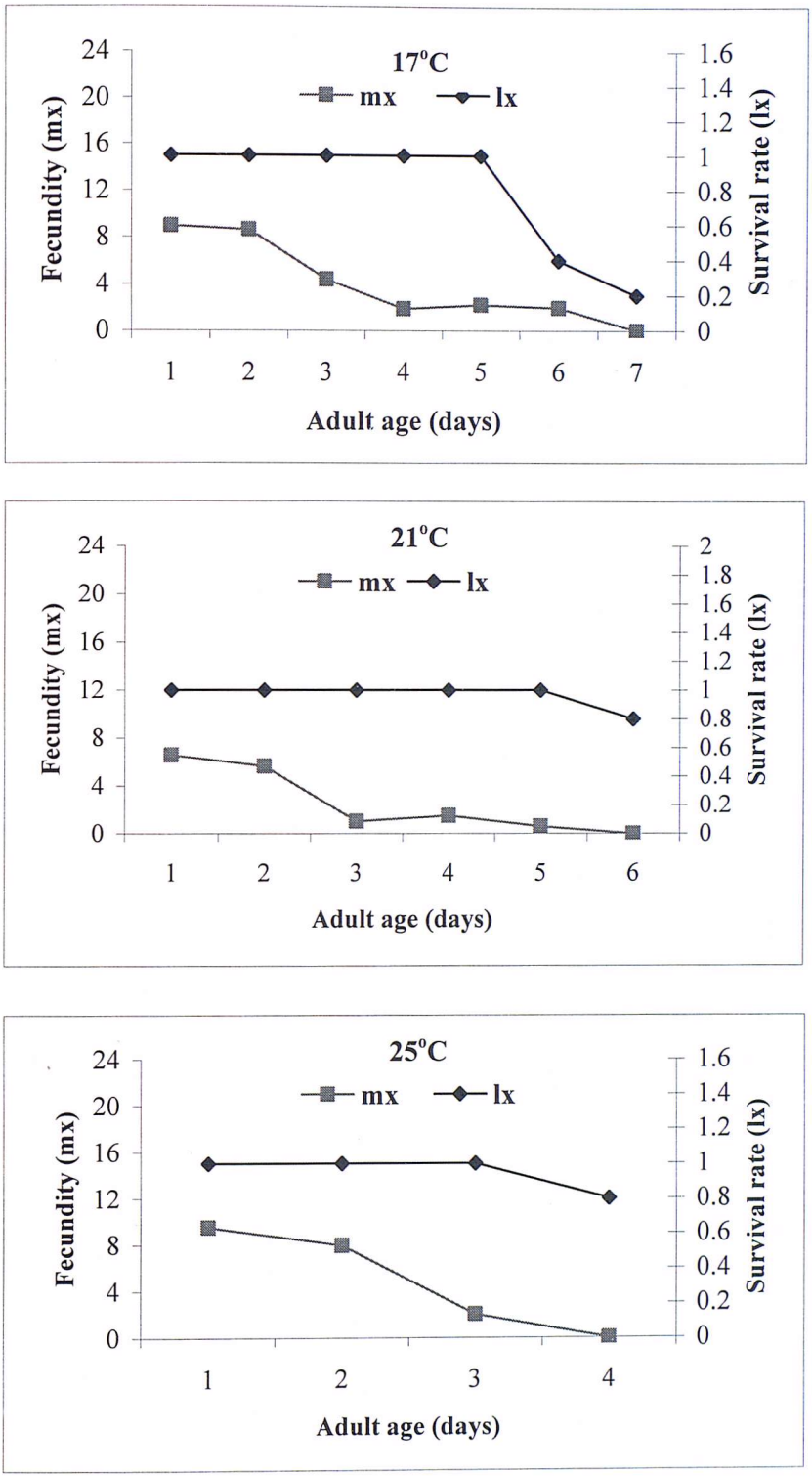


Figure 6.7 **Fecundity and survival rates of the egg parasitoid *Anagrus atomus* on the eggs of *Hauptidia maroccana* laid on Primula at different constant temperatures.**

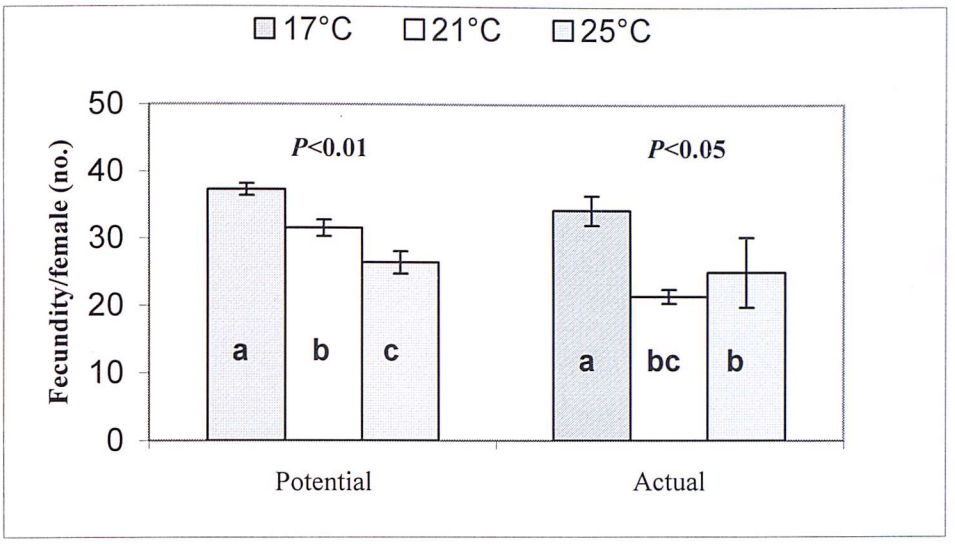


Figure 6.8 **Actual and potential fecundity of *Anagrus atomus* emerging at different constant temperatures.**

6.5.2.4 Life table parameters of *A. atomus* at different constant temperatures

The intrinsic rate of natural increase (r_m) and the associated life table parameters of *A. atomus* at different constant temperatures are presented in Table 6.7. The net reproductive rate (R_o) was the greatest at 17°C due to high total fecundity. The lower (R_o) at 21°C than that at 25°C might be due to the lowest total fecundity at 21°C (Figure 6.8). The intrinsic rate of natural increase (r_m) increased with rising temperature and hence the generation time and population doubling time was decreased. The lowest generation and doubling time was observed at 25°C because this is the optimum temperature for egg development of the host *H. maroccana*.

Table 6.7 Life table parameters of the parasitoid *Anagrus atomus* ovipositing on the egg of *Hauptidia maroccana* at different constant temperatures.

Life table parameters	17°C	21°C	25°C	
Gross reproductive rate (*GRR*)	28.05	16.36	19.50	**Female eggs/female/generation**
Net reproductive rate (R_0)	26.87	15.36	19.50	**Females/female/generation**
Capacity of increase (r_c)	0.12	0.14	0.22	**Female offspring/female/day**
Intrinsic rate of increase (r_m)	0.12	0.14	0.22	**Female eggs/female/day**
Cohort generation time (*Tc*)	27.43	19.39	13.32	**Days**
Generation time (*T*)	27.33	19.31	13.27	**Days**
Finite capacity for increase (λ)	1.13	1.15	1.25	**Female offspring/female/day**
Doubling time (*DT*)	5.76	4.90	3.70	**Days**

6.6 Discussion

6.6.1 Survival, fecundity sex ratio of *A. atomus* on the egg of *H. maroccana* laid in different host plants

The χ^2 analysis showed a significant difference in the parasitoid survival on different host plants (Table 6.1). The highest (89.31%) and the lowest (72.46%) survival were observed on cucumber and on French bean, respectively. The lowest survival in French bean might have resulted from drying up of the leaf veins, which adversely affected adult emergence.

Several studies dealing with aspects of tritrophic relationship demonstrated that different characteristic of the host and host plant involved with the localization and the acceptance of the host by parasitoid might influence the rate of oviposition such as chemical and physical characteristics of the leaf surface (Hulspas-Jordan and van Lenteren, 1978). In the present study, the total fecundity of *A. atomus* was not significantly influenced by different host plants although *A. atomus* laid comparatively more eggs in French bean than the other two host plants (Figure 6.2). The SEM study shows a wide range of differences in leaf surface topography (Chapter 10). The reason for

low oviposition rate on Cucumber and Primula might be due to the presence of dense trichomes on leaf veins, which adversely affected the walking speed and host finding by the parasitoids or it might be due to shorter egg laying periods. On the other hand, the ovipositing females of *A. atomus* lived longest (6-8 days) in French bean and the shortest (5-6 days) in Cucumber. The longevity of female parasitoids is primarily determined by their size and diet (Godfray, 1994). Here, diet alone cannot explain the observed differences in longevity of the ovipositing female since all the females were provided with the same diet and were apparently of the same size. Therefore, there might be some antibiosis effect of the host plants, which influenced the longevity of the females.

The fact that comparatively more female offspring developed from the eggs laid in French bean indicates the suitability of the host plants.

6.6.2 Life table parameters of *A. atomus* on the egg of *H. maroccana* laid in different host plants

The intrinsic rate of increase, mean generation time and population doubling time are useful indices of population growth under a given set of growing conditions. It is generally believed that shorter development time and greater total reproduction on a host reflect the suitability of the host plant tested (van Lenteren and Noldus, 1990). In the present study, the intrinsic rate of increase (r_m) and the net reproductive rate (R_o) of *A. atomus* is influenced by the host plants. The R_o and r_m of the population of *A. atomus* ranged from 15.36 to 27.73 and 0.14 to 0.119, respectively on different host plants. *A. atomus* developed on cucumber showed a higher intrinsic rate of increase (0.1991) resulting from faster development (15.35 days), thus reducing the generation time and population doubling time (3.48 days). The lowest r_m value in Primula resulted from the longest development time (18.44 days) and therefore contributed to the longest population doubling time (4.89 days). Souissi and Rii (1997) studied the life table parameters of the cassava mealybug parasitoid *Apoanagyrus lopezi* De Santis on four different host plants. They mentioned a 1.5 times higher net reproductive rate (R_o) on Talinum and Zanaga than on Faux-caoutchouc and Incoza due to the antibiosis effects in Incoza, which negatively affected the R_o.

6.6.3 Survival, fecundity, sex ratio of *A. atomus* on the egg of *H. maroccana* at different temperatures

The probability of survival of the immature stages of the parasitoid is strongly dependent on the survival of the host. Hosts may be killed through predation, starvation and exposure to unfavourable weather conditions that leads to the death of any parasitoids that are attached to or contained within the host (Jervis and Copland, 1996). Moreover, parasitoids may be more hot/cold hardy than their hosts, in which case the lethal range of temperatures for the host will determine parasitoid survival. On the other hand, parasitoids may be less hot/cold hardy than their hosts, such that they cannot tolerate the extreme of temperature that the host can tolerate, and so die as a result of thermal stress (Jervis and Copland, 1996).

In the present study, survival of the immature stages of *A. atomus* reduced with increasing temperature. Most of the mortality was caused at the pupal stage. The pupae failed to emerge and died half way to emergence due to desiccation of the outer surface of the host egg. Zarokosta (1999) reported less immature mortality of the whitefly parasitoid *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) at lower temperature. Temperature did not show any significant effects on the sex ratio of the emerging parasitoids although the sex ratio was always female biased. The highest ratio of female to male (3.7:1) was observed at 17°C.

Being a pro-ovigenic species, *A. atomus* adults began ovipositing from the first day of eclosion. The daily oviposition rates reached their highest value in the first two days of emergence and sharply declined afterwards. The lifetime fecundity schedule of the parasitoid shows that the fecundity plateau (the period of maximum egg laying when females have produced ≥50% of their total production capacity) occurred within two days of emergence. The cumulative fecundity of the parasitoid is expressed as the proportion of the highest mean total number of eggs laid per females, this total representing the maximum fecundity/fitness realised by females. The cumulative fecundity measure provides an indication of the extent to which parasitoids achieve their maximum life time fecundity under a particular condition (Jervis and Copland, 1996). On average *A. atomus* laid 34.2, 21.4 and 25 eggs at 17, 21 and 25°C, respectively, 50 percent of which was laid in first two days of emergence (Figure 6.6). Omer *et al.* (1996) also found a similar

oviposition pattern in the leafminer parasitoid *Ganaspidium utilis* Beardsley (Hymenoptera: Eucoilidae). They observed highest progeny production on day 2 and day 3 of the parasitoid's reproductive life. The decreased fecundity at high temperature may be due to the reduction in longevity of the female parasitoids. Baitha and Ram (1998) reported decreased fecundity and longevity of the egg parasitoid *Trichogramma* sp. nr. *armigera* Nagaraja with increasing rearing temperature.

The real or actual fecundity may fall slightly short of the estimate for the potential fecundity (Jervis and Copland, 1996). In the present study, the potential fecundity of *A. atomus* was always higher than the number of eggs laid during her life time at the range of temperature tested (Figure 6.8)

The adult parasitoid *A. atomus* had a variable life length at different temperatures. It lived for the longest (6-8 day) when laying eggs at 17°C and the shortest (4-5 days) at 25°C.

6.6.4 Life table parameters of *A. atomus* on the egg of *H. maroccana* at different temperatures

The determination of life history parameters requires laboratory studies, despite the fact that in the natural environment populations are subjected to more complex and fluctuating situations. Among different life table parameters the intrinsic rate of natural increase (r_m) is of great interest because it integrates the effects of all existing mortality and fertility into a single value (Doury and Rojas-Rousse, 1994). In the present study, the population of *A. atomus* reared at 17°C showed a much smaller intrinsic rate of increase than the population at 21 and 25°C (Table 6.7), despite the fact that they had the highest oviposition (34.2) and a higher survivorship (90%) of the immature stages. The reduction in r_m could be attributed to longer immature development time (26.06 days) compared to the development duration of 17.58 and 12.56 days at 21 and 25°C, respectively, which resulted in a considerably longer mean generation time (27.33 days).

The population reared at 25°C had a significantly lower maturation rate and much less egg production than those reared at 17°C (Figure 6.6) but had a higher r_m value. The

increase in r_m could be caused by the significantly faster development of immature stages (12.56 days) and a shorter reproductive period, which resulted in considerably shorter mean generation time (13.27 days) (Table 6.7).

The effect of temperature on r_m and R_0 of parasitoids has been recognized in many studies. Morales-Ramos *et al.* (1996) reported in the pteromalid *Catolaccus grandis* (Burks) the values of r_m and R_0 were affected by temperature. Similar findings were also reported by Enkegaard (1993) in aphelinid *Encarsia formosa* Gahan, where the life table parameters were affected by increasing temperature from 16 to 28°C. Huis *et al.* (1994) found a significant effect of temperature on the population increase of the trichogrammatid *Uscana lariophaga* Steffan. In the encyrtid mealybug parasitoids *Anagyrus pseudococci* (Girault), *Leptomastix dactylopii* Howard and *Leptomastidea abnormis* (Girault), the progeny production was reported to increase with rising temperature within the range of 18 to 30°C (Tingle and Copland, 1989). In the present study, 50% of the eggs have been laid within the first two days of adult emergence. These findings suggest that the biological significance of r_m is influenced to a greater extent by the fecundity of the parasitoids in the earlier part of their adult life rather than the total number of eggs laid over its life span.

Chapter 7

Effect of three different diets on the adult longevity of *A. atomus*

7.1 Introduction

The total life span or the longevity of a parasitoid is an important factor in the overall quality of a parasitoid (Bigler *et al.*, 1987). The availability of foods may play an important role determining the effectiveness of a parasitoid as population control agents (Jervis *et al.*, 1992). The nutritional quality of the food, which the parasitoids feed on, affects their life expectancy (Heimpel and Rosenheim, 1995). Nectar, pollen, homopteran honeydew, host blood or substitutes for these (e.g., diluted honey and sucrose solution) are important sources of parasitoid food (Jervis *et al.*, 1992). Naturally occurring carbohydrates such as pollen, nectar and honeydew are known to increase longevity and fecundity of adult parasitoids (Muegge and Lambdin, 1989). Honey and honeydew as a food source is reported to increase the longevity of parasitoid (Milonas and Soultani, 2000). Homopteran honeydew might have some influence on the longevity of adult *A. atomus*. Therefore this study was undertaken with the following objective.

7.2 Objectives of the experiment

The experiment was conducted to examine the effects of three adult diets comprising water, honey solution and mealybug honeydew on the longevity of the egg parasitoid *A. atomus*.

The null hypothesis:

Diet has no effect on the longevity of adult *A. atomus*.

7.3 Materials and Methods

Effects of three different diet, water only, mealybug honeydew and 50% honey solution on the longevity of adult males and females of *A. atomus* were studied in the laboratory at 21°C. The relative humidity was maintained at 85% r.h. using saturated KCl

salt solution. Individual parasitized leafhopper eggs (red egg stage) inside leaf veins were placed inside 0.5ml Eppendorf microcentrifuge tube with a piece of wet filter paper. Those tubes were then kept in the incubator at 21±1°C in a plastic box for the emergence of the parasitoid adults.

An individual newly emerged *A. atomus* adult was placed carefully inside the 2.0ml Eppendorf safe-locked microcentrifuge tube with the help of a fine paintbrush. Ten to twelve fine holes were made on the lid of the tube with a fine needle to provide aeration inside the tube. A piece of wet filter paper was placed inside the tube, which served as a source of water. In another treatment, 50% honey solution was smeared on the porous lid of the tubes so that the parasitoid could feed on it. In the other treatment, honeydew was collected from long tailed mealybug culture and diluted to 50% with water. The honeydew solution was smeared on the porous lid. An individual parasitoid was considered as one replication. Forty replications were maintained for each treatment. The tubes were placed in a plastic box and placed in an incubator at 21°C under continuous light. Humidity inside the plastic box was maintained at 85% r.h. using saturated KCl salt solution. Each tube was checked everyday and the diet in each treatment was replaced daily, until the death of the adults. If any of the adult parasitoids adhered to the water droplet or the honey solution or escaped, it was discarded from the analysis. Natural death of adult wasps was recorded and finally the mean longevity was calculated for each treatment.

The hind tibia length and the head width of the parasitoid adults were measured immediately after death to investigate the relationship of body size with life span.

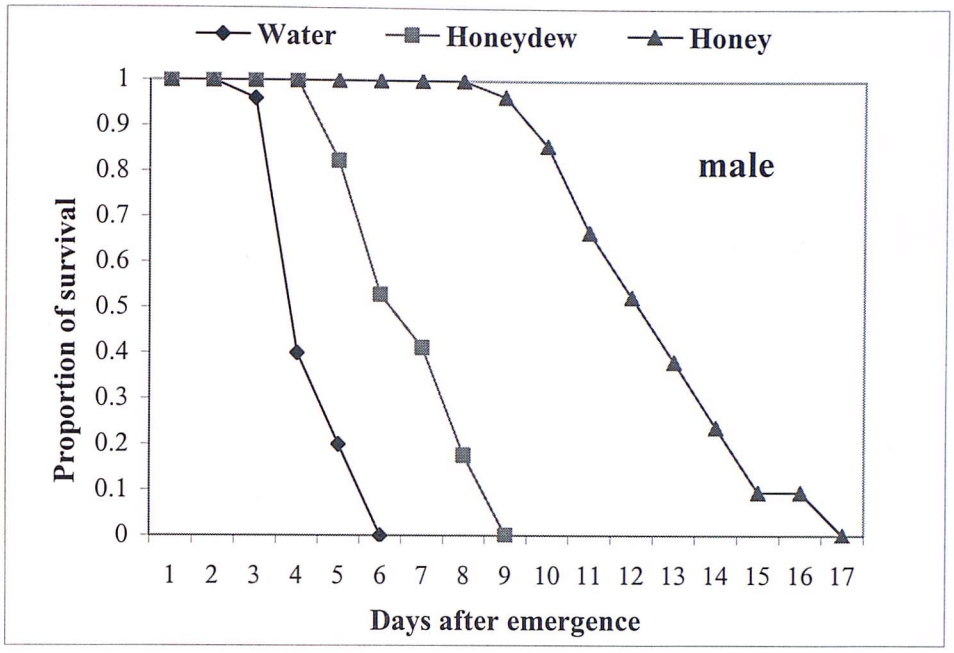
7.4 Statistical analysis

One-way ANOVA was used to compare the adult longevity on different diets in SAS. The means were compared using LSD at 5% level. Mean longevity of male and female on a particular diet was compared using a Two-sample t test.

7.5 Results

7.5.1 Survival pattern of *A. atomus*

Feeding regimes affected the adult longevity of both sexes of *A. atomus*. The survival pattern of male and female parasitoids on different diets is presented in Figure 7.1. Females were always found to live longer than the males. A similar pattern of survival was observed for both male and female in three different diets. Longer lifespan was observed when the adults were fed with 50% honey solution followed by 50% honeydew solution and water. Honey solution fed adults had a survival range of 4-12 days and 8-16 days in males and females, respectively, whereas, the survival range was only 2-6 days and 2-5 days for the male and female, respectively when fed on water.



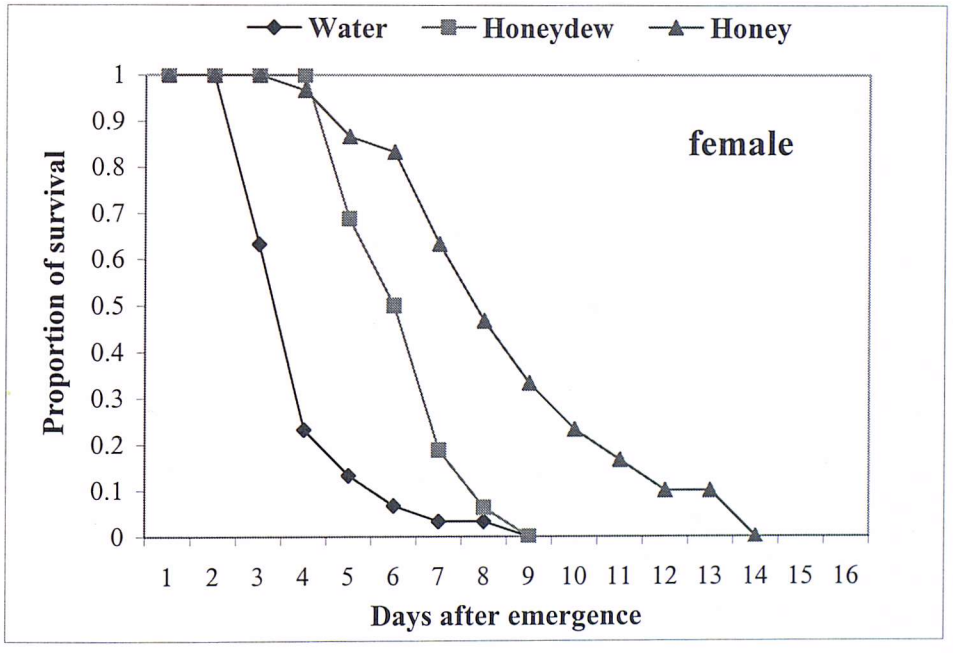


Figure 7.1 Survival patterns of male and female *Anagrus atomus* when fed on different dietary supplements in the absence of hosts.

7.5.2 Longevity of *A. atomus* on different diets

Adult longevity of both male and female *A. atomus* with different diets is presented in Table 7.1. Longevity of both male and female wasps was significantly affected by different dietary sources (male: $P<0.001$, df= 73, LSD= 1.109; female: $P<0.001$, df= 60, LSD= 1.011). Adults with access to 50% honey solution had the longest lifespan. Both male and female adults lived the shortest time when provided with only water. Females lived significantly longer than the male when fed with 50% honey solution but the longevity of male and female was not significantly different on the other two diets.

Table7.1 Effect of three different diets on adult longevity of male and female *Anagrus atomus* at 21°C.

Diet	Mean longevity of *A. atomus* (days)				Two-sample t test
	Male		Female		
	n	mean±s.e.	n	mean±s.e.	
Water	30	3.13±0.252 a A	25	3.56±0.174 a A	t= -1.39 $P>0.05$ df=49
50% honey solution	30	7.60±0.459 b A	21	11.76±0.501 b B	t= -6.12 $P<0.001$ df=45
Honeydew	16	5.44±0.316 c A	17	5.94±0.348 c A	t= -1.07 $P>0.05$ df=30
One-way ANOVA	$P<0.001$, LSD= 1.109, df=73		$P<0.001$, LSD= 1.0107, df=60		

Means in columns, followed by the same lowercase letter and in rows followed by uppercase letters are not significantly different from each other at $P\leq 0.05$
One-way ANOVA followed by Least Significant Difference test.
n = no. of insect as replicates.

7.5.3 Relationship between size and longevity of *A. atomus* adults

The regression analysis did not show any significant relationship between the size and longevity of the female parasitoid wasp (P=0.084) although the larger females appeared to live longer. Unlike females, a significant relationship was observed between longevity and size of male parasitoid wasp (P<0.05) (Figure 7.2).

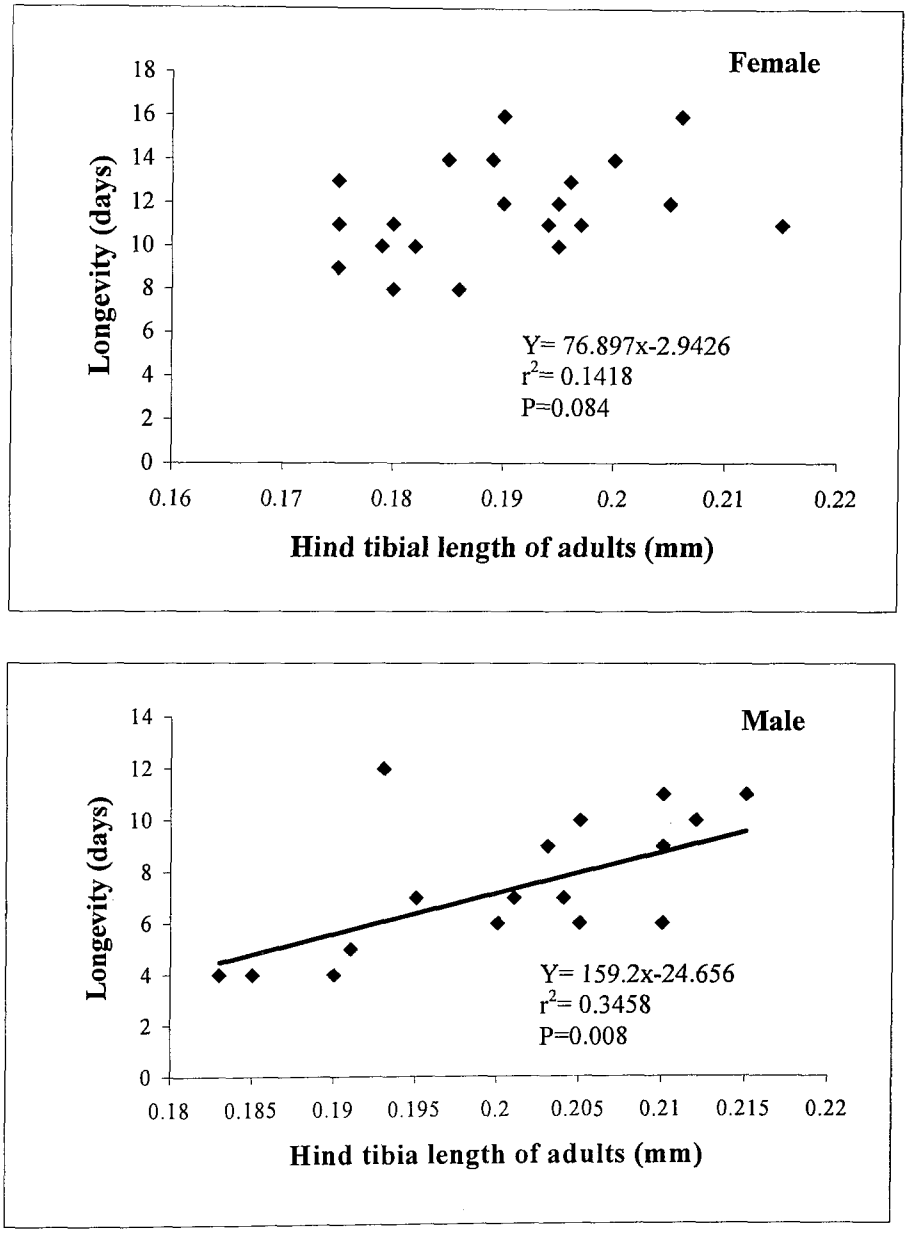


Figure 7.2 Relationship between size and longevity of male and female *Anagrus atomus* adults at 21°C.

