Introduction of *Aphidius rosae* into Australia for Biological Control of the Rose Aphid (*Macrosiphum rosae*)

By

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Mehr und mehr mußte er Abschied nehmen vom Traum,
von dem Gefühl und Genuß der unendlichen Möglichkeiten,
der tausendfältigen Zukunft ...
... stattdessen eine kleine, fordernde Wirklichkeit.

H. Hesse (Glasperlenspiel)

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where reference has been made in the text.

This thesis may be made available for loan or photocopying provided that an acknowledgment is made in instance of any reference to this work.

Jörg Kitt, January 1996

TO MY PARENTS AND FAMILY

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Summary

Macrosiphum rosae (L.) was introduced into Australia as a result of European settlement and can be considered the most serious insect pest of roses. Some native predators feed on *M. rosae*, but an effective parasitoid guild of this aphid was missing in Australia. The most promising control agent *Aphidius rosae* was introduced into South-Eastern Australia to improve biological control of rose aphids.

To improve rearing conditions and to gain a better understanding of the performance of *A. rosae* in the field, investigation of some parameters of life-history were undertaken. With increasing instar at time of parasitization, aphids became more suitable for parasitoid growth, with an overall increase in parasitoid size and fecundity from L1 to L4 and a decrease in developmental time. Host quality was dependent on aphid instar at time of parasitization. Poor plant quality and hyperparasitism had a negative effect on parasitoid growth. It can be concluded that growth of *A. rosae* was greatly susceptible to changes in nutritional quality of its host. *A. rosae* was able to exploit the whole range of instars and adults. The sex-ratio was male-biased in less suitable hosts.

Life-time fecundity increased with parasitoid size. The net life-time fecundity ranged between 144 and 790 at 18°C and increased with the size of females. The intrinsic rate of increase of *A. rosae* ranged from 0.258 to 0.323 females/female/day, compared to an average intrinsic rate of 0.319 for *M. rosae*.

Discrete steps in the process of host selection were studied to assess the host specificity of *A. rosae*. In a wind tunnel, females were strongly attracted only to roses when given a choice between uninfested roses and other plants. In a two-choice test females were not able to distinguish between aphid-infested rose shoots and uninfested rose shoots when shoots were 30 cm apart but they preferred to land on infested buds

when shoots were only 5 to 10 cm apart. Results suggest that aphid-related volatiles involved in host location are detectable by the wasp only over a short distance. A range of aphid species was exposed to *A. rosae* in choice and no-choice conditions in petri-dishes. The only aphid species parasitized, other than *M. rosae*, was *Macrosiphum euphorbiae* Thomas but oviposition did not result in offspring. *A. rosae* was considered specific to the rose aphid under given circumstances in Australia and negative environmental impacts were considered as almost non-existent.

Initial field observations were carried out to obtain a basic impression of the foraging behaviour of *A. rosae* in a patch. The wasps displayed significant differences in their activities when observations in the morning were compared with observations in the afternoon. *A. rosae* females showed higher attack rates in the morning compared to individuals observed in the afternoon. Parasitoids attacked only the edges of aphid colonies.

A. rosae established in most places released. At the release site in Adelaide, South Australia, the spread of a small population of parasitoids was monitored monthly. Seven months after the first release *A. rosae* inhabited an area of approximately 200 km^2 and was found as far as 18 km away from the nearest release site. The density of mummies decreased with increased distance from the point of release, but the spread was not random. Parasitoids spread mainly in the direction of prevailing winds.

The release of *A. rosae* was used to test the hypothesis that a threshold of about 1000 insects released at a single site and time is needed for establishment. 16, 64, 256 and 1024 mummies of *A. rosae* were