7.6 Discussion

It is evident from the results that honey solution appeared to be the best of all the diets provided. Homopteran honeydew was found to be a better source of food than water but not as suitable as honey solution. Therefore it is suggested that a carbohydrate source, perhaps from flower nectars, is necessary to increase the life span of the parasitoid. An increase in adult longevity of the parasitoid wasps fed on honey, has been reported by many authors (Ridgeway and Mahr, 1990a, 1990b; Morales-Ramos and Cate, 1992; Bernal *et al.*, 1999). Balsdon *et al.* (1996) found a significant increase in the longevity of *A. takeyanus* feeding on honey solution than those fed with only water. A 10 to 13 fold increase in longevity of *Trichogramma platneri* adults was observed in a laboratory experiment when fed with sucrose solution compared to water (McDougall and Mills, 1997). Kim and Morimoto (1995) observed a longer life span of *Microterys flavus* (Howard) when fed on honey than those fed on water or host food.

Females were deprived of hosts in the experiment so that they could not lay eggs. When comparing the longevity of host deprived females with those ovipositing in hosts it was found that the non-ovipositing females lived longer than the ovipositing females although they were provided with the same dietary supplement (honey solution). In the present study when *A atomus* was provided with hosts, females lived on average for 5.8 days (Chapter 6) while the adult longevity was doubled (11.76 days) in the absence of hosts at the same temperature with the same diet provided. The presence of hosts influences parasitoid longevity and has been reported by Drost *et al.* (1996). This higher longevity of the females in the absence of hosts might be the result of resorption and utilization of oocytes when they were starved (van Lenteren *et al.*, 1987). On the other hand, the decreased longevity of the females in the presence of hosts might either be the result of the utilization of energy during oviposition or spending more time in oviposition rather than feeding. No egg resorption was observed in *A. atomus* and such a strategy could be unwise in a pro-ovigenic species.

The honeydew of Homopteran insects contains nitrogen and free amino acids and sugar (Auclair, 1963). However, honeydew may sometimes have depressive effects on parasitoid longevity due to the presence of melezitose (Leius, 1961). The deleterious

effect of some honeydew on parasitoid life span may be due to the presence of some complex plant chemicals (Avidov *et al.*, 1970).

In the present study, it is evident that honey solution and honeydew of mealybug (Pseudococcidae) significantly increased the longevity of adult *A. atomus*. Therefore, the availability of sugar-rich food may increase the longevity of the parasitoid and increase the potential of parasitism in the field.

Chapter 8

Oviposition behaviour of *Anagrus atomus*

8.1 Introduction

In general, host selection behaviour by parasitoids can be divided into habitat selection and into a series of behaviours that lead to oviposition in or on the host (Vinson, 1998). Since oviposition is directly associated with the transmission of the female genome, natural selection acts strongly on the behaviour associated with it, resulting in finely tuned adaptations. This is especially true for optimal progeny and sex allocation in which female parasitoids upon encountering a possible host, must decide whether to accept it or not (King, 1987). Once a potential host is recognised by a parasitoid after thorough antennal examination and considered suitable, she abandons the use of antennae and probes the host with her ovipositor (Vinson, 1998). During inspection, detection of previous parasitism is an important factor in the decision of a female. Female parasitoids can perceive the changes in the chemistry of the parasitized host by inserting the ovipositor (Vinson, 1998).

However, host selection and oviposition behaviour is variable, partly because of the multitude of factors that enter into the decision making (Vinson, 1998). These factors include the effect of the physiological condition of the female (Vinson and Iwantsch, 1980a ; Nurindah *et al.*, 1999b), and her previous experience (Bjorksten and Hoffmann, 1995; Nurindah *et al.*, 1999a). Experienced females have been reported to respond sooner and exhibit more direct flights to a host-plant complex than inexperienced females (McAuslane *et al.*, 1991). As the quality of the egg as a resource decreases with time (Vinson, 1998), the host age for the egg parasitoid is an important factor in decision making for oviposition.

Studying the components of oviposition behaviour provides a more subtle means of detecting host preference by the parasitoids (Nurindah *et al.*, 1999a). Details of the oviposition process of few egg parasitoids have been studied. In many of the studies, emphasis was on *Trichogramma* species, scelionids, or encyrtids (van Dijken and Waage, 1987; Strand, 1989; Miura *et al.*, 1994), which are parasitoids of hosts that lay their eggs exposed on the substrate. In contrast, most of the Mymaridae attack host eggs that are laid

concealed within plant tissue, under scales of scale insects, or in the soil (Huber, 1986). Searching and oviposition behaviour have been described for some mymarid species attacking concealed eggs (Cronin and Strong, 1990; Moratorio, 1990; Honda and Walker, 1996; Conti *et al.*, 1996, 1997; Al-Wahaibi and Walker, 2000). No work has been published so far on the oviposition behaviour of *A. atomus*.

The present study was designed to describe the sequence of oviposition behaviour of *A. atomus* attacking unparasitized and parasitized *H. maroccana* eggs inside the leaf veins of the host plants.

8.2 Objectives of the Study

The experiment was conducted to study the different components of the oviposition behaviour of *A. atomus* and to evaluate:

i) whether previous experience alters parasitoid oviposition behaviour

ii) the behavioural activities while encountering younger and older eggs

iii) the differences among the activities while encountering unparasitized and previously parasitized eggs and

iv) the proportion of time spent in different activities while encountering young, old and parasitized hosts.

The null hypothesis:

1) Previous experience does not have any affect on parasitoid oviposition behaviour.
2) Oviposition behaviour does not vary due to different host age (younger versus older host; unparasitized versus parasitized).

8.3 Materials and Methods

Different components of the oviposition behaviour of the egg parasitoid *A. atomus* on *H. maroccana* eggs were studied in the laboratory at 21±1°C and 70% r.h. The parasitoids were collected from the mass culture in the laboratory. Upon emergence an individual parasitoid was placed inside a 0.5ml Eppendorf microcentrifuge tube with a piece of wet filter paper soaked in 50% honey solution. Those tubes were then kept in the incubator at 21±1°C in a plastic box for 24 hours.

Three different types of hosts unparasitized younger eggs (1-3 d old), unparasitized older eggs (7-9 d old) and 24 hours old parasitized eggs. Two levels of experience of the parasitoid inexperienced and experienced were used in the experiments. Younger and older eggs were collected in the same procedure described in chapter 4.

Individual male and female *A. atomus* were introduced into a 0.5 ml Eppendorf microcentrifuge tube to allow mating (Plate 8.2). Then an individual mated female parasitoid was introduced into the Petri-dish arena and its behaviour was observed under a binocular microscope while encountering younger and older eggs. The leaf alongside parasitized eggs was marked with a fine fibre tip pen. The same parasitoid was then transferred to another Petri-dish containing 24 h old parasitized eggs and was allowed to oviposit into it.

The following activities of *A. atomus* females were observed under the binocular microscope and the time spent in different activities was recorded using a stopwatch.

1) Host examination (antennal drumming): The period from the parasitoid's first coming in contact with the host by antennal drumming to the withdrawal of the antennae from the host.
2) Ovipositor positioning: Parasitoids place their ovipositor on different parts of host surface to find a suitable point for ovipositor insertion.
3) Ovipositor insertion: Time from when the parasitoid inserts half to the full length of her ovipositor inside the host and until a pumping motion of the abdomen.
4) Abdominal pumping: The parasitoid starts to pump her abdomen horizontally and with some vertical strokes to release her eggs and then withdraws her ovipositor.
5) Host examination following oviposition: After a complete bout of oviposition the parasitoid makes a turn and examines the same hosts by antennal drumming and finally leaves the host.
6) Number of turns: Turnings made during examining the same host following oviposition.

Thus the total oviposition period was calculated by summing the component 3 and 4 and the total handling time by summing 1 to 5. Parasitoids that did not show activities 1-4 were considered as unsuccessful in oviposition and were not included in the analysis. Finally nineteen parasitoids were observed to make successful ovipositions and each parasitoid was considered as a replication.

8.4 Statistical analysis

One-way ANOVA was done in SAS to compare different activities among inexperienced parasitoids and the experienced parasitoids handling younger, older and parasitized hosts. The means were compared in LSD at 5% level.

8.5 Results

8.5.1 General behaviour observed in oviposition

The oviposition behaviour of *A. atomus* is presented in Figure 8.1 and can be divided into three phases:

(A) Pre-oviposition: The oviposition phase can be defined as host examination and the acceptance of the host for oviposition. In this phase the parasitoid examined the suitability of the host by antennal drumming over the surface of the host and tried to position her ovipositor for insertion.

(B) Oviposition: The oviposition phase is defined as ovipositor insertion, oviposition and withdrawal of ovipositor after oviposition. Here the females showed different levels of ovipositor insertion and shaking and pumping of the abdomen.

(C) Post-oviposition: This phase is defined as the period from withdrawal of the ovipositor until the female leaves the host. Re-examination of the host by antennation and turning has been observed in this phase.

To monitor the host acceptance behaviour of a parasitoid it is necessary to know the likelihood that certain behaviour follows another and also to understand the effect that physiological state and experience has on this behaviour (Nurindah *et al.*, 1999b). When a parasitoid located a host she started to drum her antennae repeatedly to make contact with the surface of the egg. Once an egg was accepted she stopped walking over the leaf vein, lifted her antennae, making an almost straight angle with her wings and unsheathed her ovipositor (Plate 8.2). Then she tried to find a suitable place for inserting the ovipositor. Ovipositor insertion started by lifting the body above the substrate and placing the ovipositor to the substrate at approximately 90°. Generally during the beginning of long probes, an abdominal pumping motion occurred, with large amplitude waves running longitudinally along the abdomen. Close to the end of the majority of the long probes, vertical up and down shaking of the whole abdomen was Observed. In some cases

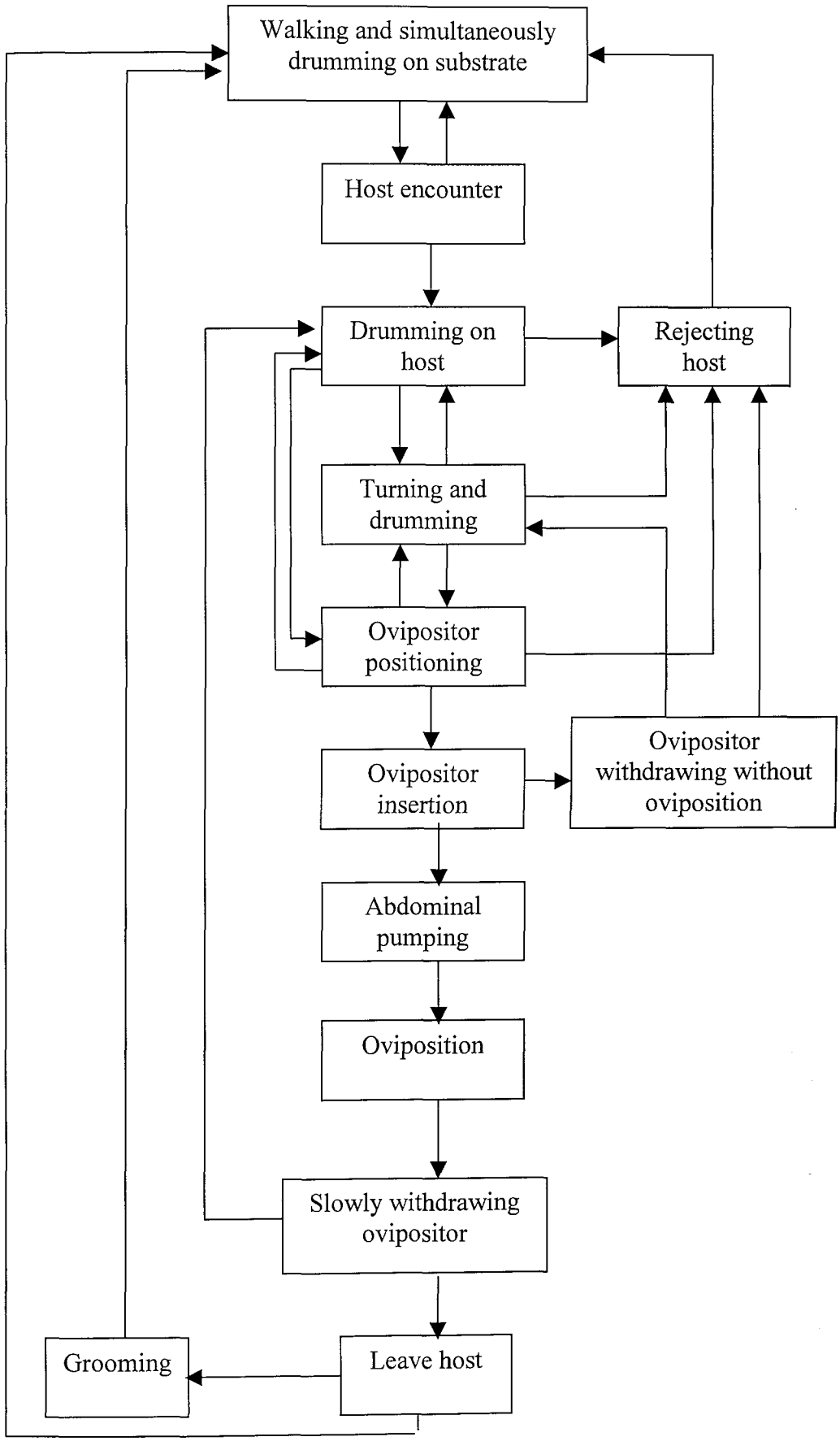


Figure 8.1 The general sequence of oviposition behaviour of the egg parasitoid *Anagrus atomus* on the egg of *Hauptidia maroccana*.

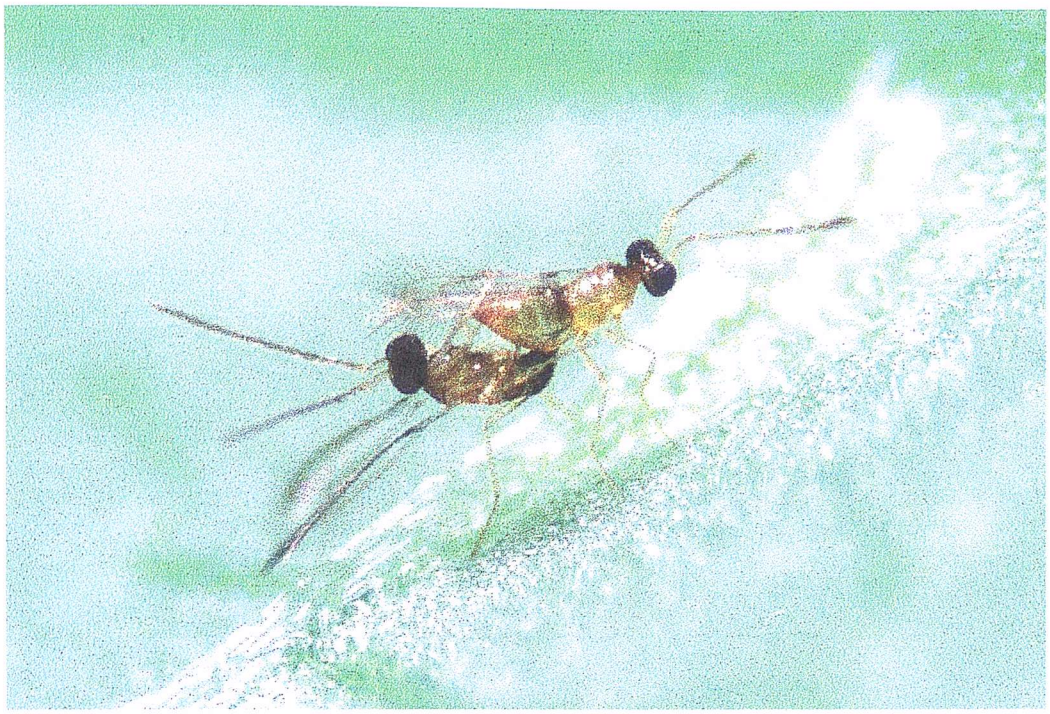


Plate 8.1: Mating posture of *Anagrus atomus* (x 50).



Plate 8.2: An ovipositing female of *Anagrus atomus* (x 75).

antennae also moved with the vertical shaking which had remained motionless at the beginning of the probe. On the other hand, parasitoids never showed a pumping motion or abdominal shaking while probing into previously parasitized hosts. Sometimes the females, being confused, made a probe into the leaf veins but this did not last long (<10 s). In some cases, the females withdrew their ovipositor from an unsuitable host after a short probe (10-40 s). No abdominal shaking was observed during those short probes. Instead, full insertion of the ovipositor was often observed in short probes. While in long probes (>50 s), the ovipositor was never completely inserted into the host.

After ovipositing, the female withdrew her ovipositor from the host and always turned 180° to re-examine before marking. The female then turned 180° and started examining and marking again. This sequence often ended after marking although the parasitoid often re-examined the marked eggs.

8.5.2 Percent duration of the components of oviposition in relation to experience and host type

The proportion of the time spent in different activities during oviposition is presented in Figure 8.1. A parasitoid spent most of its host handling time in oviposition and a major part comprised abdominal pumping in successful oviposition. Only a little time is spent in host examination while encountering an unparasitized host. On average 77 and 76% of host handling time was spent in oviposition in the unparasitized hosts by inexperienced and experienced females, respectively, whereas only 7 and 8% time was spent in host examination in unparasitized hosts by the inexperienced and experienced parasitoids. Inexperienced and experienced parasitoids spent 64% and 60% time, respectively in abdominal pumping. On the other hand, parasitoids spent 33% and 55% of the time spent in host examination and in ovipositor insertion, respectively, while encountering parasitized eggs. In contrast to unparasitized eggs, parasitoids did not show any abdominal pumping while encountering previously parasitized eggs.

Frequency distribution of probe duration by *A. atomus* is presented in Figure 8.2. The maximum frequency of probe duration was found to be 120-130 s in case of inexperienced parasitoids. But when the same parasitoids gained experience, most of the ovipositional probes were completed within 80-90 s. When encountering previously

parasitized eggs the most common frequency of probe duration was 0-10 s. The cumulative percent of the probes reached 100% at the duration of 160-170 s, 130-140 s and 30-40 s in inexperienced parasitoids, experienced parasitoids and in probing in parasitized eggs, respectively.

8.5.3 Oviposition behaviour of *A. atomus* with different experience levels and in hosts of different status

The one-way analysis of variances showed significant difference among some of the components of oviposition behaviour presented by inexperienced and experienced parasitoids in young, old and previously parasitized hosts (Table 8.1). Ovipositor positioning, total oviposition period and total host handling time was longest (10.4 s, 122.5 s and 156.5 s, respectively) in inexperienced parasitoids and shortest (3.9 s, 17.3 s and 31.8 s, respectively) when experienced parasitoids probed into previously parasitized hosts. There was no significant variation in host examination and oviposition insertion time among different experience levels and host status.

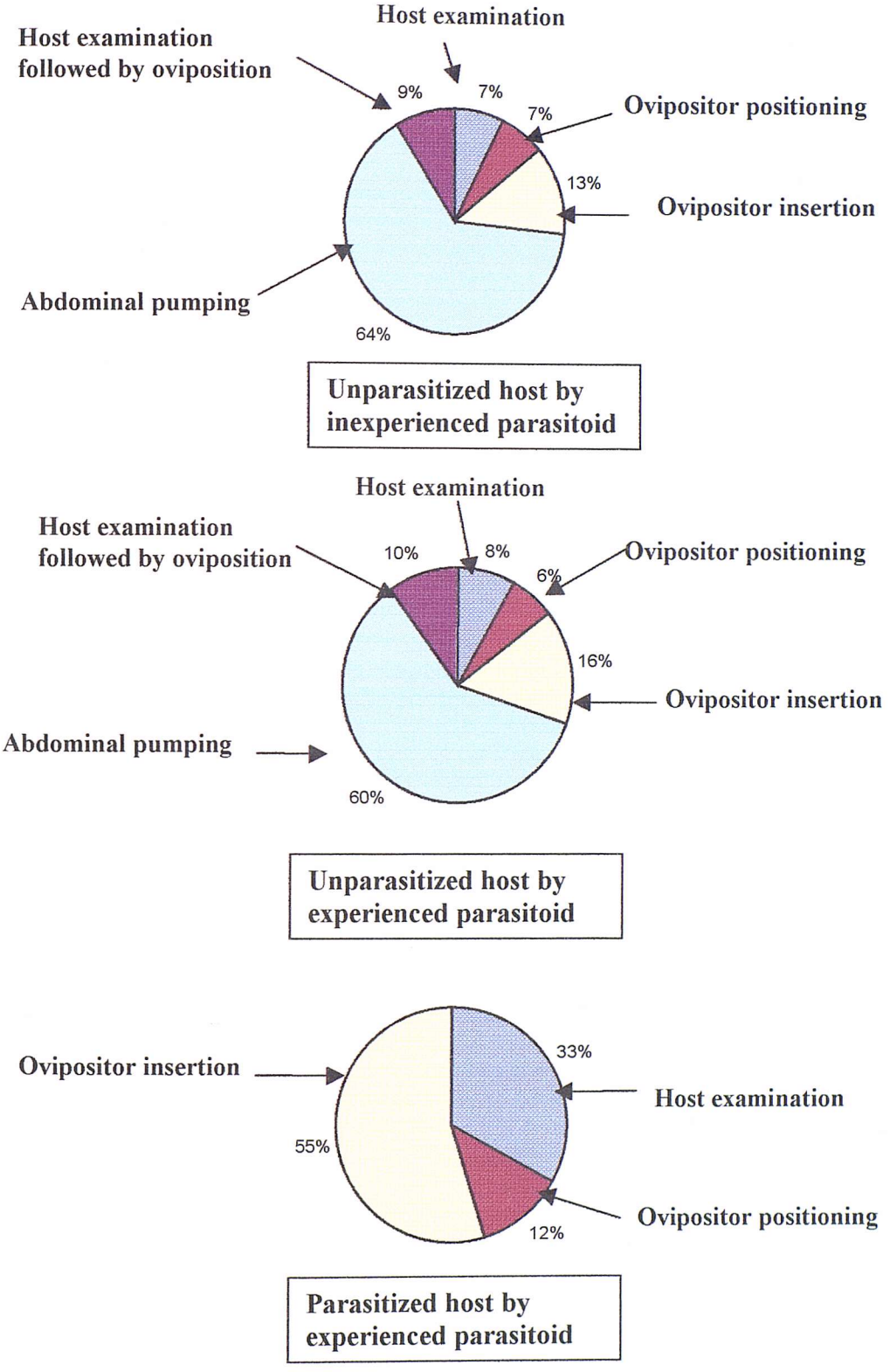
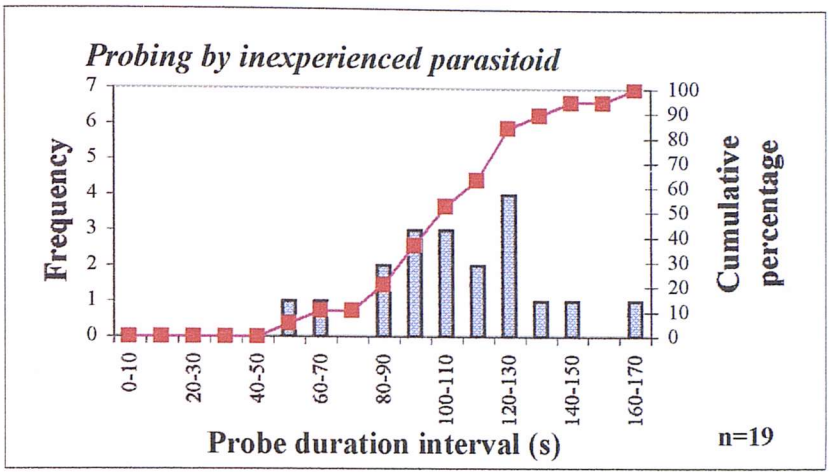
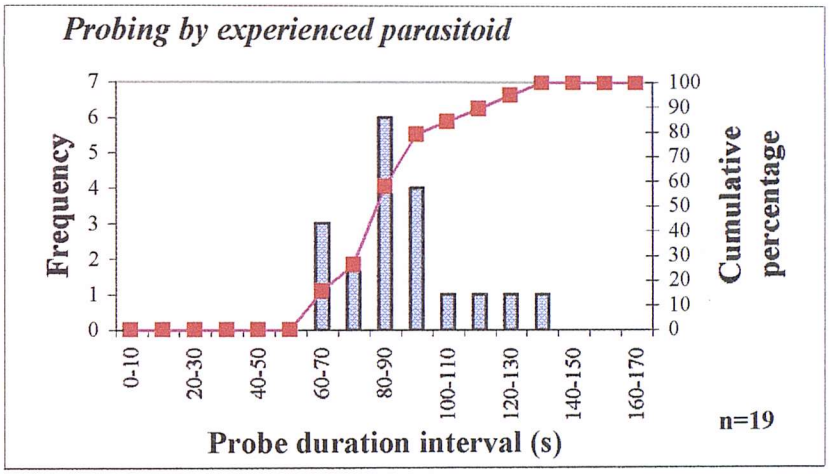


Figure 8.2 **Time spent (% of total host encounter) by *Anagrus atomus* in different activities during oviposition**





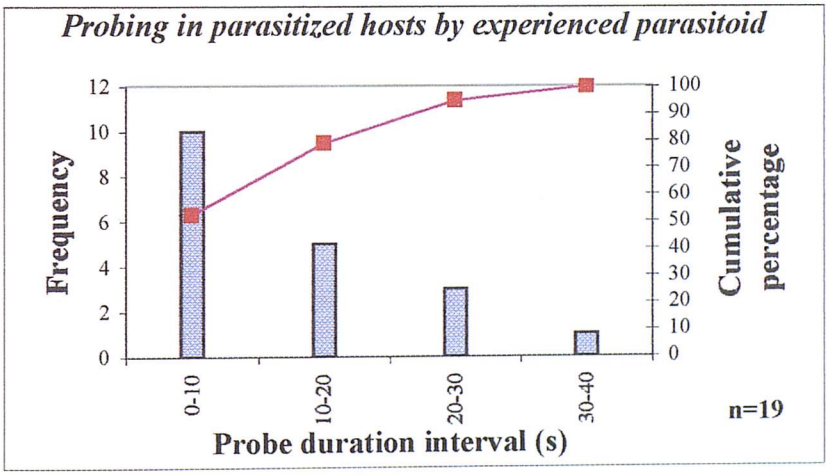


Figure 8.3 **Frequency distribution and cumulative percentage of probe duration in inexperienced and experienced *Anagrus atomus* on unparasitized and parasitized *Hauptidia maroccana* eggs.**

Table 8.1 Time (in seconds) spent in different components of the oviposition behaviour of experienced females of *Anagrus atomus* on three types of *Hauptidia maroccana* eggs.

Component	Inexperienced parasitoid on 1-3 d old eggs mean±s.e.	Experienced parasitoid on			One-way ANOVA
		Younger eggs (1-3 d old) mean±s.e.	Older eggs (7-9 d old) mean±s.e.	Parasitized eggs (24 h after parasitization) mean±s.e.	
Host examination	11.08±0.75 a	9.89±0.46 a	11.92±2.09 a	10.74±1.49 a	P=0.766, df= 72, LSD= 3.83
Ovipositor positioning	10.42±1.73 a	7.74±1.22 a	8.42±1.38 a	3.89±0.97 b	P=0.01, df= 72, LSD= 3.82
Ovipositor insertion	20.00±1.88 a	19.11±1.53 a	18.74±1.58 a	17.26±4.22 a	P=0.89, df= 72, LSD= 7.21
Abdominal pumping	100.58±7.74 a	72.89±3.73 b	81.00±7.28 b	-	P=0.01, df= 54, LSD= 18.43
Total oviposition period	122.47±7.72 a	92.00±4.42 b	100.26±8.10 b	17.26±4.21 c	P<0.001, df= 72, LSD= 17.97
Host examination following oviposition	14.11±2.33 a	12.21±2.32 a	8.16±2.31 a	-	P=0.189, df= 54, LSD= 6.57
Number of turns	1.32±0.134 a	1.42±0.139 a	1.16±0.233 a	-	P=0.565, df= 54, LSD=0.495
Total handling time	156.50±8.77 a	124.39±6.44 b	124.61±9.20 b	31.79±4.48 c	P<0.001, df= 72, LSD= 21.06

Means in rows followed by the same letters are not significantly different by LSD at 5%. (replication=19)
'-' not included in analysis.

Significant differences were observed in abdominal pumping, total oviposition period and total handling time between inexperienced and experienced parasitoids (Table 8.1). The duration of host examination, ovipositor positioning and ovipositor insertion did not show significant differences among the two levels of experience of the parasitoid. But the inexperienced parasitoids took an absolutely longer period than experienced parasitoids in all components of oviposition behaviour.

There were no significant differences in components of oviposition behaviour while ovipositing in younger and older eggs (Table 8.1). But in comparison between unparasitized and parasitized eggs, significant differences have been observed in total oviposition period and total host handling time. While probing in to previously parasitized eggs, parasitoids never showed abdominal pumping. They rejected the parasitized eggs

straight away after ovipositor insertion and neither examined the same host by antennation again nor showed any turning. While in unparasitized eggs, they turned following oviposition and spent on average 12.2 s in re-examination of the same host. Only in a few cases did they show repeated ovipositor insertion.

8.6 Discussion

The oviposition behaviour of *A. atomus* comprises host examination, ovipositor positioning, ovipositor insertion, abdominal pumping, and withdrawal of ovipositor and host examination following oviposition. As soon as a female reached a host, it started to examine the host by antennal drumming. Very short (7-8% of total handling time) host examination has been observed in *A. atomus,* which is in agreement with the findings of van Baaren *et al.* (1995). They mentioned that the host examination of two *Anaphes* species lasted for only 3-4 s. In contrast, while approaching a previously parasitized host, *A. atomus* spent on average 10.4 s which is 33% of their total handling time.

In the present study, *Anagrus atomus* spent 60-64% of their total handling time in abdominal pumping while ovipositing into unparasitized eggs, whereas they did not show any abdominal pumping when handling parasitized eggs. Total host handling time was also shorter (31.8 s) in parasitized than in unparasitized eggs (124.6 s). In two *Anaphes* species *A. victus* Huber and *A. listronoti* Huber, both abdominal pumping and the host contact following oviposition were significantly shorter when ovipositing into a previously parasitized than into an unparasitized host (van Baaren *et al.*, 1995).

In a preliminary study, it was observed that in a successful oviposition bout, female *A. atomus* took at least 50 s and always showed abdominal pumping. In some cases, spending >50 s without abdominal pumping did not result in successful oviposition. Cronin and Strong (1993) mentioned probe duration as an indicator of successful oviposition in *Anagrus delicatus* (Dozier) (Mymaridae) parasitizing the eggs of planthopper, *Prokelisia marginata* (van Duzze) (Delphacidae). In this study, probe duration is not a good indicator of oviposition by *A. atomus*. However, the presence or absence of abdominal pumping is a more robust indicator of oviposition over a wide range of environmental condition than probe duration (Al-Wahaibi and Walker, 2000). The probe duration in successful oviposition by *A. atomus* ranged from 50 to 170 s and 60

to 140 s in inexperienced and experienced parasitoids, respectively. On the other hand, in previously parasitized hosts, the probe duration lasts only 0-40 s.

Abdominal pumping near to the end of a probe is associated with oviposition into the unparasitized host egg by *A. atomus*. The association of abdominal pumping with oviposition is in agreement with findings of other studies that examined probing and oviposition processes in a number of egg parasitoids. Moratorio (1990) observed that abdominal pumping was directly correlated with oviposition in *Anagrus mutans* Walker and *A. silwoodensis* Walker, egg parasitoids of *Dicranotopis humata* (Boheman) (Delphacidae) and *Cicadella viridis* (L.) (Cicadellidae). In another mymarid genus, van Baaren *et al.* (1995) observed that oviposition by *A. victus* and *A. listronoti* in carrot weevil eggs was accompanied by specific abdominal movements. Conti *et al.* (1997) observed pumping movements by *Anaphes iole* Girault associated with oviposition in the eggs of its host. In *Trichogramma evanescens* Westwood, abdominal pumping movements were associated with oviposition in its host *Mamestra brassicae* L. (Noctuidae) towards the end of ovipositor probes (van Dijken and Waage, 1987). In the Encyrtidae, Strand (1989) also demonstrated that specific abdominal movements were associated with oviposition by *Copidosoma floridanum* Ashmead, an egg-larval parasitoid of *Pseudoplusia includens* (Walker) (Noctuidae).

A. atomus did not show any significant differences while ovipositing in younger and older eggs (Table 8.1). In contrast, van Huis *et al.* (1991) mentioned an increased handling time of older host eggs by *Uscana lariophaga*, which is attributed to the increased resistance of the egg chorion to penetration in older eggs.

Experience with a host egg prior to ovipositor probing affects the acceptance of host eggs by the female. This experience is gained through antennal contact with the host chorion, which is the first step in the process of gaining experience with the host (Nurindah *et al.*, 1999a). Kerguelen and Carde (1996) mentioned that the modification of the acceptance behaviour through experience was essentially a gradual process in which antennal contact with a pupa was a major element in *Brachymeria intermedia* (Nees), a pupal parasitoid of gypsy moth *Lymantria dispar* (L.). Experience also has a tremendous effect on the handling time for a subsequently presented host. Moreover, a complete oviposition event provides the female with the opportunity to learn or reinforce a

preference for host cues (Vinson, 1998). Therefore, it is not surprising that *A. atomus* took a shorter time during handling a host after experiencing oviposition. This type of learning is referred to as α- conditioning of experience by Vinson (1998) and is defined as an increased or improved response to stimuli to which the female has an innate sensitivity. Conti *et al.* (1997) observed the same behaviour of *Anaphes iole* Girault. They observed a significant reduction in total handling time of the parasitoids after they had gained experience. Female *Trichogramma australicum* Girault which have experienced full insertion of the ovipositor or complete oviposition differed in their handling time from the naive females and the females which have antennated and walked over the host egg (Nurindah *et al.*, 1999a). They also observed a reduction in drilling time as a result of female *T. australicum* experiencing a complete oviposition event. A decrease in handling time was also observed in experienced female of *T. maidis* which included a reduction in duration of drumming, drilling and oviposition in subsequent hosts (Wajnberg, 1989).

Parasitoids developing in insect eggs are idiobionts. Thus two different types of host selection strategies can be applied for the parasitoids that develop as idiobionts. One group attacks active hosts (parasitoids of the herbivorous larvae) which release various cues to their presence. Such parasitoids more closely resemble koinobionts in their host selection behaviour. The other group of idiobionts includes the egg and pupal parasitoids. These sessile hosts release fewer cues; and hence the egg parasitoids may face a greater challenge in locating their hosts (Vinson, 1998). However the egg parasitoids probably rely heavily on olfactory and tactile signals picked up by their antennae and ovipositors. The antennae act as receivers for signals emanating from the surface while the ovipositor probably detects signals below the surface during probes (Al-Wahaibi and Walker, 2000).

Chapter 9

Thelytokous reproduction in *Anagrus atomus*

9.1 Introduction

The most common reproductive mode in Hymenoptera is arrhenotoky, a form of parthenogenesis in which male offspring arise from unfertilized, haploid eggs and female offspring from fertilized, diploid eggs. Two other modes of parthenogenesis, deuterotoky and thelytoky may occur in some hymenopterous lineages (Luck *et al.*, 1993). With arrhenotoky, females control the sex of their offspring at oviposition by regulating the sperms' access to the egg. On the other hand, thelytokous females produce diploid female offspring from unfertilized eggs. Consequently, thelytokous lines can persist for multiple generations in the absence of males.

Two forms of thelytoky are recognized in Hymenoptera, revertible or microbe-associated thelytoky and non-revertible thelytoky (Stouthamer and Kazmer, 1994). In microbe-associated thelytoky, the symbionts involved in uniparental reproduction were first discovered in *Trichogramma* spp. (Stouthamer *et al.*, 1990; Stouthamer and Werren, 1993). This form of thelytoky has also been reported in *Muscidifurax uniraptor* (Pteromalidae) (Stouthamer *et al.*, 1993), *Encarsia formosa* (Aphelinidae) (Zchori-Fein *et al.*, 1992), *Aphytis spp* (Aphelinidae) (Zchori-Fein *et al.*, 1995) and in *Telenomus nawai* Ashmead (Scelionidae) (Arakaki *et al.*, 2000).

The microbe-associated thelytoky reverted to arrhenotoky when the females were fed with antibiotics (Zchori-Fein *et al.*, 1992, 1995; Arakaki *et al.*, 2000; Majerus and Majerus, 2000). Transition from thelytoky to arrhenotoky in some parasitoid species may be caused by inactivation or damage to symbiotic microorganisms through exposure to high temperature (Stouthamer *et al.*, 1990; Kajita, 1993; Arakaki *et al.*, 2000). Microorganisms are also found to be involved in causing thelytoky in *Encarsia formosa* Gahan by an unknown mechanism, maybe by the restoration of diploidy in the eggs to produce females (Zchori-Fein *et al.*, 1992).

Anagrus atomus can reproduce parthenogenetically, where male and female offspring developed from unfertilised and fertilized eggs, respectively (MacGill, 1934)

but thelytoky has not been reported so far in *A. atomus*. Some of the virgin females supplied from BIOWISE produced mostly female offspring without fertilization in successive generations. This population was suspected to be infected by some of the unknown microorganisms. All studies so far on parasitoids have examined synovigenic species where antibiotic fed to the adults might be used to alter the contents of eggs or the associated fluids when eggs are laid. Because *A. atomus* is pro-ovigenic all eggs are fully formed on emergence. It seemed unlikely that antibiotics would be able to cure the thelytoky.

9.2 Objectives of the study

The present study was designed to find out whether

1) antibiotic treatment can revert the population from thelytoky to arrhenotoky
2) exposure to high temperature can induce males in the further generations of a thelytokous population and
3) males produced by the use of antibiotics are fully functional.

The null hypothesis:
Antibiotic and high temperature treatments have no effects on the sex of thelytokous *A.atomus* in successive generation.

9.3 Materials and Methods

9.3.1 Parasitoid culture

The thelytokous population of *A. atomus* mentioned above was maintained at 25°C with 85% r.h. in temperature controlled illuminated incubators in the laboratory. Leaf discs of 50 mm in diameter containing leafhopper eggs were placed on agar medium in Petri-dish cages with the lower surface facing uppermost. The individual unmated female was introduced into each Petri-dish cage to parasitize leafhopper eggs for 24 hours. The petri-dish cages were put in a plastic box and kept inside an incubator at 25°C. The relative humidity inside the boxes was maintained at 85% r.h. using saturated KCl salt solution. Each Petri-dish was considered as one replication.

Ten days after laying eggs by *A. atomus*, leaves were cut into small pieces containing an individual parasitized egg (those which turned red) and placed into 0.5 ml Eppendorf safe-locking microcentrifuge tubes with a piece of wet filter paper. These tubes were placed in a plastic sandwich box and kept in temperature controlled illuminated incubators at 25°C. The humidity inside the boxes was maintained in the same way mentioned above. The tubes were checked everyday for the emergence of adult *A. atomus* and the females were used as the test insects.

9.3.2 Test Procedure

9.3.2.1 Antibiotic treatments

Three different types of antibiotics Ampicillin, Rifampicin and Penicillin were used in the experiment. To prepare the antibiotic solution, antibiotics were mixed with distilled water at a rate of 1 mg/ 1 ml of distilled water and then the solution was mixed with honey at a rate of 1 ml/ 1 ml of honey (Kajita, 1993). Newly emerged females were fed with different antibiotic solutions for 24 hours. The treated females were allowed to parasitize *H. maroccana* eggs in 50 mm diameter Petri-dish cages for 24 h and the egg were kept in the incubators at 25°C until parasitoid emergence. Five replications were maintained for each antibiotic treatment. A control treatment with 5 replications was also maintained. The number of male and female offspring in the F_1 was counted.

Five females were then randomly selected from the offspring (F_1) of the treated females and were allowed to lay eggs for 24 h following the same procedure mentioned above. The number of male and female offspring in the F_2 generations was counted to calculate the sex ratio.

9.3.2.2 Temperature treatments

Parasitoid females were exposed to high temperature (31°C) at three different development stages:

(1) Immature stages: Ten adult females were allowed to lay eggs for 24 hours. The parasitized eggs of *H. maroccana* by thelytokous females were subjected to high temperature treatment until adult emergence

(2) Pupal stages: Eight days after laying eggs when they are about to pupate, the parasitized eggs were exposed to high temperature for 48 h and then transferred again to 25°C until adult emergence. Ten replications were maintained in this case.

(3) Adult female: Newly emerged females were exposed to high temperature for 3 days and fed with 50% honey solution. Treated females were allowed to oviposit for 24 h and the parasitized leafhopper eggs were incubated at 25°C until adult emergence. Ten replications were maintained

The sex ratio in F_1 generation of the treated females was calculated on the basis of the emerging male and female wasps from each treatment. Five female wasps from F_1 of each of the three treatments were selected randomly and allowed to parasitise *H. maroccana* eggs for 24 h. Each Petri-dish was individually marked and the offspring sex ratio in F_2 was calculated to determine whether high temperature had any effect.

9.3.2.3 Male functioning

The mating potential of the antibiotic–induced males was tested in two different ways

1. Mating performance: An antibiotic-induced male along with a female was introduced into the 50 mm diameter Petri-dish cage. Behaviour of the male was observed under a binocular microscope and the mating performance was recorded. Twenty individuals from the antibiotic-induced males were used in the test to see their mating performance.

2. Viability of the sperm: Five females from the arrhenotokous (uninfected) culture were mated with antibiotic induced males. Those females along with the males were introduced into 50 mm diameter Petri-dish cages and allowed to parasitize *H. maroccana* eggs for 24 h. Each Petri-dish was considered as one replication. A control treatment was maintained using one unmated arrhenotokous female. The sex ratio of the emerging wasp from unmated and mated females was recorded.

It is assumed that the antibiotic-fed females inject some of the antibiotics from the acid glands, which enters the developing embryo. Thus, the microorganisms inside the eggs were being killed and male offsprings can be produced in succeeding generations. An attempt was made to find out how the antibiotic works in reverting the sex of infected wasps. Twenty eggs of *H. maroccana* in a leaf disc were parasitized by infected females. Twenty-four hours after parasitization, females fed with antibiotics for 24 h were allowed to venomise ten of the parasitized eggs but the females were removed before oviposition and the same females were allowed to parasitize ten of the unparasitized eggs. Thus the three types of eggs, viz. 1) parasitized by infected female, 2) parasitized by infected female but venomized by Rifampicin treated female, and 3) parasitized by Rifampicin treated female, were obtained in the same leaf disc and the eggs were marked individually. The parasitized eggs were then put into the incubator. When they pupated, the leaf was cut into small pieces containing individual eggs and put inside 0.5 ml Eppendorf safe-locking microcentrifuge tubes along with a small piece of wet filter paper. The tubes were incubated and checked everyday for adult emergence. The number of emerging male and female parasitoids was counted.

Five females were then randomly selected from the offspring of each of the three groups and were allowed to parasitize for 24 h on different leaf disc. The parasitized eggs were incubated in the same procedure. The number of male and female offspring in F_2 was counted to calculate the offspring sex ratio.

9.4 Statistical analysis

Some characteristics of arrhenotokous and thelytokous females, viz. duration of development, potential fecundity, head width and hind tibia length were compared using Two-sample t test. Effect of different antibiotics and high temperature on the sex ratio of the emerging parasitoid offspring was analysed in χ^2. In some cases, χ^2 analysis was invalid due to too few data.

9.5 Results

9.5.1 Characteristic differences between females of arrhenotokous and thelytokous *A. atomus*

The characteristic difference between thelytokous and arrhenotokous population of *A. atomus* are presented in Table 9.1. A significant difference was observed in development duration (P= 0.05, t=-1.98, df=76) between the two population of *A. atomus*. The thelytokous female developed earlier (12.5 d) than the arrhenotokous female (12.8 d). Significant differences were also observed between the longevity of thelytokous and arrhenotokous females (P< 0.001, t=-9.11, df=38). The arrhenotokous females survived twice as long as thelytokous females when provided with the same food (50% honey solution). On the other hand, two-sample t test did not show any significant differences between arrhenotokous and thelytokous population of *A. atomus* with regards to their potential fecundity and body size (head width and hind tibia length). The thelytokous *A. atomus* showed a higher intrinsic rate of increase (r_m) and shorter generation time as all the offspring were female (Table 9.2).

Table 9.1 Comparison of the characteristics of arrhenotokous and thelytokous populations of *Anagrus atomus*.

Characteristics	n	Arrhenotokous population*	n	Thelytokous population	Two-sample t test
Development duration of female (days)	185	12.9±0.064 a	40	12.5±0.097 b	P= 0.05, t=-1.98, df=76
Potential fecundity (no.)	27	26.4±1.665 a	24	25.4±0.92 a	P= 0.58, t=-0.56, df=40
Head width (mm)	33	0.176±0.0016 a	33	0.176±0.0012 a	P= 0.65, t=-0.46, df=59
Hind tibia length (mm)	31	0.188±0.0016 a	33	0.186±0.0017 a	P= 0.73, t=-0.34, df=61
Adult longevity (days)	21	11.8±0.50 a	20	5.7±0.43 b	P< 0.001, t=-9.11, df=38

*Data from Chapter 5
Means in rows, followed by the same lowercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.

n = no. of insect as replicate.

Table 9.2 Life table parameters of the thelytokous *Anagrus atomus* ovipositing on the eggs of *Hauptidia maroccana* laid on Primula at 25°C.

Life table parameters	On primula at 25°C
Gross reproductive rate (*GRR*)	25.00 **Female eggs/female/generation**
Net reproductive rate (R_o)	25.00 **Females/female/generation**
Capacity of increase (r_c)	0.25 **Female offspring/female/day**
Intrinsic rate of increase (r_m)	0.25 **Female eggs/female/day**
Cohort generation time (*Tc*)	13.11 **Days**
Generation time (*T*)	13.06 **Days**
Finite capacity for increase (λ)	1.28 **Female offspring/female/day**
Doubling time (*DT*)	2.81 **Days**

Life table was constructed considering the adult age 12.5 days on emergence and female sex ratio as 1.0.

9.5.2 Effect of different antibiotic treatments on the sex ratio of the offspring of *A. atomus* in subsequent generations

Antiobiotic treatments to the thelytokous females did not show any significant differences in the sex ratio of *A. atomus* in the F_1 generation (Table 9.3). But in F_2, χ^2 analyses showed significant differences in the sex ratio ($P<0.001$, df=3) (Table 9.4). Females whose parents had fed on Rifampicin produced 100% male offspring while without feeding with antibiotics they produced all female offspring. Ampicillin and Penicillin produced 13.73% and 14.75% males, respectively.

When the antibiotic fed females were allowed only to insert the ovipositor without laying eggs, females from those eggs produced 3.2% male offspring in F_2. On the other hand, females emerging from the eggs where the same antibiotic fed female successfully laid eggs, produced all male offspring in F_2. The infected females produced all female offspring (Table 9.5).

Table 9.3 Effect of different antibiotic treatments on the sex ratio of unmated *Anagrus atomus* in F_1 generation.

Treatment	Total emerged wasp (no.)	Male offspring (no.)	Female offspring (no.)	% Female mean±s.e.	% Male mean±s.e.
Control	71	3	68	95.77±2.39	4.22±2.39
Ampicillin	59	3	56	94.92±2.86	5.08±2.86
Penicillin	18	0	18	100.00±0.0	0.0±0.0
Rifampicin	59	0	59	100.00±0.0	0.0±0.0
χ^2 analysis	The 4x2 Chi-Square approximation was invalid due to 2 cells with expected counts less than 1.0 and 4 cells with expected counts less than 5.0.				

s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion of male
q =1-p.

Table 9.4 Effect of different antibiotic treatments on the sex ratio of unmated *Anagrus atomus* in F_2 generation.

Treatment	Total emerged wasp (no.)	Male offspring (no.)	Female offspring (no.)	% Female mean±s.e.	% Male mean±s.e.
Control	76	0	76	100.00±0.0 a	0.0±0.0 c
Ampicillin	51	7	44	86.27±4.82 b	13.73±4.82 b
Penicillin	61	9	52	85.25±4.54 b	14.75±4.54 b
Rifampicin	62	62	0	0.0±0.0 c	100.00±0.0 a
χ^2 analysis	χ^2= 186.12, df= 3, P<0.001				

Values in columns followed by the same letters are not significantly different from each others in χ^2 analysis. P value calculated from χ^2 analysis.
s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion of male
q =1-p.

Table 9.5 Comparison of offspring sex ratio of *Anagrus atomus* in F_2 produced by Rifampicin fed females, infected females and from hosts parasitized hosts by infected females but venomized by Rifampicin fed females.

Offsprings from	Total emerged wasp (no.)	Male offspring (no.)	Female offspring (no.)	% Female mean±s.e.	% Male mean±s.e.
Females fed with Rifampicin	48	48	0	0.0±0.0 b	100.0±0.0 a
Venomized eggs laid by infected females	63	2	61	96.83±2.21 a	3.17±2.21 b
Control (infected)	76	0	76	100.0±0.0 a	0.0±0.0 b
χ^2 analysis	χ^2= 177.11, df= 2, P<0.001				

Values in columns followed by the same letters are not significantly different from each others in χ^2 analysis. P value calculated from χ^2 analysis.
s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion of male
q =1-p.

9.5.3 Effect of high temperature treatment (31°C) on the sex ratio of the offspring of *A. atomus* in subsequent generations

An exposure of *A. atomus* during immature stages, pupal stages and adult stages to high temperature caused the occurrence of male offspring in the F_2 generations. More male progeny (21.9%) was obtained when high temperature was used throughout immature development. On the other hand, all female progeny was obtained from the control treatment (Table 9.6).

Table 9.6 Influence of high temperature treatment (31°C) on the sex ratio of offspring produced by *Anagrus atomus* in F_2 generation.

Treatment	Total emerged wasp (no.)	Male offspring (no.)	Female offspring (no.)	% Female mean±s.e.	% Male mean±s.e.
High temperature throughout immature stages	64	14	50	78.1±5.17 b	21.9±5.17 a
High temperature treatment for 48h at pupal stage	34	4	30	88.2±5.53 b	11.8±5.53 a
Adults treated with high temperature for 72h	26	2	24	92.3±5.23 b	7.7±5.23 a
Control (at 25°C)	65	0	65	100.0±0.0 a	0.0±0.0 b
χ^2 test	χ^2= 16.598, df= 3, P<0.001				

Values in columns followed by the same letters are not significantly different from each others in χ^2 analysis. P value calculated from χ^2 analysis.
s.e. calculated from the equation: s.e. = $100\sqrt{pq}/n$, where p= proportion of male
q =1-p.

9.5.4 Sexual functioning of the antibiotic induced males of *A. atomus*

Antobiotic induced males showed 100% mating success when allowed to mate with the arrhenotokous females. Results in Table 9.7 show that antibiotic induced males produced viable sperms and successfully transferred to the offspring. The unmated arrhenotokous females produced 100% male offspring, whereas, the females from the same population produced 78.2% and 87.5% female offspring when they were mated with arrhenotokous and antibiotic induced males, respectively.

Table 9.7 Comparison of the offspring sex ratio from arrhenotokous females mated with arrhenotokous and antibiotic induced males of *Anagrus atomus*.

Female parent	Total emerged wasp (no.)	Male offspring (no.)	Female offspring (no.)	% Female mean±s.e.	% Male mean±s.e.
Arrhenotokous unmated	62	62	0	0.00±0.00	100.00±0.00
Arrhenotokous, mated with uninfected male	87	19	68	78.2±4.43	21.8±4.43
Arrhenotokous, mated with antibiotic induced males	72	9	63	87.5±3.90	12.5±3.90
χ^2 test	χ^2= 16.598, df= 3, P<0.001				

9.6 Discussion

Differences were observed between arrhenotokous and thelytokous population of *A. atomus* with regard to development duration and longevity. Shorter development duration was observed in thelytokous females than in the arrhenotokous females. Arrhenotokous females survived twice as long as thelytokous females when provided with the same food (50% honey solution). No significant difference was observed in the wasp's potential fecundity and body size in either population. Stouthamer (1989) was able to derive arrhenotokous lines of *Trichogramma deion* and *T. pretiosum* from the thelytokous lines by antibiotic treatments and found thelytokous females less fecund than the arrhenotokous female and in two cases, arrhenotokous females produced more daughters.

In the present study, antibiotic treatment showed a remarkable effect on male production in thelytokous *A. atomus*. Rifampicin was found to be the most effective antibiotic in the induction of maleness producing 100% males in the F_2. However, the other two antibiotics, ampicillin and penicillin produced 13.7% and 14.8% males, respectively. Untreated control females produced exclusively female progeny. This result is in agreement with the observations of Kajita (1993). He reported male occurrence in thelytokous *Encarsia formosa* Gahan when treated with Rifampicin and tetracycline

hydrochloride. These two antibiotics inhibit RNA and protein synthesis, respectively. On the other hand, ampicillin, penicillin G and sulfamethoxazole do not inhibit RNA and protein synthesis, and therefore, did not show any effect of male production (Kajita, 1993). The thelytokous *Telenomus nawai* Ashmead produced 27.1% male progeny when treated with Tetracycline (Arakaki *et al.*, 2000).

Bacteria of the genus *Wolbachia* were identified as the causative agent of thelytoky in many parasitoid species that could be treated by feeding the female parents with antibiotics or by heat treatments. The parthenogenetic strain (thelytokous) of *Trichogramma* wasps successfully reverted to the production of male progeny after antibiotic treatment (Stouthamer *et al.*, 1990). Their subsequent work identified that cytoplasmically inherited bacteria of the genus *Wolbachia* were associated with that form of thelytoky (Stouthamer *et al.*, 1993).

The exposure of thelytokous *T. nawai* during the pupal stage to a high temperature (35°C) was reported to produce 16.4% males whereas in the control treatment, only 0.1% males were produced (Arakaki *et al.*, 2000). This may be due to the high temperature having a harmful effect on the development of reproductive organs in the pupal stages.

In the present study, antibiotic induced male *A. atomus* showed 100% mating success and produced viable sperms. These males when mated with arrhenotokous females produced female offspring. Stouthamer *et al.* (1990) reviewed the functionality of heat induced males in thelytokous species of *Trichogramma* and reported them as non-functional. Even when the females of *Encarsia formosa* were fed with antibiotics, the males obtained from those females were not sexually functional (Zchori-Fein *et al.*, 1992). In another study, Zchori-Fein *et al.* (1995) reported successful induction of male offspring from the uniparental lines of two species of the genus *Aphytis* when treated with rifampicin. Those males produced viable sperms which successfully transferred to the female spermatheca. However the sperms were not viable as they failed to produce any female after fertilization. Kajita (1993) reported that the males induced from treatment with Rifampicin and tetracycline hydrochloride showed mating behaviour in *Encarsia formosa* Gahan. Arakaki *et al.* (2000) observed functional males in *T. nawai* from the antibiotic treated thelytokous females. Males could successfully fertilize arrhenotokous females and produced 19.1% males. This appears to be the result of successful mating and

fertilization. Arrhenotokous wasps usually show few male offspring after mating. On the other hand, *Wolbachia*-infected females exclusively produced female progeny after mating with the tetracycline-induced males, suggesting that the eggs developed parthenogenetically and sperm did not participate in egg development (Arakaki *et al.*, 2000).

Thelytoky in a biological control agent may confer disadvantages such as low genetic variability, difficulties in studying the genetics and, thus, limitations in genetic improvement (Zchori-Fein *et al.*, 1992). However, the thelytokous *A. atomus* could gain higher reproductive potential than the arrhenotokous ones because they are producing all female offspring without having any significant differences in their potential fecundity. The results from antibiotic and heat treatments to the thelytokous population strongly suggests that some unknown micro-organisns infecting the thelytokous *A. atomus* are the causative agents of parthenogenesis. Treatment with antibiotics or high temperature exposure allowed the males to mate successfully with the females and successful insemination occurred. Thus the transition from thelytoky to arrhenotoky in *A. atomus* might be caused by inactivation or damage to symbiotic microorganisms through exposure to high temperature or feeding with antibiotics. Further studies are needed to confirm which microorganisms are involved in thelytoky in this parasitoid.

Thelytokous strains may have the intrinsic advantage that, since they produce no male offspring, all of their progeny go on to attack new hosts directly. This factor may also be important in mass rearing, and at low population densities, where sexual strains may be disadvantaged because female may find it difficult to find mates (Quicke1997). The thelytoky observed in these studies may not be seen as detrimental. All parasitoids produced were females and hence capable of attacking host eggs. While longevity is reduced, it is still long enough for the pro-ovigenic species to lay all of its egg load. The size and general activity did not seem to be impaired.

Chapter 10

Scanning electron microscope study of plant physical characteristics and their influences on searching behaviour of *Anagrus atomus*

10.1 Introduction

Chemical and physical characteristics of the plant can mediate the behaviour of parasitoids and predators of herbivores (Eigenbrode and Espelie, 1995). Plant structural complexity like cuticular thickness, glandular and non-glandular trichomes can play a significant role in host encounter by parasitoid wasps (Dicke, 1996; Lukianchuk and Smith, 1997). When a parasitoid lands on a plant, plant surface characteristics, mainly trichomes alter their walking speed (Hulspas-Jordaan and van Lenteren, 1978), inhibit their ability to locate a host (Keller, 1987; Kashyap *et al.*, 1991) and finally, reduce adult survival (Obrycki and Tauber, 1984). Plant volatiles can act as repellents and trichome and trichome exudates inhibit the movement of the wasp on the plant (Romeis *et al.*, 1998).

Plants with trichomes and their secretions can impede the movement of parasitoids, often affecting searching time and weakening the parasitoid functional response (Sütterlin and van Lenteren, 1997; Romeis *et al.*, 1999; Clercq *et al.*, 2000; Lovinger *et al.*, 2000). Efficiency of several parasitoid species were affected negatively by high trichome densities. A higher walking speed of *Encarsia formosa* was observed on a hairless cucumber variety than on a variety with intermediate trichome density (van Lenteren *et al.*, 1995). The searching behaviour of the egg parasitoid *Trichogramma exiguum* Pinto and Platner was found to be affected by surface chemistry among different plant species (Keller, 1987). Thus understanding the natural enemy response to host plant resistance characteristics is important for successful integration of tactics used in pest management

Hauptidia maroccana is a polyphagus insect and feeds and lay eggs on a wide range of host plants with a variety of trichomes and semiochemicals. *Anagrus* are considered polyphagous parasitoids, yet many are limited to a few plant species although some host leafhoppers have a broad plant range. For example, Stiling (1980) found high rates of parasitism by an *Anagrus* species associated with three *Eupteryx* leafhopper

species on stinging nettle, but little or no parasitism on surrounding weedy vegetation containing *Eupteryx*. Chantarasa-ard *et al.* (1984) found that *Anagrus incarnatus* Haliday parasitized *Harmalia albicollis* Motschulsky eggs on rice in the laboratory, but they did not detect parasitism in the field when host eggs were deposited in weeds. These examples indicate that *Anagrus* species may have an inherent attraction to specific plant volatiles. Since the searching efficiency of predators and parasitoids is influenced by the presence of trichomes and the semiochemicals in the host plants, the success of biological control may be influenced by the characteristics of different host plants.

10.2 Objectives of the study

The present study was undertaken to

1) identify the characteristic difference of the host plant surface on the basis of types and density of the trichomes.
2) determine the effect of different plant species on searching efficiency of *A. atomus* on five different host plants.

The null hypothesis:

1) Different host plants are similar in trichome types and density.
2) Searching behaviour of *A. atomus* does not vary on different host plants.

10.3 Materials and Methods

10.3.1 Scanning electron microscope (SEM) studies of the abaxial surface of five host plant species

A scanning electron microscope study of the following five host plants of *H. maroccana* was done to find the characteristic differences in the abaxial surfaces of the leaf:

1. Cucumber, *Cucumis sativus* L. (var. Bush champion)
2. Tomato, *Lycopersicon esculentum* Mill. (var. Alisa Graig)
3. French bean, *Phaseolus vulgaris* L. (var. Prince)
4. Primula, *Primula vulgaris* Huds. (var. Mixed colour) and
5. Sweet pepper, *Capsicum annum* L. (var. Worldbeater)

Fully expanded leaves of the above mentioned host plants, which were free from any kind of infestation were collected from the glass house. The samples were put inside a plastic box and immediately transferred to the electron microscopy unit for the preparation of specimens. The preparation of specimens consisted of the following stages.

10.3.1.1 Drying

In the electron microscope, specimens are exposed to a very high vacuum. This causes the water-containing specimens to dry out and collapse. To prevent this, biological specimens are usually dried in a special way, which minimises distortion. The common method is called critical point drying.

10.3.1.2 Fixation

After drying, the specimen is immersed in a chemical fixative solution, which kills the cells, and preserves them. Glutaraldehyde, mixed with a phosphate buffer, is a common fixative. Samples of 10 mm across were cut carefully from the leaves without damaging them. They were immersed in the 2.5% Glutaraldehyde in phosphate buffers for twelve hours. After this period the specimens were placed in two changes of phosphate buffer alone for ten minutes to remove surplus fixative.

10.3.1.3 Dehydration

During this process water is gradually removed from the specimen, and replaced with acetone. The specimen is dipped in different dilutions of acetone in the following procedure:

In 50% acetone for twenty minutes
In 70% acetone for twenty minutes or left in the fridge overnight
In 80% acetone for twenty minutes
In 90% acetone for twenty minutes
In 100% acetone for twenty minutes
In 100% acetone for twenty minutes
In 100% acetone for twenty minutes

10.3.1.4 Critical point drying

The specimen is sealed in a 'Critical point drying apparatus'. Here acetone is replaced with liquid CO_2. The temperature and pressure are then increased until the 'critical point' for CO_2 is reached. Under this condition CO_2 is a vapour. In fact, while the temperature and pressure are rising, the specimen is gradually drying, but in such a way that no damaging surface tension forces occur within it.

The critical point dryer specimen container is filled with 100% acetone. The specimens are transferred to the container quickly to avoid desiccation. The drying process takes a minimum of two hours. On completion the specimens must be kept dry and dust free.

10.3.1.5 Attachment to stub

An adhesive such as Araldite® or a special colloidal silver mixture is used to mount the specimen securely on a metal 'stub'. This acts as a specimen holder in the SEM.

10.3.1.6 Gold-coating

The specimen, adhesive and stub are coated with a very thin layer of gold in a 'sputter coater'. This is necessary because otherwise an electrical charge would build up within the specimen while it was being examined in the SEM. Instead, gold conducts the charge away to earth.

The gold-coated specimens were loaded into a Hitachi SS430 Scanning Electron Microscope and observed at different magnifications. The photographs were taken by a built in camera at various magnifications.

10.3.2 Searching behaviour of *A. atomus* on the abaxial surface of the leaves of five host plant species

The behavioural difference during searching for hosts by *A. atomus* on five different host plants were observed under laboratory conditions at room temperature (25±1°C). Fifty mm diameter Petri-dish cages were used in the experiment. Leaf discs of 50 mm diameter from the healthy plants were placed on 1% agar medium with the lower surface facing uppermost. Twenty-four hours old mated, inexperienced females were used in this experiment. The wasps were provided with 50% honey solution prior to using in the experiment. A single female wasp was released onto the leaf disc.

Different activities of the parasitoid were monitored through a JVC camera with a 50 mm macro lens, attached to the computer and parasitoid behaviour was recorded on video tapes using the computer program 'Inchworm' (Varley *et al.*, 1994). Parasitoid behaviour on each leaf disc was recorded for 30 minutes. Moreover, the behaviour of *A. atomus* was also recorded on a Primula leaf disc containing *H. maroccana* eggs to find whether the presence of the host alters its searching behaviour. Recording was carried out between 0900-1400 hours to avoid any diurnal variations. Illumination was provided with a fibre optic at 55.5 W/ m^2, with both arms placed 10-12 cm over the arena. The same parasitoid was used on different leaves and considered as one replication but each leaf disc was used only once. Ten replications were done for each plant species.

The videotapes were replayed and the direction of walking of the parasitoid was traced by clicking on the right mouse button. Any parasitoid that stayed at least 15 seconds continuously on the leaf surface was accepted as one replication. The computer program 'Inchworm' gave the following information

1. Net speed (mm/sec)
2. Meandering (degrees turned/mm) and
3. Absolute turn (degrees turned/sec)

10.4 Statistical analysis

One-way analysis of variance was done in SAS to compare different activities during searching by *A. atomus* on different host plant leaves and the leaves of *P. vulgaris* containing eggs of *H. maroccana*. Activities on leaf lamina and leaf veins on different host plants were compared using two sample t-tests in MINITAB. Means were compared by LSD at 5% level.

10.5. Results

10.5.1 Structural differences of the abaxial surfaces of host plant leaves

A great variation has been observed in the types, density and the arrangements of trichomes on the abaxial surface of the leaves of five plant species on the basis of the SEM study (Plate 10.1-10.9). Trichome density on the abaxial surfaces of the fully expanded leaves is presented in Table 10.1. Trichome density was always higher on leaf veins than on leaf lamina. Among the five plant species, the highest (348/mm^2) and lowest (6/mm^2) trichome density was observed in Primula and Sweet pepper, respectively (Table 10.1). The characteristic differences in the shapes of the trichomes are discussed below.

Both glandular and non-glandular erect trichomes were found on the leaf lamina of Cucumber leaf but glandular trichomes were absent on leaf veins (Plate 10.5). The density of non-glandular trichomes in Cucumber was observed as 3.9 and 69.4 trichomes per mm^2 on leaf lamina and leaf veins, respectively. Shipp and Whitfield (1991) observed 489.35 trichomes on a 4 cm diameter leaf disc of *Cucumis sativus* L. 'Sandra'.

Both erect and glandular trichomes were found in Primula, 85% of which were non-glandular. The head of the glandular trichomes were single celled. Trichomes were densely arranged on both leaf lamina and leaf veins (Plate 10.6).

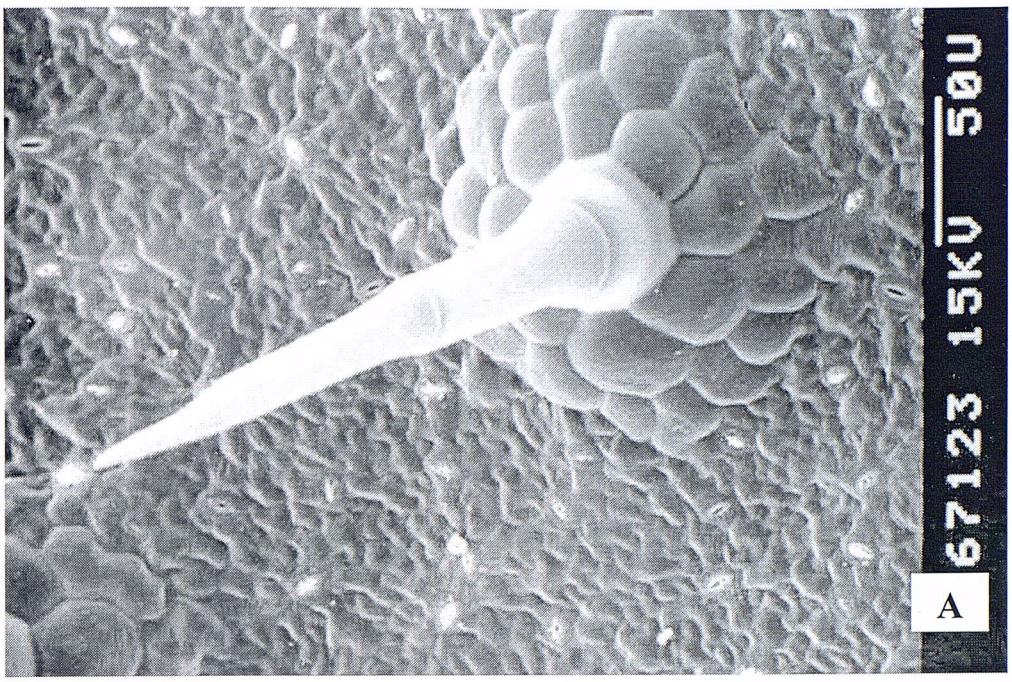
The abaxial surface of French bean leaves was uniformly covered with glandular and hooked trichomes at almost equal proportions on both leaf lamina and leaf veins (Plate 10.7).

Both glandular and non-glandular trichomes were observed on Tomato leaves. The head of the glandular trichomes was four celled (Plate 10.8). In Tomato, 97% of the trichomes were non-glandular. Leite *et al.* (1999) also mentioned 90.3% and 9.7% of non-glandular and glandular trichomes, respectively in Tomato.

Only glandular trichomes were found in Sweet pepper and the trichomes were present only on leaf lamina. The head of the trichomes was two celled (Plate 10.9). Shipp and Whitfield (1991) observed 16.9 trichomes on a 4 cm diameter leaf disc of *Capsicum annum* L. 'Plutona'.

Table10.1 Trichome density on leaf vein and leaf lamina of the five different host plants under SEM study.

Host plant	Trichome density (no./mm^2)				
	On leaf vein		On leaf lamina		
	Glandular	Non-glandular	Glandular	Non-glandular	Total
Cucumber	11.6	34.7	0	8.9	55.2
Tomato	0	69.4	1.9	3.9	75.2
French bean	62.5	83.3	2.3	7.3	155.4
Primula	8.7	291.9	11.9	35.8	348.3
Sweet pepper	4.3	0	1.9	0	6.2



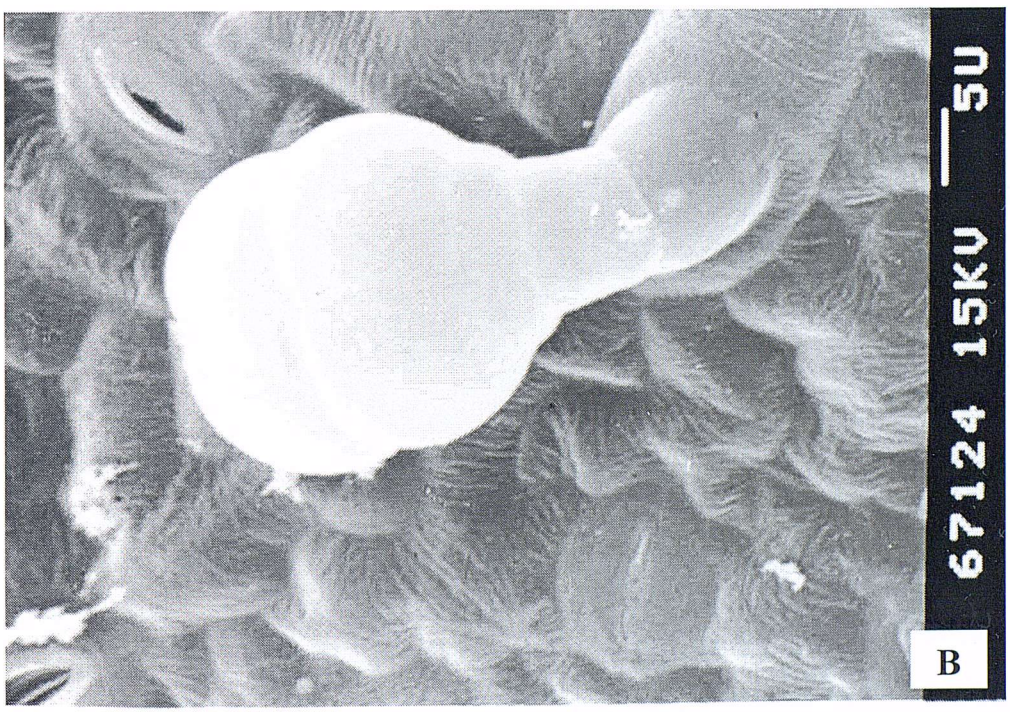


Plate 10.1: **Trichome found on the abaxial surface of Cucumber leaf A) multi cellular erect and B) glandular.**

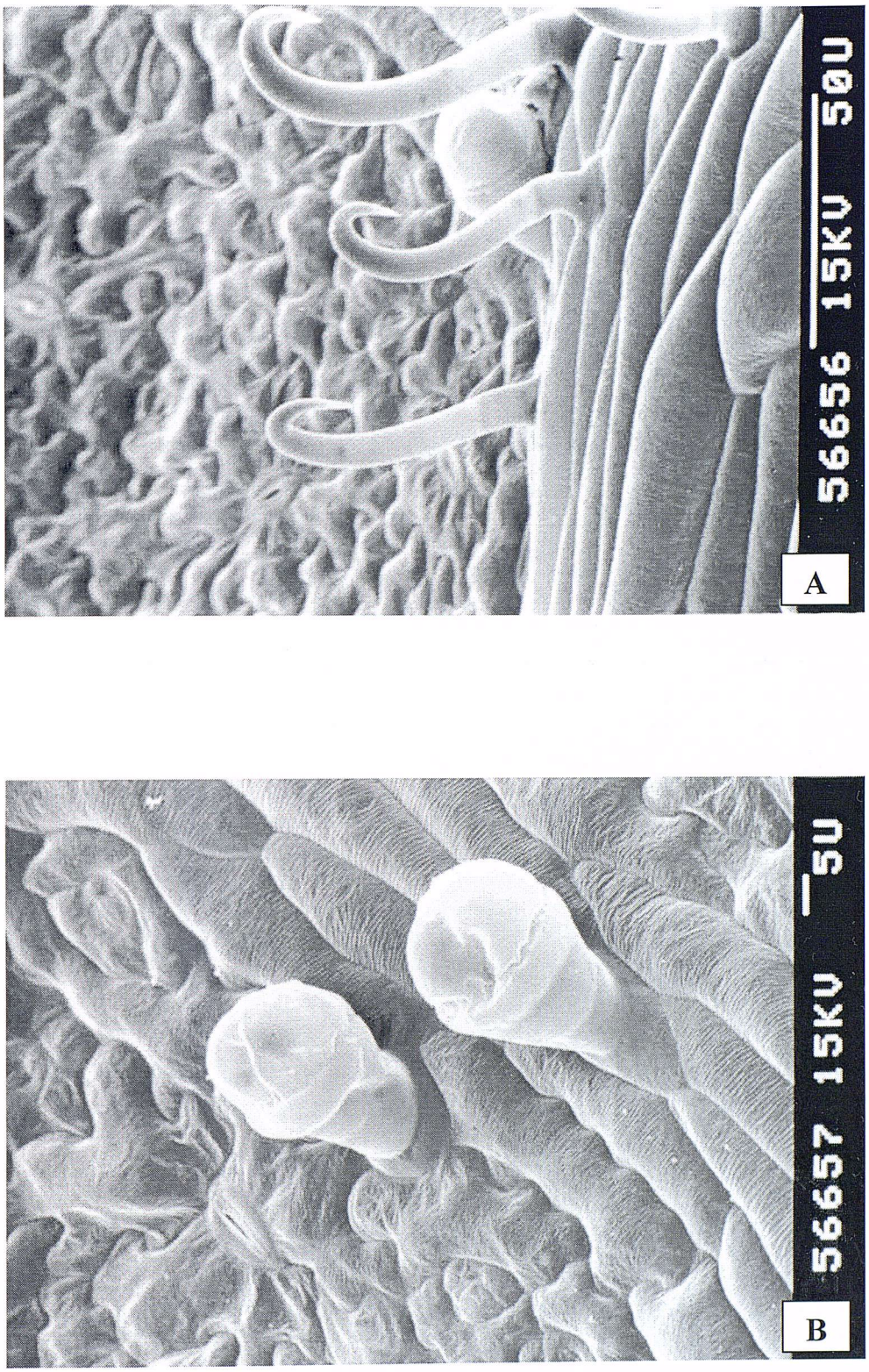


Plate 10.2: **Trichome found on the abaxial surface of French bean leaf A) hooked shaped and B) glandular.**

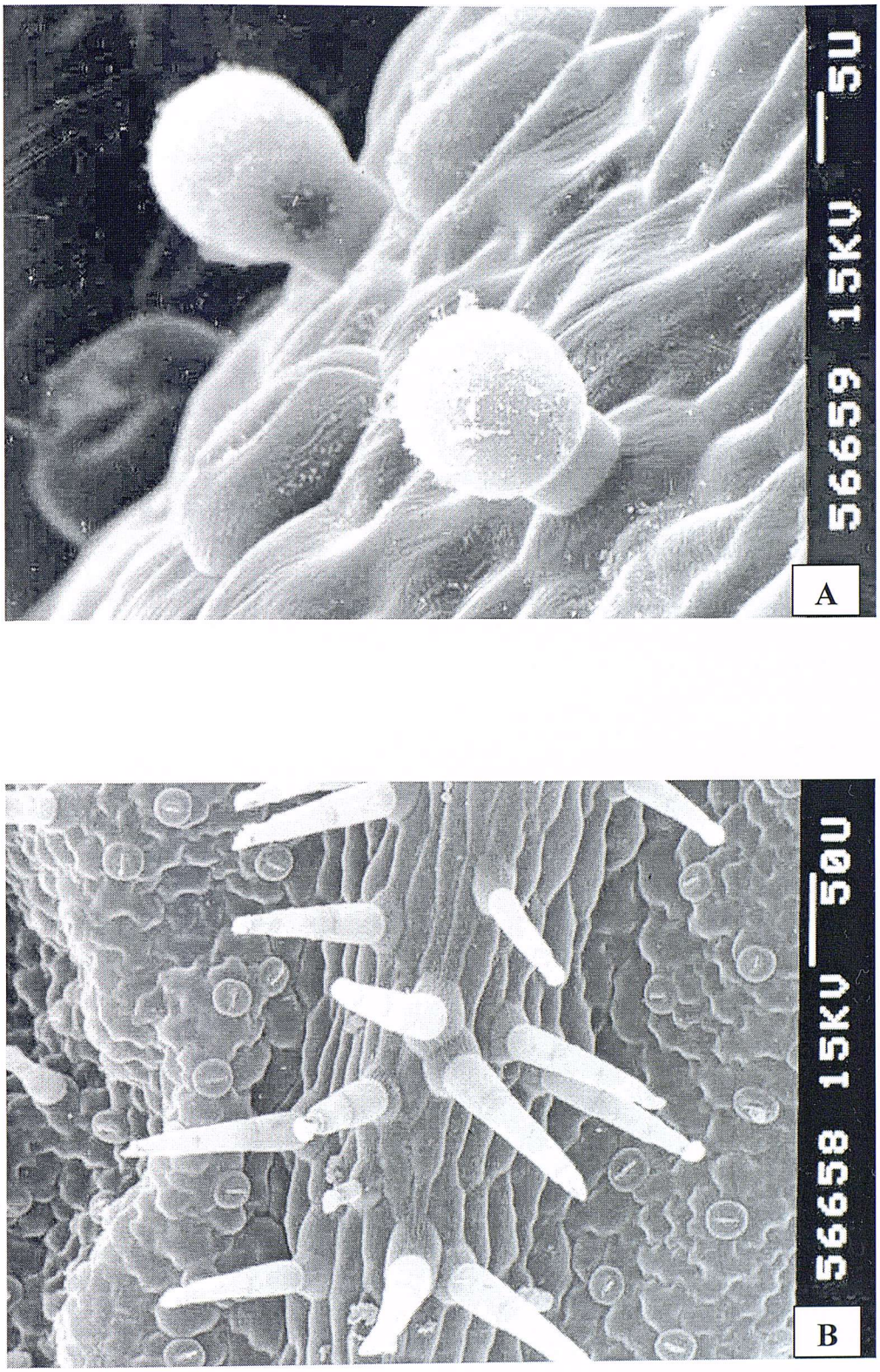


Plate 10.3: **Trichome found on the abaxial surface of Primula leaf A) glandular and B) multi cellular erect.**

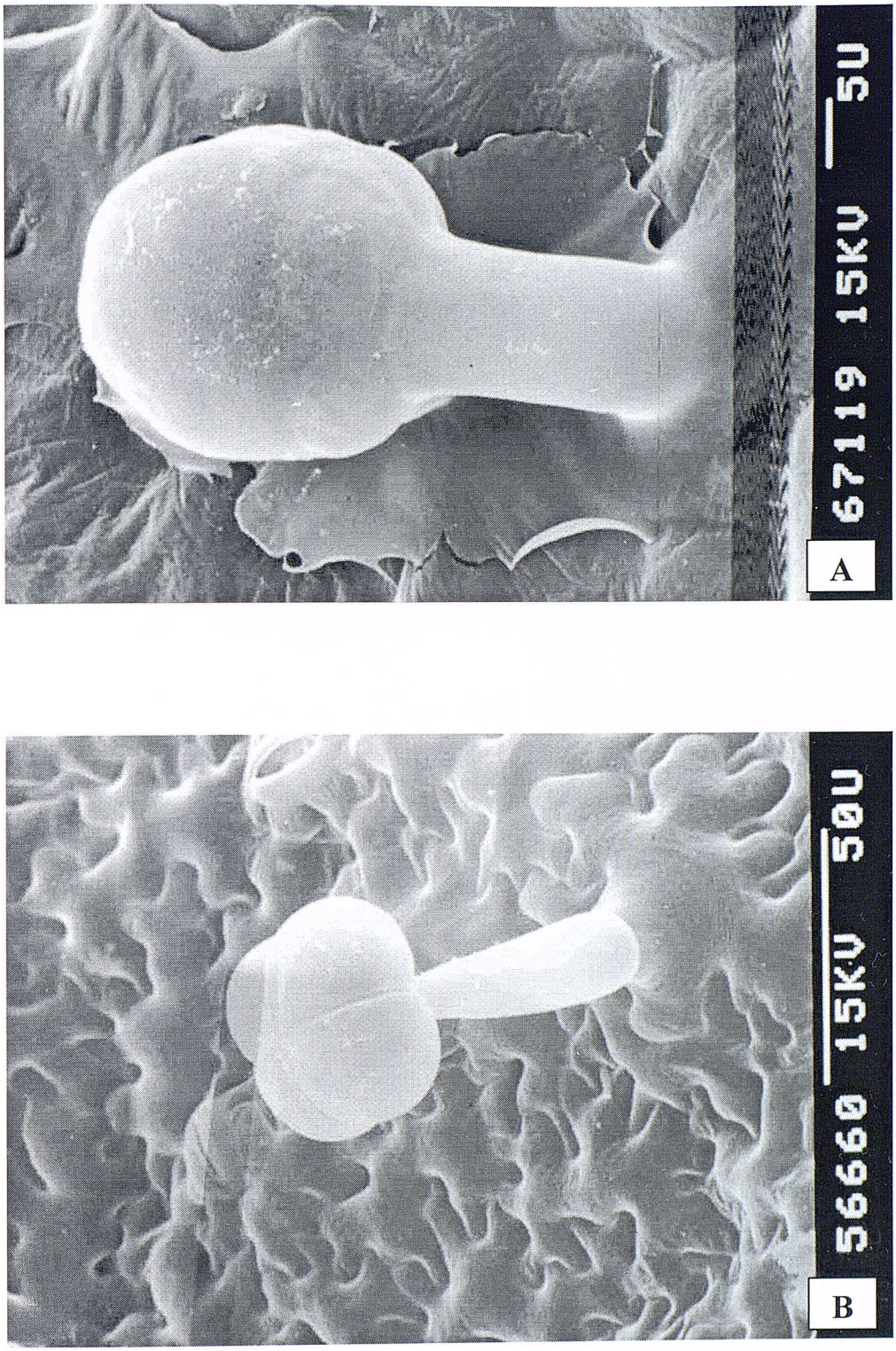


Plate 10.4: **An individual trichome found on the abaxial surface of A) Sweet pepper and B) Tomato.**

Leaf lamina



Leaf vein

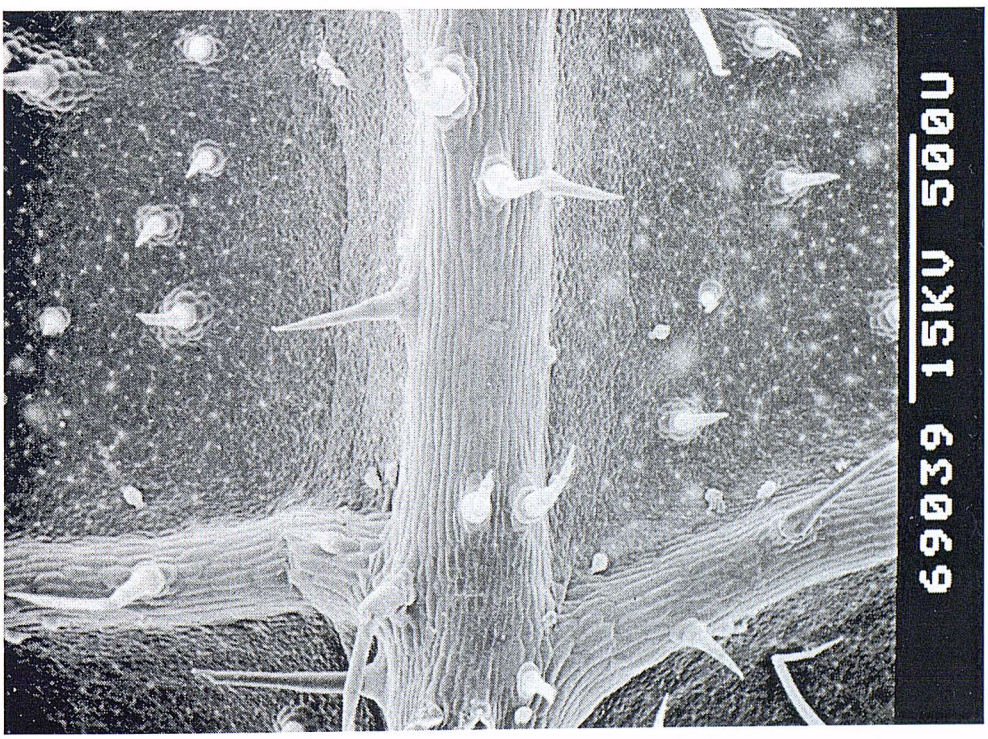
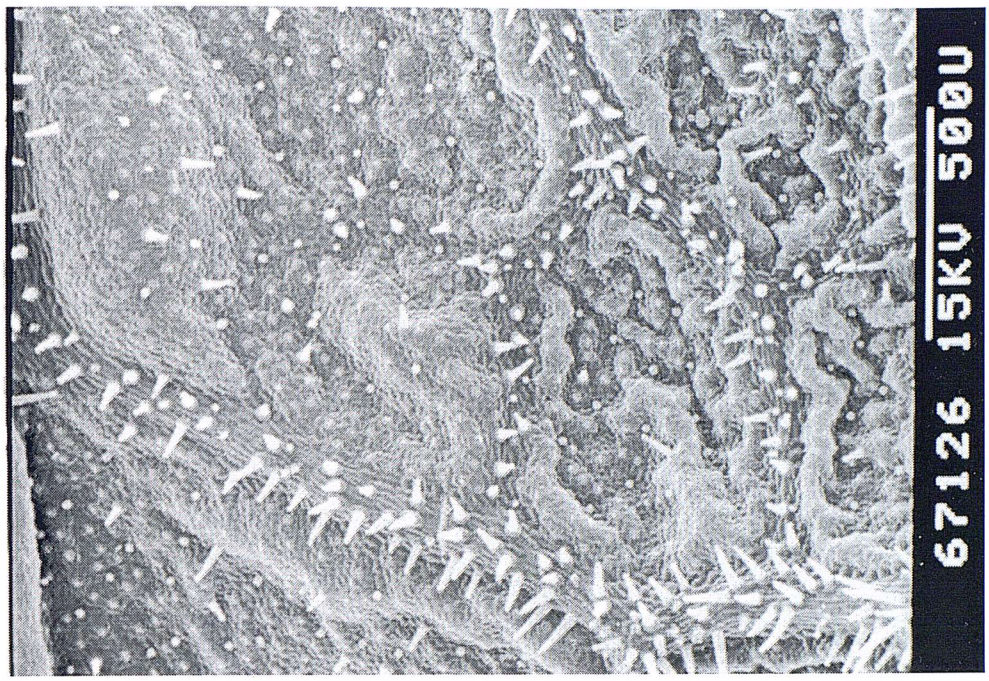


Plate 10.5: Arrangement of trichomes on leaf lamina and veins of the abaxial surface of Cucumber leaf under SEM study.

Leaf lamina



Leaf vein

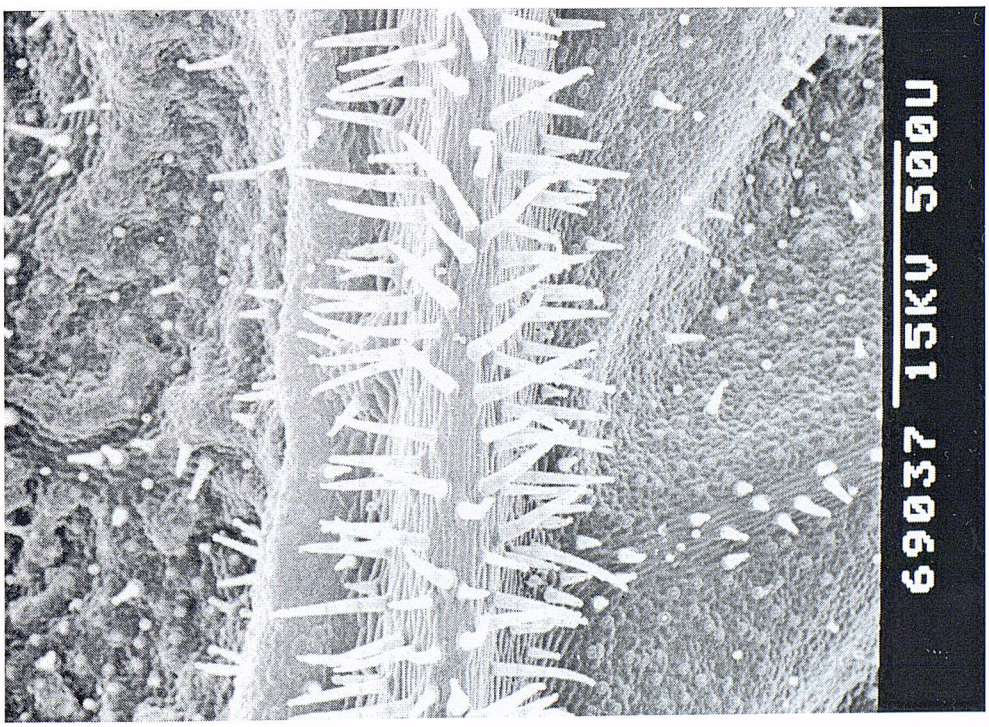
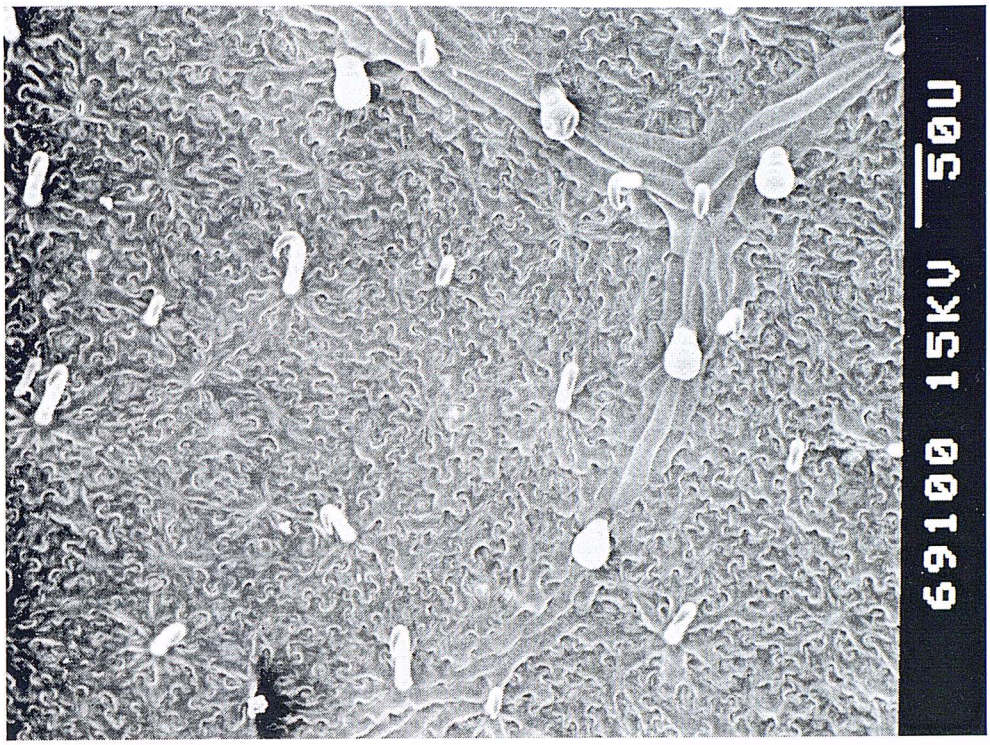


Plate 10.6: **Arrangement of trichomes on leaf lamina and veins of the abaxial surface of Primula leaf under SEM study.**

Leaf lamina



Leaf vein

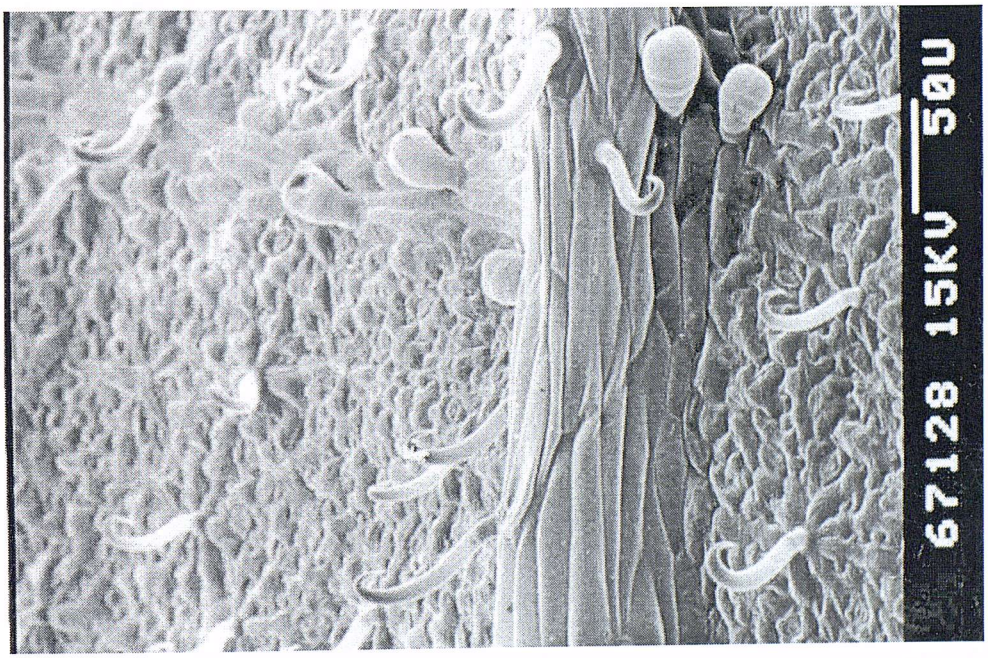
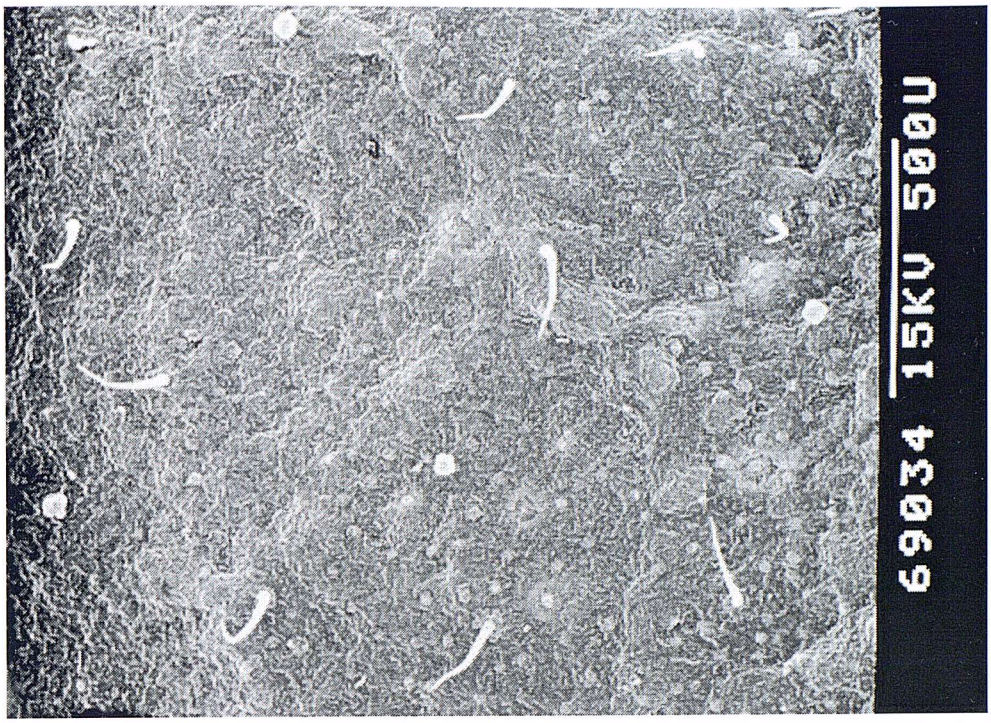


Plate 10.7: **Arrangement of trichomes on leaf lamina and veins of the abaxial surface of French bean leaf under SEM study.**

Leaf lamina



Leaf vein

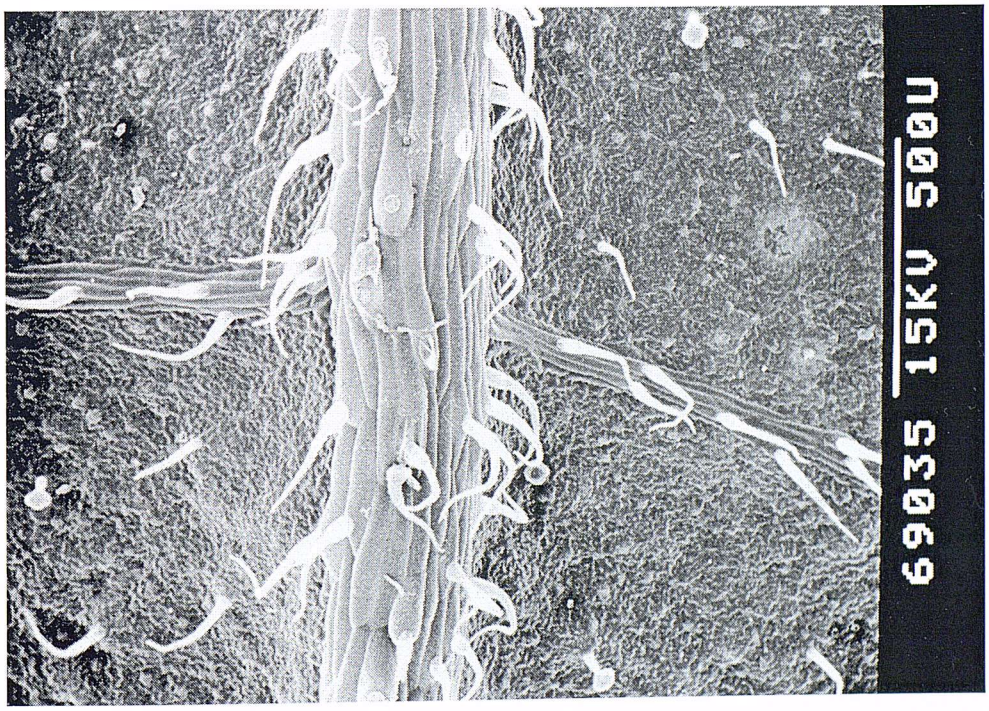
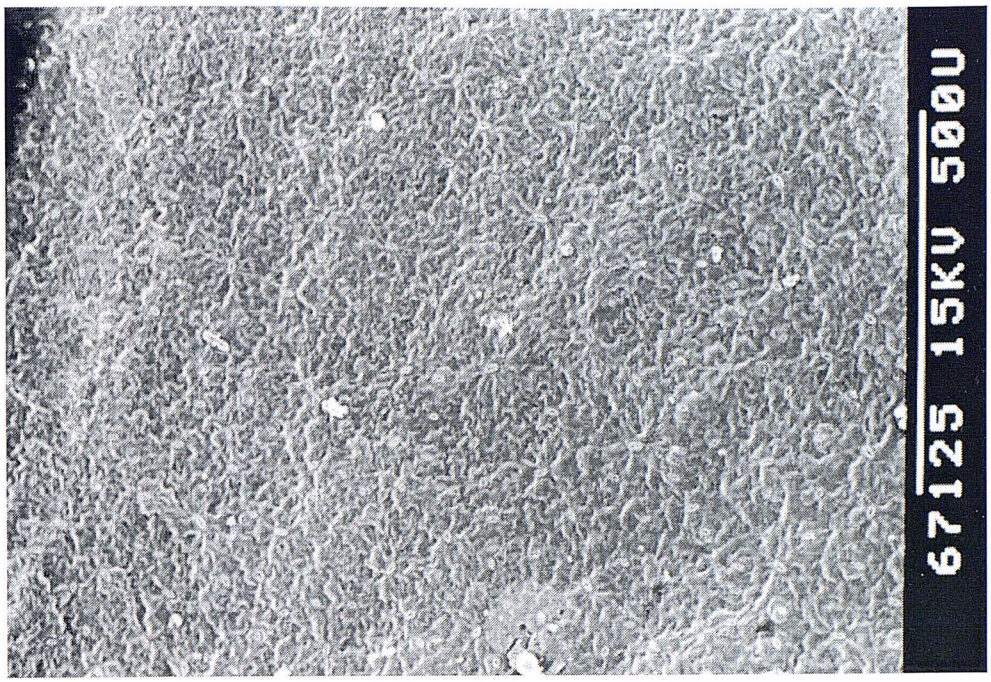


Plate 10.8: **Arrangement of trichomes on the surface and veins of the abaxial surface of Tomato leaf under SEM study.**

Leaf lamina



Leaf vein

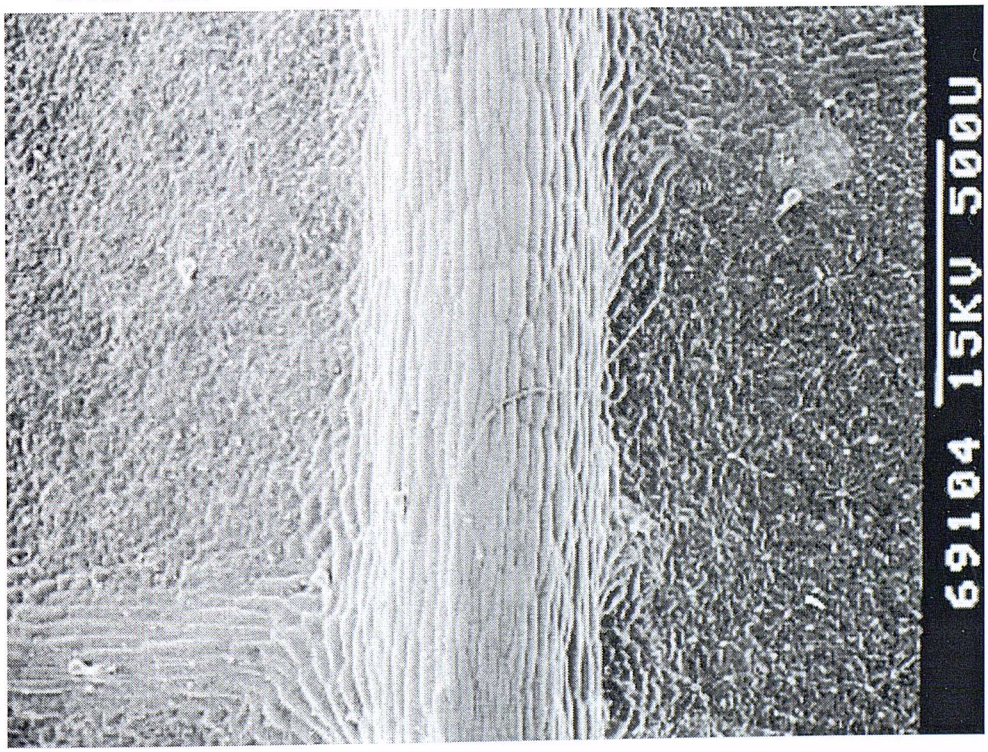


Plate 10.9: **Arrangement of trichomes on leaf lamina and veins of the abaxial surface of Sweet pepper leaf under SEM study.**

10.5.2 Searching behaviour of *A. atomus* on the abaxial surface of the leaves of five host plant species

When the parasitoids were introduced onto the leaf disc without host eggs they started to move in any direction, changed direction frequently and apparently randomly, sometimes reaching the Petri-dish wall. They then walked alongside the wall and then again would change direction. But when leaves contain host eggs, parasitoids started drumming with their antennae immediately after introduction onto the leaf discs and never left the leaves.

10.5.2.1 Net walking speed

A significant difference was observed in the net walking speed of the parasitoid on the leaf vein ($P<0.05$, LSD= 0.39, df= 26) and leaf lamina ($P<0.001$, LSD= 0.59, df= 32) of five plant species. Net speed was the highest (1.21 and 2.51 mm/s) on Sweet pepper and lowest (0.72 and 1.1 mm/s) on Primula along the leaf vein and leaf lamina, respectively (Table 10.2). Net speed was lower on leaves containing host eggs than on a clean leaf. Among two regions of the leaf, the net speed was always higher on the leaf lamina than on the leaf veins in the five host plant species.

10.5.2.2 Absolute turning (degrees/s)

No significant difference was observed in the absolute turning (degrees/s) of the parasitoid on leaf vein and leaf lamina of five different host plants even when the leaves contained host eggs (Table 10.3). The Two-sample t test did not show any significant difference in absolute turning between leaf lamina and leaf veins on the five host plant species.

10.5.2.3 Meandering (Degrees/mm)

Meandering (Degrees/mm) of the parasitoid showed a significant difference on different plant species both on leaf vein ($P<0.001$, LSD= 27.96, df= 24) and leaf lamina ($P<0.001$, LSD= 17.02, df= 32) among the five host plants. The highest and lowest meandering was observed on Primula and Sweet pepper, respectively. Moreover, the turning was higher on the leaf containing host eggs than on the clean leaf (Table 10.4).

Table 10.2 Net walking speed of the egg parasitoid *Anagrus atomus* on the leaf surface of different host plants.

Host Plant	Net walking speed (mm/s)				Two-sample t test
	n	Leaf vein	n	Leaf lamina	
Cucumber	6	0.98±0.078 ab A	6	1.14±0.119 cd A	NS t =1.13, df= 8
Tomato	-	-	6	1.39±0.161 bc	-
French bean	7	0.81±0.089 bc B	7	1.85±0.219 b A	$P<0.01$ t= 4.39, df=7
Primula	8	0.72±0.042 bc B	7	1.10±0.069 cd A	$P<0.001$ t= 4.68, df=10
Sweet pepper	5	1.21±0.087 a B	7	2.51±0.338 a A	$P<0.05$ t= 2.80, df=9
Primula with host egg	5	0.50±0.087 c A	5	0.68±0.085 d A	NS t= 4.39, df=7
One-way ANOVA		$P<0.0$ LSD= 0.39 df= 26		$P<0.001$ LSD= 0.59 df= 32	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P\leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

Table 10.3 Absolute turning (degrees/sec) of the egg parasitoid *Anagrus atomus* during searching for eggs on the surface of different host plants.

Host Plant	Absolute turning (degrees/s)				Two-sample t test
	n	Leaf vein	n	Leaf lamina	
Cucumber	6	65.15±6.44 a A	6	55.67±4.55 a A	NS t=-1.20, df=7
Tomato		-	6	52.62±3.08 a A	
French bean	5	61.23±7.38 a A	7	59.20±4.77 a A	NS t= -0.23, df=7
Primula	8	66.88±4.17 a A	7	59.10±3.69 a A	NS t= -0.32, df=7
Sweet pepper	5	57.03±5.79 a A	7	49.19±4.37 a A	NS t= -1.08, df=7
Primula with host egg	5	54.67±5.20 a A	5	57.78±3.43 a A	NS t= 0.50, df=7
One-way ANOVA		*P*= 0.75 LSD= 16.92 df= 24		*P*=0.43 LSD= 11.99 df= 32	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

Table 10.4 Meandering (degrees/mm) of the egg parasitoid *Anagrus atomus* during searching for eggs on the surface of different host plants.

Host Plant	Meandering (degrees/mm)				Two-sample t test
	n	Leaf vein	n	Leaf lamina	
Cucumber	6	56.67±6.32 bc A	6	54.47±6.33 b A	NS t= -0.25, df=9
Tomato	-	-	6	40.13±3.21 bc	*P*<0.001 t= 4.68, df=10
French bean	7	66.49±4.78 bc B	7	35.83±4.86 cd A	*P*<0.001 t= -4.50, df=11
Primula	6	83.78±6.28 b B	7	55.48±6.35 b A	*P*<0.01 t= -3.17, df=12
Sweet pepper	5	43.53±7.49 c B	7	21.61±3.20 d A	*P*<0.05 t= -2.69, df=5
Primula with host egg	5	121.86±21.11 a A	5	89.58±10.82 a A	NS t= -1.36, df=5
One-way ANOVA		*P* <0.001 LSD= 27.96 df= 26		*P* <0.001 LSD= 17.02 df= 32	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates.

10.6 Discussion

Variation was observed in trichome densities among different host plants. Glandular and non-glandular trichomes were found in every host plant except Sweet pepper where non-glandular trichomes were absent.

The dense trichomes on the abaxial surface of leaves can provide protection against herbivore attack in many ways. The hooked trichomes in French bean can catch the arthropods, immobilising them and causing death from dehydration and starvation before they can escape. On the other hand, the glandular trichomes, which may protect the plant from herbivorous attack, secrete sticky exudates when ruptured.

The glandular trichomes found in Tomato have a four-celled head (Plate 10.5B). These trichomes are known as a type A Gibson's and are common in various Solanaceous species and release sticky exudates when ruptured by insects (Jeffree, 1986). These highly specialised secretory cells synthesise and accumulate a large variety of terpene oils and other essential oils whereas the principal toxic component has been identified as 2-tridecanone (Dimock and Kennedy, 1983).

Plant characteristics can play an important role in the searching behaviour of natural enemies of herbivores. Plant feature, such as leaf toughness or hairiness, or increased trichome density on leaves, make the parasitoid less effective in finding hosts (van Driesche and Bellows, 1996). One of the characteristics that can negatively influence the behaviour is the presence of leaf hairs (Kleijn *et al.*, 1997). The results of the present study indicate that leaf surfaces of different host plants influenced walking speeds and turning of the egg parasitoid *A. atomus*. The variation in the walking behaviour on the leaf surface of different plant species may be due to the morphological differences in leaf structure, particularly trichome forms and density.

The highest net walking speed of *A. atomus* was observed on both leaf vein (1.21 mm/s) and leaf lamina (2.51 mm/s) of Sweet pepper among five host plant species. Parasitoids showed the lowest meandering (21.6-43.5 degrees/mm) on Sweet pepper among different plant species. On the other hand, parasitoids walked more slowly (0.72-1.11 mm/s) and showed more meandering (55.5- 83.8 degrees/mm) on Primula. This

might be due to the high trichome density on Primula, which prevented the parasitoid walking a straight path and led to more turning. These results agree with the findings of Sütterlin and van Lenteren, (1997) who observed the highest walking speed (0.73mm/s) of *Encarsia frmosa* Gahan on hairless Sweet pepper leaves and the lowest on Cucumber (0.2mm/s) with stiff and large hairs in a high density. Van Lenteren *et al.* (1995) had also mentioned a higher walking speed of *E. formosa* on hairless (0.60mm/s) than on hairy (0.40mm/s) cultivars of Cucumber. This may illustrate that leaf hairiness in general hampers parasitoid activity.

The presence of glandular trichomes on the leaf surface can adversely affect the searching behaviour of parasitoid wasps. Lovinger *et al.* (2000) reported that the egg parasitoid *A. nigriventris* Girault, spent less time, showed fewer oviposition probes and tended to fly off the stems of alfalfa with glandular trichomes than on alfalfa without glandular trichomes. Romeis *et al.* (1998) mentioned that the movement of *Trichogramma chilonis* Ishii on pigeon pea pods and calyxes was inhibited by long trichomes and they were trapped by sticky trichome exudates. In another study Romeis *et al.* (1999) observed *T. chilonis* to spend longer time on chickpea leaves when the acidic trichome exudate was washed off than on unwashed leaves.

Anagrus atomus stayed most of the time either on the lid or on the side of the Petri-dish when they were introduced onto Tomato leaf discs. The parasitoid wasp never walked over the leaf veins and walked fast on the leaf lamina and fled to the lid of the Petri-dish. This might be due to the presence of the secondary plant metabolite 'Tomatin' or the glandular trichome exudates that repelled the parasitoid wasp. Farrar and Kennedy (1991) found that the glandular trichome exudates of the resistant wild Tomato *Lycopersicon hirsutum glabratum,* which contains 2-tridecanone acted as a repellent and harmed the egg parasitoid *Telenomus sphingis* (Ashmead) at high concentrations, and could entangle it, repel it or reduce its searching efficiency or even kill the parasitoid by poisoning.

Parasitoid behaviour was reported to change with the presence of hosts on a leaf. The egg parasitoid *A. nigriventris* Girault spent more time on plants containing host eggs and the proportion of fast walking time was significantly greater on plants without host

eggs (Al-Wahaibi and Walker, 2000). The parasitoid *Encarsia formosa* stayed longer on leaves where they could recognise hosts or host products (van Lenteren *et al.*, 1996). In the present study, *A. atomus* walked slowly and made more turns per mm on the leaves containing host eggs.

Leaf surface characteristics were reported to affect the searching and predation efficiency of predators (Kleijn *et al.*, 1997; Siddiqui *et al.*, 1999). The dispersal of the predatory mite *Phytoseiulus persimilis* was reported to be hampered by the glandular trichomes on tomato leaves (Walter, 1996). Kleijn *et al.* (1997) observed a decreased walking speed and predation rate of the predatory mites *Phytoseiulus persimilis* Athias-Henriot with the increase in leaf hair on the underside of the *Gerbera jamesonii* leaves. Krips *et al.* (1999) also found a decreased walking speed and predation rate of *P. persimilis* on *G. jamesonii* with high trichome densities. Clercq *et al.* (2000) reported that the presence of glandular trichomes on the leaf surface interfered with the searching efficiency and the predation ability of the predator *Podisus nigripinus* (Dallas).

The results of the present study indicate that structural variation of plant surfaces significantly affected the searching rates of *A. atomus* on different plant species. This means that habitat structure influences the searching success of a parasitoid and it should be considered during application of a biological control programme. However, the results of laboratory studies may not directly predict the magnitude of effects of plant surface characteristics on parasitoid behaviour in the field. More laboratory as well as field studies are needed to confirm the predicted effects of leaf surfaces and parasitoid age on oviposition behaviour and the rate of parasitism by *A. atomus*.

Chapter 11

Study of the host discrimination and superparasitism behaviour *by Anagrus atomus*

11.1 Introduction

In parasitic insects, host discrimination is the ability of a female to distinguish parasitized from unparasitized hosts. This behaviour is named self-discrimination when a female recognises hosts parasitized by herself, conspecific discrimination when it concerns hosts parasitized by another female of the same species, and interspecific discrimination when a female recognises hosts parasitized by another species (Mackauer, 1990). Parasitoid females can discriminate between already parasitized and unparasitized hosts and usually avoid ovipositing in the parasitized hosts (van Lenteren, 1981; Bakker *et al.*, 1985). This recognition is done with the help of markers left on or in the hosts by the ovipositing females, which are usually species-specific and are hence usually effective only to conspecific females (Bakker *et al.*, 1985). In addition, female wasps can discriminate between host parasitized by them (Volkl and Mackauer, 1990; Holler *et al.*, 1991; Ueno, 1994; Ueno and Tanaka, 1994). This ability is called 'self-host discrimination' or 'self-recognition' (Holler *et al.*, 1991).

Discrimination between self- and conspecific parasitized hosts implies that females mark their hosts and that this marker varies between individuals (Mackauer, 1990). The markers employed by some parasitoid species are individual-specific and so allow females to recognize self-parasitized hosts (Volkl and Mackauer, 1990; Holler *et al.*, 1991; van Dijken *et al.*, 1992; Ueno, 1994; Ueno and Tanaka, 1994).

Several theoretical and empirical works have shown that superparasitism may be an adaptive and positive reproductive strategy of parasitoids under certain circumstances when unparasitized hosts are scarce (van Alphen and Visser, 1990; Weisser and Houston, 1993; Godfray, 1994). Adaptive superparasitism theory does not assume that female parasitoids failed to recognize parasitized hosts. Recognition of prior parasitism is viewed to be always advantageous, but it does not necessarily result in avoidance of superparasitism. The theory considers that a female parasitoid should decide after

recognition and prior to parasitism of a host to superparasitize or not, based on the relative benefits and costs associated with rejecting versus accepting that host for oviposition. Thus discrimination of whether or not female parasitoids recognize prior parasitism when they superparasitize is crucial to consider whether superparasitism is their positive strategy (Ueno, 1999). Some female parasitoids mark the hosts they parasitize with pheromonal cues on the surface or inside. These marks allow other females to discriminate between unparasitized and parasitized hosts, and to avoid the latter (Godfray, 1994). But in some cases, the second female appears to be killing the eggs of the previous females, which is commonly known as ovicide. The Strand and Godfray (1989) model predicts that ovicide is more likely to evolve when the competitive advantage of the first clutch under superparasitism is large. This increases the relative pay-off of ovicide. The model also predicts that ovicide is more likely when unparasitized hosts are rare. This makes host rejection a relatively unfavourable strategy. Finally, ovicide is more likely when the time taken to perform ovicide is small and the costs of ovicide is reduced.

However, the behavioural activities of a female parasitoid during oviposition on unparasitized hosts may differ from those during superparasitism. Therefore, positive superparasitism can be detected by a detailed and direct observation of the behaviour of ovipositing females (Ueno, 1999). No study has examined so far the behavioural differences *of A. atomus* during oviposition on parasitized versus unparasitized hosts. In the current study, experiments were designed with a number of objectives.

11.2 Objectives of the experiment

The objectives of the experiments were to assess:

a) Discrimination ability of parasitoids at different time intervals after oviposition.
b) Whether the parasitoid can discriminate between self and conspecific parasitized hosts
c) Superparasitism by self and conspecific parasitoids.
d) Survival of parasitoid offspring in self and conspecifically superparasitized hosts.

Null hypothesis:

1) Discrimination of the parasitized hosts by *A. atomus* does is the same at different hours after oviposition.
2) *Anagrus atomus* could not discriminate self and conspecifically parasitized hosts.

11.3 Materials and Methods

Three to five day-old eggs of *H. maroccana* were used in this experiment. To obtain the desired ages of eggs, healthy potted plants of French bean at the two leaves stage were placed in the mass culture of leafhopper. A large number of hoppers was maintained inside the perspex cage to ensure plenty of host eggs. After seventy two hours of infestation, the plants were removed from the perspex cage containing 1-3 day-old eggs. Leaf discs of 20 mm diameter containing leafhopper eggs were placed on agar medium in Petri-dish cages with their lower surface facing uppermost and kept in the incubator at 25°C for 48 hours prior to use in the experiment. Thus after 48 hours the eggs attained the age of 3-5 days.

Twelve-hour-old mated female parasitoid wasps, fed with 50% honey solution were used in the experiment. A single female parasitoid wasp was introduced into the leaf disc arena carefully with the help of a fine paintbrush and the activities were observed under a binocular microscope. The females start searching for hosts immediately after introduction onto the leaf surface. Oviposition by females was confirmed on the basis of time spent between insertion into and withdrawal of the ovipositor from the host. The female wasp was removed after she had parasitized ten eggs. The position of *H. maroccana* eggs inside leaf veins was drawn on a piece of paper and the position of the parasitized eggs were marked. Unparasitized eggs were destroyed with a sharp needle keeping five unparasitized eggs with those ten parasitized eggs for the following study. The leaf disc was then placed inside the incubator at 25°C. The female wasp was carefully taken out from the leaf disc and put inside an Eppendorf microcentrifuge tube and kept in the incubator to be used in the self-specific superparasitism study and provided with 50% honey solution. The same procedure was followed to study conspecific superparasitism except twelve-hour-old mated parasitoid wasps fed with 50% honey solution were used in the experiment to observe their discrimination and superparasitism behaviour on the previously parasitized hosts.

Self and conspecific host discrimination and superparasitism behaviour of *A. atomus* were studied at three different hours after oviposition (12, 24 and 72 h). Five replications (Petri-dishes) were maintained for each of the six situations. In each replication, the behaviour of *A. atomus* was studied in ten previously parasitized and five unparasitized eggs.

The following behaviour of the female wasp was recorded for both self and conspecific parasitoids until it had encountered all of the ten parasitized eggs.

1. Drumming of antennae
2. Antennal rejection
3. Rejection after ovipositor insertion
4. Repeated ovipositor insertion
5. Ovicidal action
6. Time spent during ovipositor insertion
7. Parasitoid survival in parasitized and superparasitized hosts

Previously parasitized eggs where superparasitism had occurred were marked individually. Hosts in each Petri-dish were incubated at 25°C and the same procedure was followed to estimate the percentage of adult emergence as in Chapter 5.

11.4 Statistical analysis

The ability to discriminate between healthy and already parasitized hosts by self-specific and conspecific parasitoids was evaluated by the number of eggs rejected after antennation and after ovipositor insertion out of the total eggs encountered. Data on parasitoid survival, host discrimination and other activities of the self and conspecific parasitoid at different time interval were analysed with χ^2 test.

11.5 Results

11.5.1 Host discrimination by the egg parasitoid *A. atomus* in self and conspecific superparasitism

The ability of *A. atomus* to discriminate between unparasitized hosts and those that have already been parasitized by the self and conspecific wasps over a range of oviposition time intervals (12, 24 and 72 h) was evaluated and the results are shown in Table 11.1, Table 11.2 and Figure 11.1. In a preliminary observation, the female wasps were found to take on average 64.9±2.7 s for a successful oviposition ranging from 51.9-77.9 s. Therefore, when the parasitoids spent at least 50 s after ovipositor insertion this was considered as successful oviposition and less than that was considered as probing rejection. Results in Table 11.1 show that the antennal rejection is significantly different at different time intervals between first parasitization and the second time encounter for self (χ^2= 49.164, P< 0.000, df= 2) and conspecific (χ^2= 5.906, P= 0.05, df= 2) parasitoids. The selfspecific parasitoids rejected 68% of the parasitized host by antennal examination at 12 h of parasitization. Whereas, conspecific parasitoids rejected only 22% of the hosts after antennal examination. The detection of previous parasitization by both self and conspecific parasitoids decreased with the time of previous parasitization. Twenty-four hours after first parasitization, the discrimination ability decreased to 12% and 8% for self and conspecific parasitoids. On the other hand, probing rejection increased when the time interval of previous parasitization increased. Probing rejection increased from 32% at 12 h to 84% at 72 h after parasitization for selfspecific parasitoids. In conspecific parasitoids, probing rejection was observed 60% and 78% at 12 h and 72 h after parasitization, respectively.

Table 11.1 Host discrimination by antennal examination in self and conspecific parasitism by *Anagrus atomus* at different time intervals after oviposition.

Parasitoid	Total parasitized host encountered (no.)	Antennal host rejection at different hours after oviposition (no.)			χ^2 test
		12 h	24 h	72 h	
Self specific	50	34 (68)	6 (12)	6 (12)	χ^2= 49.164 *P*< 0.001 df= 2
Conspecific	50	11 (22)	4 (8)	4 (8)	χ^2= 5.906 *P*< 0.05 df= 2
χ^2 test		χ^2= 21.374 *P*< 0.001 df= 1	χ^2= 0.444 *P*= 0.505 df= 1	χ^2= 0.444 *P*= 0.505 df= 1	

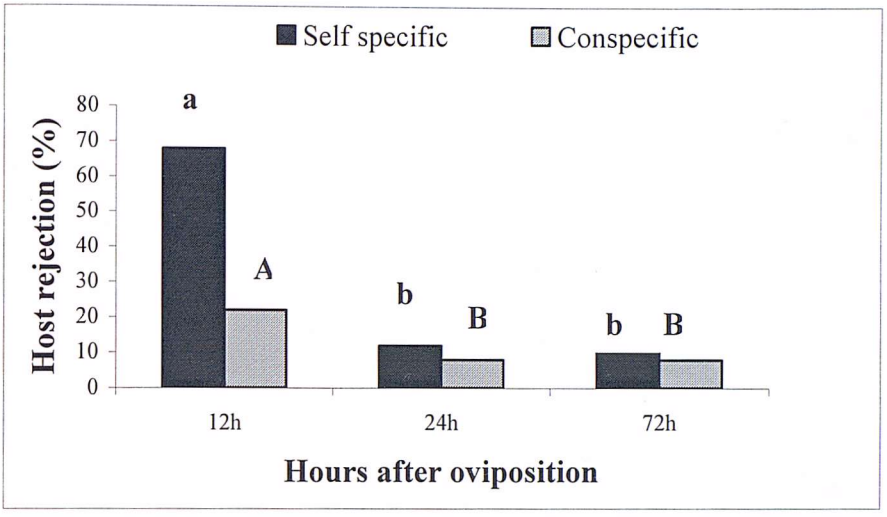
Figures in parentheses are the percent values.

Table 11.2 Host discrimination after ovipositor insertion in self and conspecific parasitism by *Anagrus atomus* at different time intervals after oviposition.

	Total parasitized host encountered (no.)	Probing rejection at different hours after oviposition (no.)			χ2 test
		12 h	24 h	72 h	
Self specific	50	16 (32)	43 (86)	42 (84)	χ^2= 42.615 *P*< 0.001 df= 2
Conspecific	50	30 (60)	31 (62)	39 (78)	χ^2= 4.38 *P*= 0.112 df= 2
χ^2 test		χ^2= 7.89 *P*< 0.01 df= 1	χ^2= 7.484 *P*< 0.01 df= 1	χ^2= 0.585 *P*= 0.444 df= 1	

Figures in parentheses are the percent values.

Discrimination by antennal examination



Discrimination by ovipositor insertion

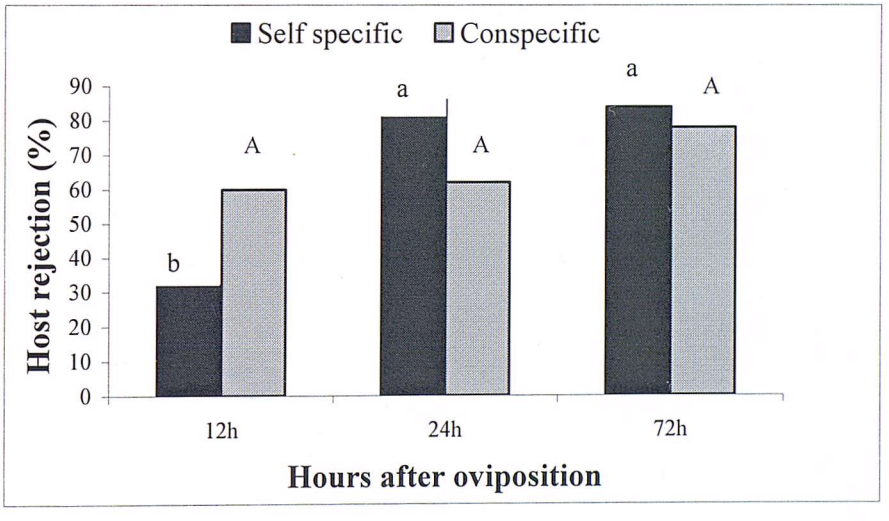


Figure 11.1 **Host discrimination by *Anagrus atomus* in self and conspecific superparasitism at different time intervals after oviposition.**

11.5.2 Oviposition and superparasitism by self and conspecific parasitoids of *A. atomus*

The percent of superparasitism was very low (2-4%) when the parasitoid was ovipositing into eggs parasitized by herself but the conspecific parasitoids superparasitized 18%, 30% and 10% of the previously parasitized eggs at 12, 24 and 72 h, after parasitization, respectively (Table 11.3). Moreover, the conspecific parasitoids showed repeated ovipositor insertion into the host from different angles. The intensity of repeated ovipositor insertion increased when the time interval of parasitization increased. The conspecific parasitoids inserted the ovipositor repeatedly in 46% of the 72 h old parasitized hosts, whereas, the self-specific parasitoids showed repeated ovipositor insertion only in 6% of the 72 h old parasitized hosts (Table 11.4).

The percentage distribution of the total encounters by *A. atomus* which resulted in rejection, attempts at oviposition or successful oviposition in healthy and previously parasitized hosts by self and conspecific parasitoids are represented schematically in Figure 11.2 and 11.3. Both self and conspecific parasitoids accepted 100% of the healthy hosts for oviposition when provided with 12, 24 and 72 hours old parasitized hosts. Oviposition success was always higher in conspecific parasitoids than in selfspecific parasitoids at all time intervals after the first oviposition.

Table 11.3 Superparasitism by self and conspecific parasitoids of *Anagrus atomus* at different time intervals after parasitization.

	Total host encountered (no.)	Hosts superparasitized at different hours after parasitization (no.)			χ^2 test
		12h	24h	72h	
Self specific	50	0	1 (2)	2 (4)	χ^2= 0.344 *P*= 0.558 df= 2
Conspecific	50	9 (18)	15 (30)	5 (10)	χ^2= 6.938 *P*< 0.05 df= 2
χ^2 test		χ^2= 9.89 *P*< 0.01 df= 1	χ^2= 14.583 *P*< 0.001 df= 1	χ^2= 1.382 *P*= 0.240 df= 1	

Figures in parentheses are the percent values.

Table 11.4 Repeated ovipositor insertion by self and conspecific parasitoids of *Anagrus atomus* at different time intervals after parasitization.

	Total parasitized host encountered (no.)	Hours after parasitization			χ^2 test
		12h	24h	72h	
Self specific	50	0	3 (6)	3 (6)	χ^2= 3.125 *P*= 0.21 df= 2
Conspecific	50	4 (8)	8 (16)	23 (46)	χ^2= 22.435 *P*< 0.001 df= 2
χ^2 test		χ^2= 4.167 *P*< 0.05 df= 1	χ^2= 2.554 *P*= 0.110 df= 1	χ^2= 20.79 *P*< 0.001 df= 1	

Figures in parentheses are the percent values.

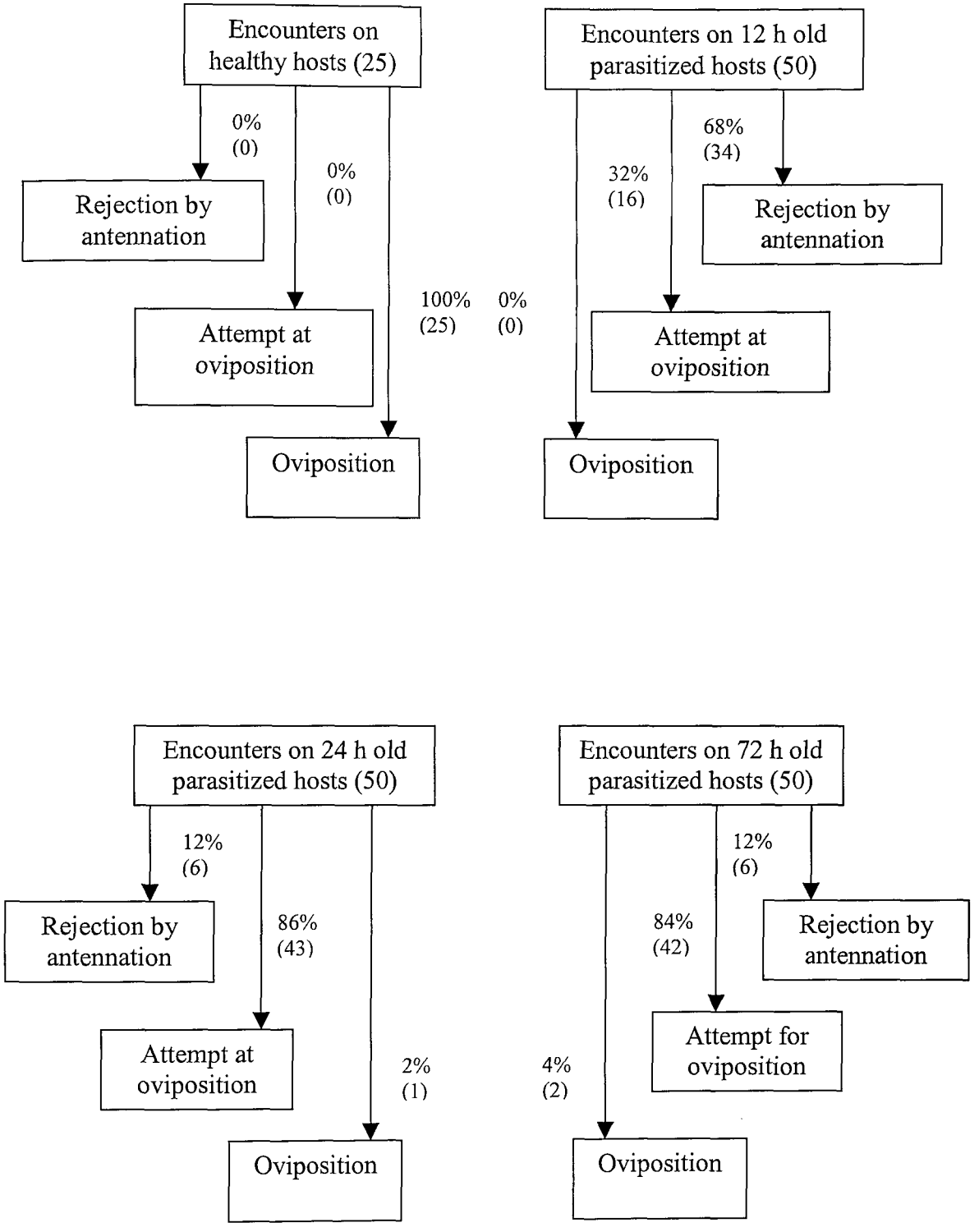


Figure 11.2 **Self-specific superparasitism behaviour by the egg parasitoid *Anagrus atomus* during oviposition in healthy hosts and parasitization in 12, 24 and 72 h old parasitized hosts. Figures in parentheses indicate the number of hosts of each quality.**

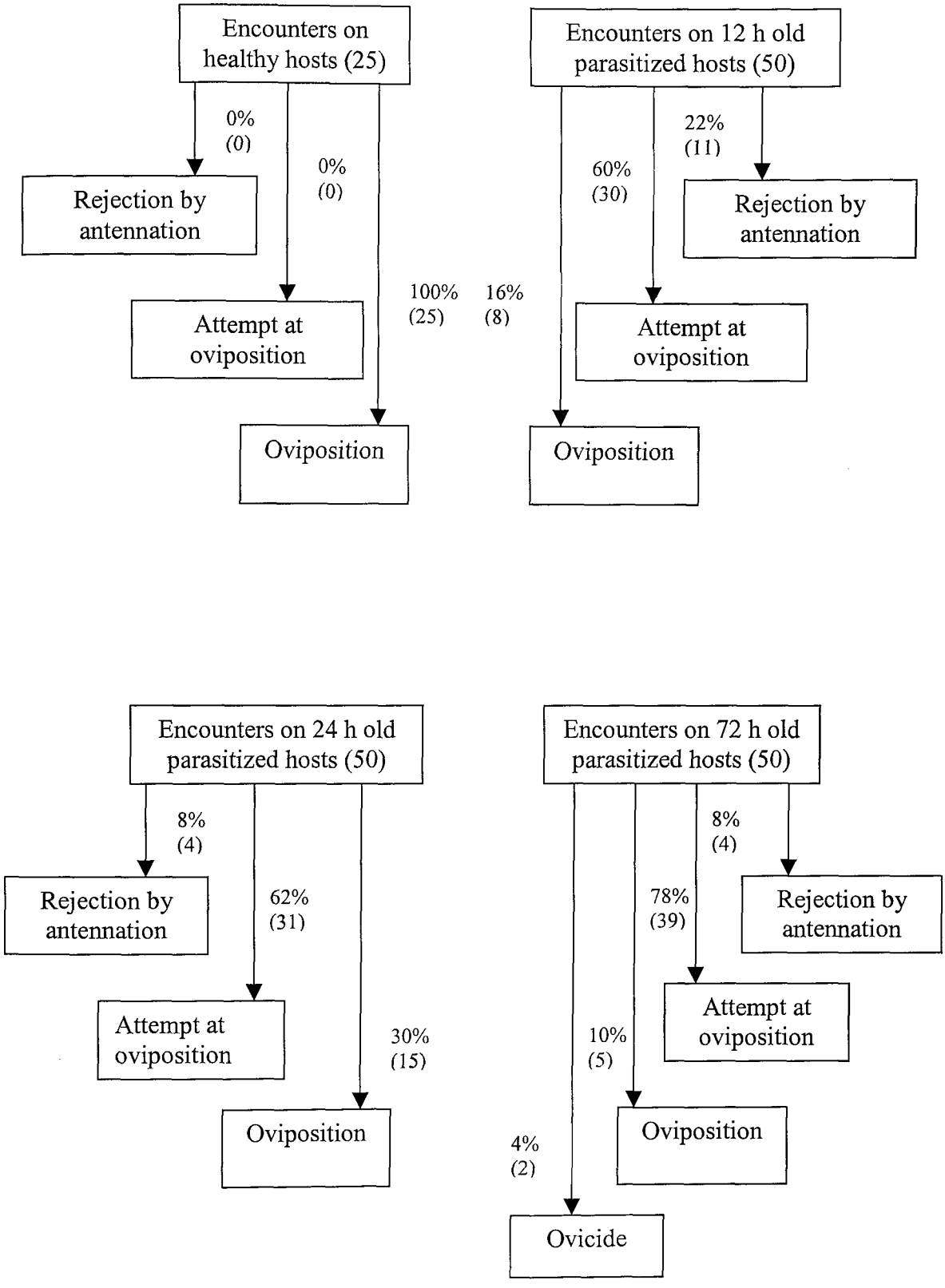


Figure 11.3 **Conspecific superparasitism behaviour by the egg parasitoid *Anagrus atomus* during oviposition in healthy hosts and parasitization in 12, 24 and 72 h old parasitized hosts . Figures in parentheses indicate the number of hosts of each quality.**

111.5.3 Parasitoid survival in superparasitized hosts

The offspring survival from the superparasitized eggs of *H. maroccana* by the egg parasitoid *A. atomus* at different time intervals after parasitism is presented in Figure 11.4. In the present study selfspecific parasitoid did not superparasitize 12 h old parasitized eggs. But when 24 and 72 h old parasitized eggs were offered, they superparasitized 2 and 4% of the eggs, respectively and 100% of the parasitoid offspring emerged from those superparasitized eggs. On the other hand, superparasitism in conspecific parasitoids varied from 10-30% when parasitized eggs were offered at different time intervals after parasitism and the survival of parasitoid offspring varied from 62.5- 93.3%.

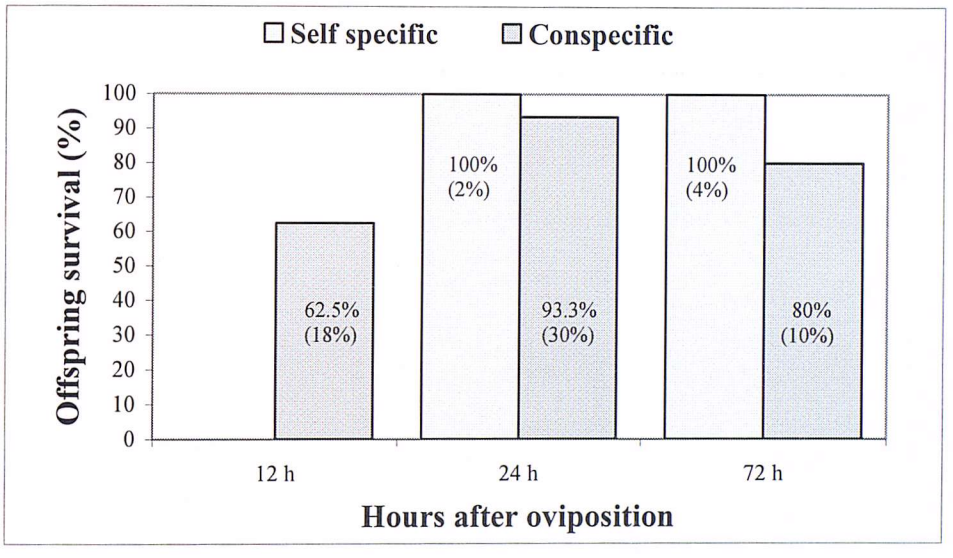


Figure 11.4 **Parasitoid survival in the host superparasitized by self and conspecific parasitoids of *Anagrus atomus* at different time intervals after parasitization (χ^2= 0.351, *P*= 0.839, df=2 for conspecific parasitoid). Values on the bars represent % survival and in parentheses % superparasitism.**

11.6 Discussion

11.6.1 Host discrimination by the egg parasitoid *A. atomus* in self and conspecific superparasitism

Host discrimination is a common phenomenon in hymenopteran parasitoids (van Lenteren, 1981; van Alphen and Visser, 1990). Host discrimination by the parasitoid occurs after detection of either an individual external pheromone by antennal drumming, or an internal pheromone, which can be recognized by chemosensors located at the tip of the ovipositor (van Baaren *et al.*, 1994). These chemosensors respond during ovipositor insertion to chemical changes associated with parasitism in the host (Ueno, 1999).

Results show that female *A. atomus* can discriminate between unparasitized and previously parasitized hosts through antennal examination and by inserting the ovipositor into the host depending on the time after the previous parasitization. In self-discrimination, host rejection was mostly done by antennal examination, suggesting that the females recognize their own oviposition through an external marker probably deposited immediately after oviposition. In self-discrimination, *A. atomus* rejected 68% of the parasitized host by antennal examination at 12 h after parasitization. In contrast, in conspecific discrimination, female *A. atomus* rejected only 22% of the conspecifically parasitized hosts after antennal examination and the rejection was mostly done after ovipositor probing. A similar observation was found in the solitary endo-parasitoid *Pimpla nipponica* Uchida, where the female parasitoids accepted a majority of conspecifically parasitized hosts as oviposition sites after external examination, while they rejected self-parasitized hosts without probing (Ueno and Tanaka, 1996). In *Encarsia formosa*, van Roermund and van Lenteren (1995) reported 95% and 85% antennal rejection in self and conspecific parasitoids, respectively. Some of the parasitoid females can learn to recognize external markings left by the conspecifics as in the egg parasitoid *Anaphes victus* (Hymenoptera: Mymaridae). At the beginning of oviposition sequence, *Anaphes victus* rejected eggs after insertion of the ovipositor but, after some encounters with parasitized hosts, they started rejecting them after antennal drumming (van Baaren and Boivin, 1998). On the other hand, when females attacked parasitized hosts, many of them inserted the ovipositor into the host more than once during a single host encounter. Some females drilled only once and then rejected or accepted parasitized hosts for

oviposition, but the others drilled into hosts repeatedly and then rejected or accepted parasitized hosts for oviposition (Ueno, 1999).

The rejection after antennal examination by *A. atomus* in both self and conspecific discrimination was found to decrease with the time interval of previous parasitization. One possible explanation is that wasps cannot distinguish the parasitized host after a certain period of time following the first oviposition, because the marker cue left by the first female seems to be unstable and the efficacy of both external and internal marker cue reduce with time. Ueno and Tanaka (1996) suggested that the volatile chemical markers applied by females during oviposition degrade with time. The results of the present study agree with Chow and Mackauer's (1986) model, which describes time-dependent host discrimination in the aphid parasitoid *Ephedrus californicus* Baker.

On the other hand, probing rejection increased when the time interval of previous parasitization increased. Since the egg of *A. atomus* hatch approximately 3 days after oviposition, the changes in the host haemolymph acts as an internal marker. Probing rejection reached 84 and 78% at 72 h after parasitization for selfspecific and conspecific-discriminations, respectively. This result suggests that the female *A. atomus* respond to changes in host quality associated with the developing larva inside the parasitized host and reject the host after insertion of the ovipositor. This result is in agreement with Mackauer's (1990) hypothesis, that the changes in host quality associated with the developing parasitoid embryo or larva can serve as an internal marker and also lead to host rejection.

11.6.2 Oviposition and superparasitism by self and conspecific parasitoids of *A. atomus*

Direct observation of superparasitism behaviour showed that an individual female *A. atomus* oviposited into previously parasitized hosts even after recognizing prior parasitism, supporting the hypothesis that superparasitism is a positive host selection strategy in parasitoid. In the present study, only 2-4% superparasitism was observed in self specific parasitoids while it varied from 10-30% in conspecific parasitoids at different time interval after the previous parasitism. Moreover, the conspecific parasitoids showed

repeated ovipositor insertion almost always before ovipositing into the hosts when 72 h old hosts were offered. Females of *Encarsia formosa* avoided 86% of the conspecific eggs during superparasitism which increases up to 100% in self-superparasitism (van Roermund and van Lenteren, 1995). Ueno (1999) described repeated ovipositor insertion as an action related to the recognition of prior parasitism in the solitary parasitoid *P. nipponica.*

11.6.3 Parasitoid survival in superparasitized hosts

Although 100% of the parasitoid offspring emerged from the superparasitized eggs by conspecific parasitoids it is difficult to draw any conclusions as the number of observation was very small. In contrast, the survival of parasitoid offspring in the 72 h old superparasitized eggs by conspecific parasitoids is 80%. This might be due to the ovicidal action by the conspecific parasitoids. Ovicide is best known in ectoparasitoids from the families Ichneumonidae and Bethylidae, which either eat conspecific eggs or use their ovipositor to destroy them (Strand and Godfray, 1989). It is much less commonly reported in endoparasitoids, which may be due in part to the greater difficulty of demonstrating it experimentally, but also because of the inherent constraints on mechanisms to achieve it (Strand and Godfray, 1989). In the egg parasitoids there are more difficulties associated with locating the parasitoid egg floating inside the host egg, and to hold and destroy it. However in the present study, *A. atomus* showed 4% ovicide (Figure 11.3). The repeated ovipositor insertion into the host from different angles reveals that the conspecific *A. atomus* could detect the presence of developing larva of the first parasitoid and tries to kill them, which was not observed in selfspecific parasitoids. More studies should be done to confirm the ovicide in *A. atomus*.

Chapter 12

General discussion and conclusion

12.1 Development of *H. maroccana* in relation to temperature and host plant

Climatic factors such as temperature, humidity and photoperiod have a profound influence on insect populations, either directly or by their influence on other organisms in the ecosystem (Hagen *et al.*, 1984). Different biological attributes of *H. maroccana* were studied on different host plants at different constant temperatures. Temperature and host plant had significant effects on the development, immature survival, relative growth rate, adult longevity and different life table parameters of *H. maroccana*. Duration of nymphal development shortened with increasing temperature within the temperature range 17 to 25°C. Among the different host plants studied, the longest nymphal duration was observed on Primula.

Typically, developmental rate increases from zero at a low temperature threshold reaching a maximum at an optimal temperature, then decreases rapidly to zero at an upper lethal temperature (Lactin *et al.*, 1995). The rate of development of *H. maroccana* during egg and nymphal stages was linearly related to a range (17 to 25°C) of temperatures. The linear regression analyses of egg and nymphal development versus temperature showed a good fit (r^2=0.88 to 0.99 and 0.97 to 1, respectively) on different host plants. The linear trend of increase in developmental rate with increasing temperature has been reported in many other insect species (Wagner *et al.*, 1984; Soultani, 1997). Extreme temperature causes a decrease in developmental rate resulting in a departure of developmental rate from a linear trend (Liu and Tsai, 1998).

In order to accurately resolve the relationship of temperature and developmental rate, the frequency of observation should be maximized, particularly in the study of brief events such as development of individual instars (McDonald *et al.*, 1998). In the absence of field data and information on environmental factors, the use of more complex, non-linear models of developmental rate (van Rijn *et al.*, 1995) to predict general patterns across a wide geographical area is likely to offer a significant improvement over the linear model used here.

There was a significant difference in the percentage of stage-specific survival from 1st instar nymph to total survival at different temperatures. The lowest survival of all instars observed at 27°C indicated that the upper developmental threshold of *H maroccana* nymphs was between 25 to 27°C.

Host plants with poor nutritional quality slow down the rate of development of herbivore at different stages of their growth and affects the rate of survival of herbivores at various life stages (van Driesche and Bellows, 1996). Therefore, the percentage of immature survival to adulthood can be taken as an indication of differences in host quality for *H. maroccana* development. This percentage varied significantly among the five host plants used in the experiment. French bean was found to be the least suitable compared to the other four at all temperatures with lowest nymphal survival (26-75%) (Table 3.14). On the other hand, Primula was found to be the most favourable host plant with 81-100% nymphal survival. Survival was higher in the early developmental stages and decreased as development proceeded on different host plants. The differences in the suitability of the plants seems to indicate the presence of some internal factors, which make them less attractive for feeding during post-embryonic development. These factors can be either nutritional or morphological. The SEM study of different host plants shows a great variation in the leaf topography (Chapter 10). Therefore, the morphological differences of the host plants adversely affected the development of *H. maroccana*.

A greater relative growth rate of *H. maroccana* was observed at high temperature due to the decreased adult weight and faster development. Females had a slower RGR than the males. Adult longevity decreased with increasing temperature.

12.2 Host age effects on development, survival, size and offspring sex ratio of *A. atomus*

Host quality is considered as an important factor, which influences the reproductive strategies in parasitoids (Strand, 1986). Reduced quality often causes a decline in progeny survival or adult size, which in turn affects other fitness measures such as fecundity and longevity (Charnov *et al.*, 1981). For solitary species of parasitoids, host quality is generally assumed to be determined by host size; because larger hosts contain more resources, they are supposed to be of higher quality than smaller hosts (Charnov *et*

al., 1981; Charnov, 1982, Hardy, 1994). On the other hand, in gregarious parasitoids the number of eggs deposited in a host depends on numerous factors including host size (Schmidt and Smith, 1985), physiological status of the female and patch quality (Skinner, 1985).

The present study examined the development time, immature survival, potential fecundity, adult size and offspring sex ratio of the egg parasitoid *A. atomus* in the laboratory at constant temperature. Older hosts eggs appeared to be larger in size but this study failed to show a relationship between age and size. However, host eggs may change their nutritional value with age as the embryo develops in the same way that growing hosts change quality with size. Certainly, development of both sexes was faster in older eggs. Based on empirical data, a number of workers have formulated predicted models that describe immature parasitoid development and adult oviposition decisions where host size was often used as an index of host quality (Charnov, 1982; Charnov and Skinner, 1985; Mackauer and Sequeira, 1993). These models have considerable application for parasitoids attacking static resources (= idiobiont parasitoids). Because hosts parasitized by idiobionts do not grow after they are parasitized, clutch size decisions of the female parasitoid will have a great influence on the size of its offspring (Godfray, 1994).

The ability to assess the size of the host at the time of attack and allocate a specific sex by depositing male eggs in smaller hosts and female eggs in large hosts is widespread in hymenopteran parasitoids (Godfray, 1986; King, 1987). Since size has a larger effects on the fecundity of females than males in most parasitic Hymenoptera (Heinz, 1991), it has been shown on many occasions that idiobiont parasitoids selectively lay male eggs on small hosts- presumably because being small does not disadvantage a male offspring as much as it would a female one (Quicke, 1997). King (1987) suggested that female biased sex broods facilitate the use of parasitoid wasps as biological control agents, because a few males are able to fertilize many females. In this case the production of female-biased broods decreases the competition among sons while increasing the number of potential mates for the remaining sons (Harvey, 2000). In the present study, more female offspring developed from the older eggs, indicating that fertilized eggs were deposited in older hosts. The varied sex ratio of *A. atomus* with different host age reflects their preference for host age as a natural choice for allocating progeny sex. A high female sex ratio in

older hosts suggests that host size alone does not determine the decision of *A. atomus* to deposit diploid or haploid eggs. Other qualities like physiological condition of the parasitoid and greatest potential for parasitoid growth of the host stage irrespective of its size might have contributed to more female offspring from larger hosts. Despite being a koinobiont parasitoid, a higher proportion of females was observed to emerge from larger hosts of the aphid parasitoid *Lysiphlebia mirzai* Shuja-Uddin (Pandey and Singh, 1999). Higher allocation of males in smaller hosts in *Metaphycus helvolus* (Compere) was reported by Lampson *et al.* (1996) and Jahan (1997). Egg parasitoids may accept or reject hosts based on quality and may reject hosts or exhibit modified strategy of sex and progeny allocation in utilizing them (van Huis and Rooy, 1998). Eggs laid by *Callosobruchus maculatus* (Fabricius) were larger on pigeonpea and hence the egg parasitoid *Uscana lariophaga* Steffan deposited more fertilized eggs in the eggs laid on pigeonpea than on other host plants (van Huis and Rooy, 1998).

As the availability of different host stages within the population varies with time, the potential value of a host fluctuates, and parasitoids might be expected to be dynamic in their foraging strategy in order to maximize their reproductive potential (Godfray, 1994). In the present study, potential fecundity of *A. atomus* did not vary significantly with host age. Oviposition in the younger hosts resulted in delayed parasitoid development but produced larger parasitoid offspring.

12.3 Development of *A. atomus* in relation to temperature and host plant

The optimum range of temperature or humidity for development, reproduction and survival of a candidate biological control agent may be different from that of the pest, and natural enemies may either fail to establish or prove ineffective due to the direct or indirect effects of climate in the area of introduction (Jervis and Kidd, 1996b). In this study, the development of the egg parasitoid *A. atomus* was greatly influenced by the temperature range 17 to 27°C. Development duration of both sexes decreased with increasing temperature. Males always developed faster than the females. The favourable temperature for the development of *A. atomus* was found to be in the range of 17-25°C.

The predicted lower threshold for development of the female parasitoid (9.6°C) was a little higher than its host (7.09°C), which is an important biological factor for the parasitoid species. Both male and female *A. atomus* require 200 degree days, which was 171 for its host.

The success of a predator or a parasitoid in a biological control system depends on its ability to reproduce in sufficient numbers to control the pest population. One measure of the potential success of a predator or parasitoid is its fecundity, that is the number of progeny a female can produce (Hentz *et al.*, 1998). The extrinsic factors such as temperature, humidity, photoperiod and nutrition are examples of key environmental elements that may affect the fecundity. The potential fecundity of *A. atomus* was found to decrease with an increase in the development temperature and showed a significant linear relationship ($y= -1.4406x+ 61.866$, $r^2= 0.9955$, $P<0.001$).

In general, larger parasitoids within a species have more eggs and a positive correlation between potential fecundity and the size of the parasitoid is a common phenomenon in Hymenoptera (Rosenheim and Rosen, 1992; Croft and Copland, 1993; Lampson *et al.*, 1996). A positive linear relationship was observed in the potential fecundity and the size of *A. atomus*. This finding has an important implication for foraging models, since larger females may obtain greater fitness returns per host compared with smaller females, they can also utilize a series of hosts in rapid succession (O'Neill and Skinner, 1990). The mean longevity of the adult *A. atomus* was found to decrease with increasing temperature.

Host plants were found to affect significantly the development, size and potential fecundity of *A. atomus*. Parasitoids were larger in size, more fecund and took a longer time to develop in the eggs laid on Primula making this the potential plants for commercial rearing.

12.4 Life table parameter study of *A. atomus*

The information from demographic parameters is considered essential for evaluation of the efficacy of an insect's natural enemies. In this study, temperature was

found to influence the demographic parameters of the egg parasitoid *A. atomus*. Increasing temperature from 17°C to 25°C leads to a reduction in the generation time (T), population doubling time (DT), and to an increase in the intrinsic rate of natural increase (r_m).

In nature, numerous factors such as moisture, temperature, photoperiod, natural enemies, inter- and intra-specific competition may modify the reproductive potential of a parasitoid. The present study was conducted in a controlled environment. Therefore, further study would be necessary to develop predictions of the population dynamics of *A. atomus* under different conditions, e.g. cycling temperature and different humidity levels.

In the present study, the r_m value of the parasitoid (0.14) is higher than that of the host *H. maroccana* (0.033) at the same temperature, indicating the ability of *A. atomus* to multiply rapidly under controlled conditions. These results suggest that the parasitoid *A. atomus* could be a valuable biocontrol agent for controlling *H. maroccana*.

Hulspas-Jordan and van Lenteren (1978) argued that different characteristics of the host and host plants involved in the localization and the acceptance of the host by parasitoids might influence the rate of oviposition. French bean was found the most preferred host plant by *A. atomus*. The high trichome density in Cucumber and Primula adversely affected the host finding and oviposition by *A. atomus*.

12.5 Adult longevity of *A. atomus* with different diets

The results of the present study provided useful information on the survival of adult *A. atomus* on different diets. Adults of both sexes lived longer when provided with honey solution. Therefore, the provision of supplemental foods appeared to be an important means of promoting the longevity of *A. atomus* and would thus reduce the frequency of augmentative release of the parasitoids in controlling *H. maroccana*. Honey as a dietary constituent increased the longevity of both males and females of *Colpoclypeus florus* (Walker) (Milonas and Soultani, 2000). Van Lenteren *et al.* (1987) reported that *Encarsia formosa* Gahan can survive for a prolonged period without a host when other food sources are present.

Homopteran honeydew is a potential source of adult parasitoid food and can be used as a food source by many species (Cross and Moore, 1992). Spraying of food can also extend the effectiveness of field releases of predators, for example, *Chrysoperla carnea* Stephens and *Hippodamia species* in alfalfa fields (Hagen, 1986). Kim and Morimoto (1995) reported that the longevity of *Microterys flavus* (Howard) a primary parasitoid of *Protopulvinaria mangiferae* (Green) lived the longest when fed on honey. The honey fed adult females lived for 45 days, whereas the longevity of the adult females was only 3.9 and 4.7 days when fed on water and host food. The availability and accessibility of food sources, such as nectar or honeydew in a target area, strongly affect parasitoid retention and host finding efficacy. For parasitoids to maintain high reproductive success it is important that disruption of their foraging process is minimal so that most of their time and energy can be allocated to host finding (Lewis *et al.*, 1998).

In addition to longevity when food is present, female parasitoids stay longer and parasitize more hosts than if food is not available (Lewis *et al.*, 1998). Therefore the role of food, as well as the mechanisms governing the foraging for food, should be well understood before any biological control strategy is implemented. This knowledge is necessary for designing agro-ecosystems in which optimal food is available for parasitoids and other beneficial insects. Food sources should be innately attractive to beneficial insects and/or should provide them with reliable and detectable cues which they can learn to associate with the presence of food (Jervis and Kidd, 1996b).

In natural enemies conservation systems, plants providing food sources should be planned with a crop plant. Furthermore, interplanting a crop and alternative food plants allows a more complex agricultural landscape and stimulates diversity and effectiveness of natural enemies. More work needs to be done to find a suitable formulation for supplemental food sprays for parasitoid augmentation.

12.6 Oviposition behaviour of *A. atomus*

The oviposition behaviour of insect parasitoids has been intensively studied in many parasitoids for its role in the host-parasitoid system (Bjorksten and Hoffmann, 1995; Nurindah *et al.*, 1999a, 1999b; Al-Aahaibi and Walker, 2000; Karamaouna and Copland, 2000). Godfray (1994) pointed out that after a parasitoid locates a host, it is faced with a sequence of decisions concerned with oviposition. The parasitoid may use the host for oviposition, or the host may be ignored or the host may be used for food. *Anagrus atomus* performed a sequence of intensive behaviour after encountering unparasitized and parasitized eggs of *H. maroccana* including examination (antennation and probing), ovipositor positioning, ovipositor insertion, abdominal pumping and host rejection after or without oviposition (Figure 8.1). When an egg was found suitable after examination, parasitoids tried to oviposit into it.

Among different components, parasitoids spent a very little time in host examination (7-8%). The majority of the time in successful oviposition was spent in abdominal pumping (60-64%). van Roermund and van Lenteren (1995) mentioned that host stage, host quality and the physiological state of the parasitoid may influence the female's decision to reject, oviposit, or feed on the host. The findings of the present experiment suggests that *A. atomus* could successfully discriminate between unparasitized and parasitized hosts. Females usually oviposited in unparasitized hosts and rejected the parasitized ones. They did not show abdominal pumping behaviour in previously parasitized eggs. Van Baaren *et al.* (1995) observed a great variation in abdominal pumping of *Anaphes victus* Huber a solitary egg parasitoid of Carrot weevil, *Listronotus oregonensis* (LeConte) while encountering unparasitized and parasitized hosts. The duration of abdominal pumping was always shorter in parasitized hosts. The presence or absence of abdominal pumping was described as a more robust indicator of oviposition than probe duration (Al-Wahaibi and Walker, 2000). Probe duration is likely to be

affected by the toughness and thickness of the plant tissue, the ambient temperature and other environmental factors. On the other hand, the abdominal vibrations associated with oviposition are probably a result of the muscle contractions that send the eggs down the ovipositor and would be expected to be less affected by these environmental factors (Al-Wahaibi and Walker, 2000). Since all eggs of *H. maroccana* are embedded in plant tissue this complicates the process for the parasitoid in finding the hosts. Such parasitoids probably rely heavily on olfactory and tactile signals picked up by their antennae and ovipositors. The antennae act as receivers for signals emanating from the surface while the ovipositor detects signals below the surface during probes.

An important factor affecting *A. atomus* oviposition behaviour is host experience. Experienced females spent less time in handling the hosts. Similar results were obtained with other egg parasitoids and are a consequence of learning, which modifies a parasitoids behaviour in its response to host and plant stimuli (Conti *et al.,* 1997). Previous oviposition experience reduced host handling time of *A. atomus*. Total host handling time (including: host examination, oviposition and host encounter following oviposition) was relatively shorter (124.4 s) in experienced parasitoids than in inexperienced parasitoids (156.5 s). Nurindah *et al.* (1999a, 1999b) observed a reduction in host drilling time as a result of complete oviposition experience by the females of *Trichogramma australicum*. *Anagrus atomus* did not show any behavioural differences while handling younger and older eggs although Huis *et al.* (1991) mentioned that prolonged drilling by *Uscana laripophaga* Steffan in the older eggs of the host *Callosobruchus maculatus* (Fabricius) was probably due to an increased thickness or hardness of the chorion of the developing eggs.

12.7 Thelytoky in *A. atomus*

Because of the haplo-diploid genetic system of sex determination in Hymenoptera, sex ratio in parasitoid wasps can vary considerably. Male biased sex ratios in parasitoid mass rearing are undesirable, because males produced in excess of those required for ensuring that all females are fertilized, are useless for biological control. Thus, the study of sex allocation in insect parasitoids is an important area of study not only for those interested in evolutionary ecology, but also for practitioners of biological control (van Alphen and Jervis, 1996). Generally, no direct consideration is given to sex ratio as the

characteristics of an effective natural enemy. However, sex ratio directly contributes to the intrinsic rate of increase and could indirectly contribute to several other attributes of effective natural enemies such as competitive ability, interference among parasitoids and aggregation on host patches (Hall, 1993).

Thelytokous reproduction was observed in *A. atomus* as some of the individuals were producing all female offspring without being mated. Those females were expected to be infected by some unknown microorganisms. Microbe associated thelytoky was reported in many parasitoid species (Zchori-Fein *et al.*, 1992, 1995; Arakaki *et al.*, 2000) which was successfully cured by antibiotic and high temperature treatments. The lifetable analysis showed a higher intrinsic rate of population increase and shorter population doubling time of thelytokous *A. atomus*, which suggest a higher reproductive potential of the species in biological control.

Antibiotic treatment showed a remarkable effect on male production in thelytokous *A. atomus*. Rifampicin was found to be the most effective antibiotic in the induction of males, producing 100% males followed by the two other antibiotics, ampicillin and penicillin which produced 13.7% and 14.8% males, respectively.

Heat treatments to the thelytokous *A. atomus* throughout the developmental stages, during pupal and adult stage showed some induction of males in the F_2 generation. The males produced by antibiotic treatments were able to mate with the females and found to produce viable sperms.

It is suspected that the antibiotics are passed in to the host eggs when antibiotic fed females laid eggs. The females in F_1 are now cured of the infections and hence producing male offspring in F_2. The is not yet identified. Therefore, more studies should be done to identify the microbes and to find the mechanisms involved in their cure.

12.8 Host plant effect on searching behaviour of *A. atomus*

The host plants of arthropod pests may affect parasitoids and predators directly or indirectly, through multitrophic interactions. Direct plant effects may involve simple mechanisms such as reduced parasitoid searching efficiency caused by trichomes.

Multitrophic effects often involve complex interactions that are not well understood, and their impact on natural enemies and biological control are difficult to predict (Bottrell *et al.*, 1998).

Host plant resistance and biological control are fundamental to an integrated pest management approach. Plant features very often influence foraging success and reproductive fitness of natural enemies. Spatial dispersion of plants can affect the ability of herbivores and natural enemies to locate and, in some cases, successfully colonize the plants. Plant features such as leaf toughness or hairiness, which in some cases defend plants against herbivores, may also affect natural enemies (van Driesche and Bellows, 1996). Increased trichome density on leaves is associated for some parasitoids with reduced walking speed and lowered foraging rates, making them less effective at finding hosts (Hua *et al.*, 1987). In the present study variation in trichome shape and density on the abaxial surfaces of different host plants influenced walking speeds and turning of the egg parasitoid *A. atomus*. Highest net walking speed and lowest meandering of *A. atomus* was observed on both leaf vein and leaf lamina of Sweet pepper among five host plant species. On the other hand, parasitoids walked more slowly and showed more meandering on Primula. This might be due to the high trichome density on Primula, which prevented the parasitoid walking a straight path and led to more turning.

The searching behaviour of parasitoids of herbivorous insects or phytophages, particularly information from plants in their searching process indicates the importance of tritrophy (Ananthakrishnan, 1999). Plants may therefore be considered as a dynamic component of insect-plant-natural enemy interactions. The multitrophic interaction of host plants, arthropods and natural enemies are still not well understood (Bottrell *et al.*, 1998). Therefore, knowledge of such interactions is important for conducting foreign exploration to obtain species of natural enemies adapted to attack the target pest on the target crops; for planning conservation and augmentation programmes; and for guiding changes made to crop plants through plant breeding programmes (van Driesche and Bellows, 1996).

12.9 Host discrimination and superparasitism in *A. atomus*

Host discrimination is important for parasitoids because it prevents wastage of eggs, save time and enables the parasitoid to leave a patch where most of the hosts are parasitized (Hofsvang, 1990). However, when the unparasitized hosts are unavailable or scarce, it may be advantageous for a female to superparasitize if the probability of her offspring surviving competition is greater than zero (Bakker *et al.,* 1985). Host discrimination occurs after detection of either an individual external pheromone by antennal drumming, or an internal pheromone, and is recognized by the sensilla at the tip of the ovipositor (van Baaren *et al.*, 1994).

Egg parasitoids tend to mark hosts externally, which are therefore more readily detected by females inspecting a potential host than an internal one (Nufio and Papaj, 2001). In the present study, both self and conspecific parasitoid successfully discriminated between unparasitized and parasitized eggs at different hour intervals after the previous parasitization. Self and conspecific parasitoids rejected 68% and 22%, respectively of the parasitized eggs after antennal examination 12 h after the previous parasitization. Antennal rejection decreased with the time interval since previous parasitization in both self and conspecific parasitoids. The behaviour of *A. atomus* during discrimination differs from that of *A. mutans* and *A. silwoodensis* Walker in the fact that both of the latter do not appear to mark the eggs externally and they recognize parasitized eggs only after drilling, probably because of the presence of internal markers (Moratorio, 1990). In contrast, *A. atomus* rapidly discriminates between parasitized and unparasitized eggs during external examination and rejected the marked eggs. This is in agreement with the findings of Conti *et al.* (1997) where *Anaphes iole* Girault could easily discriminate between unparasitized and parasitized eggs of *Lygus hesperus* Knight by antennal examination. Females of *Pimpla nipponica* Uchida were reported to accept the majority of conspecifically parasitized hosts as oviposition sites after external examination, while they rejected self-parasitized hosts without probing (Ueno and Tanaka, 1996).

Rejection of self-parasitized hosts usually followed a thorough external examination of the hosts but, in some cases, contacting any part of the host was enough to allow recognition of a self-parasitized host. Thus the marking pheromone seems to be a non-persistent chemical because of their declining nature of effectiveness with time. On

the other hand, probing rejection increased from 32% at 12 h to 84% at 72 h after parasitization in self-specific parasitoids. Nelson and Roitberg (1993) suggested that markers deposited within the host as a factor associated with developing eggs or larvae may also degrade over time or disappear within the haemolymph and reduce the ability of a parasitoid to discriminate. Recognition of self parasitism may also involve some memory of the previous oviposition and more work is needed on this aspect.

The present result shows that the rejection ability of the parasitoid *A. atomus* varies with the length of time interval between attacks. Therefore, it is suggested that host discriminatiion is time-dependent and appears to be induced by a marking cue (internal/external) immediately after oviposition and then by modification in host quality associated with parasitoid development. This result supports the model of Chow and Mackauer (1986), where the host discrimination in the aphid parasitoid *Ephedrus califirnicus* was induced by two different stimuli, which act independently. During the early phase of parasitism, the reaction was to an external marker, possibly a pheromone, in which the deterent effect of the marker was greatest shortly after oviposition and declines with the time interval after the previous parasitism. During the late phase of parasitism, the reaction to change in host quality was caused by the developing parasitoid's egg or larva.

Several theoretical and empirical works have shown that superparasitism may be an adaptive and positive reproductive strategy of parasitoids under certain circumstances when unparasitized hosts are scarce (Weisser and Houston, 1993; Godfray, 1994). Adaptive superparasitism theory does not assume that a female parasitoid failed to recognize parasitized hosts. Recognition of prior parasitism is viewed to be always advantageous, but it does not necessarily result in avoidance of superparasitism. The theory considers that a female parasitoid should decide after recognition and prior to parasitism of a host to superparasitize or not, based on the relative benefits and costs associated with rejecting versus accepting that host for oviposition. Thus discrimination of whether or not female parasitoids recognize prior parasitism when they superparasitize is crucial in considering whether superparasitism is their positive strategy (Ueno, 1999).

This behaviour of discrimination differs among experienced and inexperienced parasitoids. Inexperienced females generally accept parasitized hosts, while under the

same experimental conditions experienced females reject them, and the gained fitness by superparasitism is different for the two types of females (van Alphen *et al.* 1987). These authors also concluded that it is adaptive for inexperienced females to accept previously parasitized hosts, because inexperienced females lack information about, for instance, host density and competing female density, and hence it is the best strategy for them to superparasitize. On the other hand, Henneman (1995) suggested that while discrimination against parasitized hosts does not require prior experience with unparasitized hosts, it was not possible to conclude that recognition of parasitized hosts was even partly innate. At the beginning of an oviposition sequence by *Anaphes victus,* an egg parasitoid of *Listronotus oregonensis*, females reject eggs after insertion of the ovipositor but, after some encounters with parasitized hosts, they start rejecting them after antennal drumming (van Barren and Boivin, 1998).

Ovicide is known in several parasitoid species and these parasitoids kill conspecific eggs by stinging them with the ovipositor (Price, 1970; Strand and Godfray, 1989; Hardy and Blackburn, 1991). Ovicide followed by oviposition may be considered as a special case of superparasitism in which a female increases the probability of her offspring's survival by killing a previous female's eggs. It may only evolve when parasitoid females can discriminate between parasitized and unparasitized hosts, and find and kill previously laid eggs (Netting and Hunter, 2000). Perhaps due to constraints on the ability of parasitoids to find previously laid eggs, the occurrence of ovicide is far less well documented than superparasitism and has been mentioned as an ovipositional strategy in only a few parasitoids. However, the present study suggests that when females attacked parasitized hosts, many of them inserted the ovipositor into the host more than once during a single host encounter. Some females drilled only once and then rejected or accepted parasitized hosts for oviposition, but the others drilled into hosts repeatedly and then rejected or accepted parasitized hosts for oviposition, which was more common in conspecific parasitoids.

The self-specific parasitoid of *A. atomus* did not show any ovicidal action but the conspecific parasitoid did ovicide in only 4% of the hosts. In contrast, *E. formosa* Gahan could not discriminate between self-parasitized and conspecific parasitized hosts which resulted in the death of their own offspring (Netting and Hunter, 2000). The occurrence of ovicide among endoparasitoids may be more widespread than the present observations.

However, Mayhew (1997) speculated that ovicide should be favoured in ectoparasitoids with large, easily located eggs that can be found quickly and killed.

12.10 General Conclusion

Selection of natural enemies to be mass reared for biological control is a complex issue. Often, the natural enemies most likely to be selected for these biological control programmes are those that can be easily mass-produced (Heinz and Parrela, 1994). Moreover, the awareness of the involvement of the host plant, insect and natural enemies in effective and sustainable pest management strategies has revolutionized the concept of biological control of phytophagus insects. Therefore, it is essential to know more about the population dynamics of the pest and the natural enemy in relation to temperature and host plants (Drost *et al.*, 1998).

The present studies investigated the influence of temperature and host plants on different aspects of developmental biology of *H. maroccana* and its egg parasitoid *A. atomus*. Although the data obtained in the laboratory are not necessarily directly applicable to the field situation, they represent the range for developmental time in the field. In nature, the population dynamics of an insect in the field is dependent on many factors such as optimal weather, presence of natural enemies, intra- or interspecific competition, presence of food and oviposition site. However, the results on developmental rate, threshold temperature and day degree required for completing a generation obtained in this study should be useful in predicting the field developmental time and dynamics of the parasitoid. Thelytoky was observed in *A. atomus* for the first time, which may increase the potential of the parasitoid as a biocontrol agent. These investigations will enable us to evaluate the potential of *A. atomus* for future field release and will also provide vital information for rearing this parasitoid in the laboratory, a critical step in any field release programme.

Knowledge of these parameters in conjunction with demographic analyses would help to develop population models that can provide a much-needed theoretical framework for biological control of Glasshouse leafhopper. In addition, research efforts should be continued to develop new and innovative approaches to biological control, including the manipulation of natural enemies, their behaviour through semiochemicals and the development of improved rearing techniques of mass production and release.

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Appendix

Appendix 1: Weight gained by *Hauptidia maroccana* during nymphal development on different host plants at different constant temperatures.

Host plants	Weight gained (pooled sex) (mg/mg/day) mean±SE (n)				One-way ANOVA
	17°C	21°C	25°C	27°C	
Cucumber	0.566±0.012 ab A (17)	0.529±0.009 b B (15)	0.443±0.013 b C (15)	0.475±0.012 a C (10)	df=53 *P*<0.001 LSD=0.0346
Tomato	0.591±0.018 a A (19)	0.485±0.015 c BC (12)	0.505±0.010 a B (20)	0.450±0.015 b C (12)	df=59 *P*<0.001 LSD=0.0441
French Bean	0.599±0.020 a A (18)	0.522±0.011 b B (12)	0.522±0.014 a B (11)	0.451±0.014 ab C (14)	df=51 *P*<0.001 LSD=0.0478
Primula	0.538±0.012 b A (18)	0.531±0.009 b A (17)	0.447±0.012 b B (17)	0.452±0.011 ab B (18)	df=66 *P*<0.001 LSD=0.032
Sweet pepper	0.478±0.008 c B (18)	0.577±0.012 a A (20)	0.452±0.009 b BC (10)	0.429±0.014 b C (19)	Df=64 *P*<0.001 LSD=0.0341
One-way ANOVA	df=85 *P*<0.001 LSD=0.0415	df=72 *P*<0.001 LSD=0.0431	df=68 *P*<0.001 LSD=0.0355	df=31 *P*=0.25 LSD=0.0397	

Means in columns, followed by the same lowercase and in rows followed by the same uppercase letters are not significantly different from each other at $P \leq 0.05$. One-way ANOVA followed by Least Significant Difference test.
n = no. of insects as replicates

Appendix 2: Data format used in a QBASIC programme (Jervis and Copland, 1996) to calculate life table parameters of the glasshouse leafhopper *Hauptidia maroccna*. The number of days for which data has been obtained is typed in line 290. Lines 300 onwards contain mean values (separated by commas) of the maternal age (x), age specific survival (lx) and age specific fecundity (mx).

```
10 REM program to calculate life table parameters (MJWC 1994)
15 CLS
20 DIM x (100), k (100), m (100): REM dimension arrays (n<100)
30 READ n
35 FOR h = 1 TO n: READ x(h), k(h), m(h): NEXT h: REM  read data
40 PRINT "calculating data"
50 ct = 0: mx = 0: ro = 0: j = 1: REM set variables
60 REM ---------- cumulative calculations
70 FOR h = 1 TO n
80 ct = ct + x(h) * k(h) * m(h): mx = mx + m(h): ro = ro + k(h) * m(h)
90 NEXT h
100 rc = LOG (ro) / (ct / ro): rm = rc: REM calculate rc
110 crm = 0: FOR h = 1 TO n
115 crm = crm + k (h) * m (h) * EXP (-x (h) * rm): NEXT h
120 REM ---------- iterative substitution for rm
130 IF ABS (1 - crm) < .00001 THEN GOTO 180: REM accurate to 4 dec points
140 IF crm > 1 THEN rm = rm + ABS (1 - crm) / x(1)
150 IF crm < 1 THEN rm = rm – ABS (1 - crm) / x(1)
160 PRINT j: j = j + 1: GOTO 110
170 REM ---------- display results
180 PRINT "gross reproductive rate     (GRR)   ="; mx
190 PRINT "net reproductive rate       (Ro)    ="; ro
200 PRINT "capacity for increase       (rc)    ="; rc
210 PRINT "intrinsic rate of increase  (rm)    ="; rm
220 PRINT "cohort generation time      (Tc)    ="; ct / ro
230 PRINT "generation time             (T)     ="; LOG(ro) / rm
240 PRINT "finite capacity for increase (lambda) ="; EXP(rm)
250 PRINT "doubling time               (DT)    ="; LOG(2) / rm
260 END
270 REM
280 REM ---------- data
290 DATA 7:              REM number of days data
300 DATA 44.73, .85, 2.01: REM x (day), lx (survival), mx (fecundity)
310 DATA 49.73, .85, 1.86: REM                  female offspring
320 DATA 54.73, .85, 1.02:
330 DATA 59.73, .73, 0.93:
340 DATA 64.73, .61, 0.94:
350 DATA 69.73, .49, 0.18:
360 DATA 74.73, .24, 0.35:
```

Result:

Gross reproductive rate	= 7.2899
Net Reproductive rate	= 5.581
Capacity of increase	= 0.0327
Intrinsic rate of increase	= 0.0334
Cohort generation time	= 52.4260
Generation time	= 51.5680
Finite capacity for increase	= 1.0339
Doubling time	= 20.7892

Appendix 3. Data format used in a QBASIC programme (Jervis and Copland, 1996)to calculate life table parameters of the egg *parasitoid Anagrus atomus* on the egg of glasshouse leafhopper *Hauptidia maroccana*. The number of days for which data has been obtained are typed in line 290. Lines 300 onwards contain mean values (separated by commas) of the maternal age (x), age specific survival (lx) and age specific fecundity (mx).

3.1 Life table analysis at 17°C using Primula as host plant

```
10 REM program to calculate life table parameters (MJWC 1994)
15 CLS
20 DIM x(100), k(100), m(100):          REM dimension arrays (n<100)
30 READ n
35 FOR h = 1 TO n: READ x(h), k(h), m(h): NEXT h: REM  read data
40 PRINT "calculating data"
50 ct = 0: mx = 0: ro = 0: j = 1:       REM set variables
60 REM ---------------------------------- cumulative calculations
70 FOR h = 1 TO n
80 ct = ct + x(h) * k(h) * m(h): mx = mx + m(h): ro = ro + k(h) * m(h)
90 NEXT h
100 rc = LOG(ro) / (ct / ro): rm = rc:          REM calculate rc
110 crm = 0: FOR h = 1 TO n
115 crm = crm + k(h) * m(h) * EXP(-x(h) * rm): NEXT h
120 REM ---------------------------------- iterative substitution for rm
130 IF ABS(1 - crm) < .00001 THEN GOTO 180: REM accurate to 4 dec points
140 IF crm > 1 THEN rm = rm + ABS(1 - crm) / x(1)
150 IF crm < 1 THEN rm = rm - ABS(1 - crm) / x(1)
160 PRINT j: j = j + 1: GOTO 110
170 REM ---------------------------------- display results
180 PRINT "gross reproductive rate     (GRR)   ="; mx
190 PRINT "net reproductive rate       (Ro)    ="; ro
200 PRINT "capacity for increase       (rc)    ="; rc
210 PRINT "intrinsic rate of increase  (rm)    ="; rm
220 PRINT "cohort generation time      (Tc)    ="; ct / ro
230 PRINT "generation time             (T)     ="; LOG(ro) / rm
240 PRINT "finite capacity for increase (lambda) ="; EXP(rm)
250 PRINT "doubling time               (DT)    ="; LOG(2) / rm
260 END
270 REM
280 REM ---------------------------------- data
290 DATA 7:             REM number of days data
300 DATA 26.1, 1, 8.96: REM x (day), lx (survival), mx (fecundity),
310 DATA 27.1, 1, 8.64: REM                 female offspring
320 DATA 28.1, 1, 4.40:
330 DATA 29.1, 1, 1.89:
340 DATA 30.1, 1, 2.20:
350 DATA 31.1, 0.4, 1.96:
360 DATA 32.1, 0.2, 0:
```

Result:

Gross reproductive rate	= 28.05
Net Reproductive rate	= 26.874
Capacity of increase	= 0.1199
Intrinsic rate of increase	= 0.1204
Cohort generation time	= 27.4333
Generation time	= 27.3267
Finite capacity for increase	= 1.1279
Doubling time	= 5.7553

3.2 Life table analysis at 21°C using Primula as host plant

```
10 REM program to calculate life table parameters (MJWC 1994)
15 CLS
20 DIM x(100), k(100), m(100):          REM dimension arrays (n<100)
30 READ n
35 FOR h = 1 TO n: READ x(h), k(h), m(h): NEXT h: REM  read data
40 PRINT "calculating data"
50 ct = 0: mx = 0: ro = 0: j = 1:       REM set variables
60 REM ---------------------------------- cumulative calculations
70 FOR h = 1 TO n
80 ct = ct + x(h) * k(h) * m(h): mx = mx + m(h): ro = ro + k(h) * m(h)
90 NEXT h
100 rc = LOG(ro) / (ct / ro): rm = rc:          REM calculate rc
110 crm = 0: FOR h = 1 TO n
115 crm = crm + k(h) * m(h) * EXP(-x(h) * rm): NEXT h
120 REM ---------------------------------- iterative substitution for rm
130 IF ABS(1 - crm) < .00001 THEN GOTO 180: REM accurate to 4 dec points
140 IF crm > 1 THEN rm = rm + ABS(1 - crm) / x(1)
150 IF crm < 1 THEN rm = rm - ABS(1 - crm) / x(1)
160 PRINT j: j = j + 1: GOTO 110
170 REM ---------------------------------- display results
180 PRINT "gross reproductive rate     (GRR)   ="; mx
190 PRINT "net reproductive rate       (Ro)    ="; ro
200 PRINT "capacity for increase       (rc)    ="; rc
210 PRINT "intrinsic rate of increase  (rm)    ="; rm
220 PRINT "cohort generation time      (Tc)    ="; ct / ro
230 PRINT "generation time             (T)     ="; LOG(ro) / rm
240 PRINT "finite capacity for increase (lambda) ="; EXP(rm)
250 PRINT "doubling time               (DT)    ="; LOG(2) / rm
260 END
270 REM
280 REM ---------------------------------- data
290 DATA 6:             REM number of days data
300 DATA 18.44, 1, 6.58: REM x (day), lx (survival), mx (fecundity),
310 DATA 19.44, 1, 5.65: REM                female offspring
320 DATA 20.44, 1, 1.03:
330 DATA 21.44, 1, 1.5:
340 DATA 22.44, 1, 1.6:
350 DATA 23.44, .8, 0:
```

Result:

Gross reproductive rate	= 16.36
Net Reproductive rate	= 15.36
Capacity of increase	= 0.1409
Intrinsic rate of increase	= 0.1415
Cohort generation time	= 19.3912
Generation time	= 19.3092
Finite capacity for increase	= 1.1519
Doubling time	= 4.8994

3.3 Life table analysis at 25°C using Primula as host plant

```
10 REM program to calculate life table parameters (MJWC 1994)
15 CLS
20 DIM x(100), k(100), m(100):          REM dimension arrays (n<100)
30 READ n
35 FOR h = 1 TO n: READ x(h), k(h), m(h): NEXT h: REM  read data
40 PRINT "calculating data"
50 ct = 0: mx = 0: ro = 0: j = 1:       REM set variables
60 REM ---------------------------------- cumulative calculations
70 FOR h = 1 TO n
80 ct = ct + x(h) * k(h) * m(h): mx = mx + m(h): ro = ro + k(h) * m(h)
90 NEXT h
100 rc = LOG(ro) / (ct / ro): rm = rc:          REM calculate rc
110 crm = 0: FOR h = 1 TO n
115 crm = crm + k(h) * m(h) * EXP(-x(h) * rm): NEXT h
120 REM ---------------------------------- iterative substitution for rm
130 IF ABS(1 - crm) < .00001 THEN GOTO 180: REM accurate to 4 dec points
140 IF crm > 1 THEN rm = rm + ABS(1 - crm) / x(1)
150 IF crm < 1 THEN rm = rm - ABS(1 - crm) / x(1)
160 PRINT j: j = j + 1: GOTO 110
170 REM ---------------------------------- display results
180 PRINT "gross reproductive rate     (GRR)   ="; mx
190 PRINT "net reproductive rate       (Ro)    ="; ro
200 PRINT "capacity for increase       (rc)    ="; rc
210 PRINT "intrinsic rate of increase  (rm)    ="; rm
220 PRINT "cohort generation time      (Tc)    ="; ct / ro
230 PRINT "generation time             (T)     ="; LOG(ro) / rm
240 PRINT "finite capacity for increase (lambda) ="; EXP(rm)
250 PRINT "doubling time               (DT)    ="; LOG(2) / rm
260 END
270 REM
280 REM ---------------------------------- data
290 DATA 4:             REM number of days data
300 DATA 12.7, 1, 9.52: REM x (day), lx (survival), mx (fecundity),
310 DATA 13.7, 1, 7.96: REM                 female offspring
320 DATA 14.7, 1, 2.02:
330 DATA 15.7, 0.8, 0:
```

Result:

Gross reproductive rate	= 19.5
Net Reproductive rate	= 19.5
Capacity of increase	= 0.2231
Intrinsic rate of increase	= 0.2239
Cohort generation time	= 13.3154
Generation time	= 13.2673
Finite capacity for increase	= 1.2509
Doubling time	= 3.6959

3.4 Life table analysis at 21°C using French bean as host plant

```
10 REM program to calculate life table parameters (MJWC 1994)
15 CLS
20 DIM x(100), k(100), m(100):          REM dimension arrays (n<100)
30 READ n
35 FOR h = 1 TO n: READ x(h), k(h), m(h): NEXT h: REM  read data
40 PRINT "calculating data"
50 ct = 0: mx = 0: ro = 0: j = 1:       REM set variables
60 REM ---------------------------------- cumulative calculations
70 FOR h = 1 TO n
80 ct = ct + x(h) * k(h) * m(h): mx = mx + m(h): ro = ro + k(h) * m(h)
90 NEXT h
100 rc = LOG(ro) / (ct / ro): rm = rc:          REM calculate rc
110 crm = 0: FOR h = 1 TO n
115 crm = crm + k(h) * m(h) * EXP(-x(h) * rm): NEXT h
120 REM ---------------------------------- iterative substitution for rm
130 IF ABS(1 - crm) < .00001 THEN GOTO 180: REM accurate to 4 dec points
140 IF crm > 1 THEN rm = rm + ABS(1 - crm) / x(1)
150 IF crm < 1 THEN rm = rm - ABS(1 - crm) / x(1)
160 PRINT j: j = j + 1: GOTO 110
170 REM ---------------------------------- display results
180 PRINT "gross reproductive rate     (GRR)   ="; mx
190 PRINT "net reproductive rate       (Ro)    ="; ro
200 PRINT "capacity for increase       (rc)    ="; rc
210 PRINT "intrinsic rate of increase  (rm)    ="; rm
220 PRINT "cohort generation time      (Tc)    ="; ct / ro
230 PRINT "generation time             (T)     ="; LOG(ro) / rm
240 PRINT "finite capacity for increase (lambda) ="; EXP(rm)
250 PRINT "doubling time               (DT)    ="; LOG(2) / rm
260 END
270 REM
280 REM ---------------------------------- data
290 DATA 7:             REM number of days data
300 DATA 17.25, 1, 9.88: REM x (day), lx (survival), mx (fecundity),
310 DATA 18.25, 1, 7.42: REM                female offspring
320 DATA 19.25, 1, 3.55:
330 DATA 20.25, 1, 3.2:
340 DATA 21.25, 1, 3.68:
350 DATA 22.25, 0.8, 0:
360 DATA 23.25, 0.6, 0:
```

Result:

Gross reproductive rate	= 27.73
Net Reproductive rate	= 27.73
Capacity of increase	= 0.1781
Intrinsic rate of increase	= 0.1798
Cohort generation time	= 18.6507
Generation time	= 18.4832
Finite capacity for increase	= 1.1969
Doubling time	= 3.8559

3.5 Life table analysis at 21°C using Cucumber as host plant

```
10 REM program to calculate life table parameters (MJWC 1994)
15 CLS
20 DIM x(100), k(100), m(100):          REM dimension arrays (n<100)
30 READ n
35 FOR h = 1 TO n: READ x(h), k(h), m(h): NEXT h: REM  read data
40 PRINT "calculating data"
50 ct = 0: mx = 0: ro = 0: j = 1:       REM set variables
60 REM ---------------------------------- cumulative calculations
70 FOR h = 1 TO n
80 ct = ct + x(h) * k(h) * m(h): mx = mx + m(h): ro = ro + k(h) * m(h)
90 NEXT h
100 rc = LOG(ro) / (ct / ro): rm = rc:          REM calculate rc
110 crm = 0: FOR h = 1 TO n
115 crm = crm + k(h) * m(h) * EXP(-x(h) * rm): NEXT h
120 REM ---------------------------------- iterative substitution for rm
130 IF ABS(1 - crm) < .00001 THEN GOTO 180: REM accurate to 4 dec points
140 IF crm > 1 THEN rm = rm + ABS(1 - crm) / x(1)
150 IF crm < 1 THEN rm = rm - ABS(1 - crm) / x(1)
160 PRINT j: j = j + 1: GOTO 110
170 REM ---------------------------------- display results
180 PRINT "gross reproductive rate     (GRR)   ="; mx
190 PRINT "net reproductive rate       (Ro)    ="; ro
200 PRINT "capacity for increase       (rc)    ="; rc
210 PRINT "intrinsic rate of increase  (rm)    ="; rm
220 PRINT "cohort generation time      (Tc)    ="; ct / ro
230 PRINT "generation time             (T)     ="; LOG(ro) / rm
240 PRINT "finite capacity for increase (lambda) ="; EXP(rm)
250 PRINT "doubling time               (DT)    ="; LOG(2) / rm
260 END
270 REM
280 REM ---------------------------------- data
290 DATA 5:             REM number of days data
300 DATA 15.35, 1, 12.17: REM x (day), lx (survival), mx (fecundity),
310 DATA 16.35, 1, 8.12: REM                  female offspring
320 DATA 17.35, 1, 1.96:
330 DATA 18.35, 1, .93:
340 DATA 19.35, 0.4, 0
```

Result:

Gross reproductive rate	= 23.18
Net Reproductive rate	= 23.18
Capacity of increase	= 0.1983
Intrinsic rate of increase	= 0.1991
Cohort generation time	= 15.9742
Generation time	= 15.9150
Finite capacity for increase	= 1.2203
Doubling time	= 3.4821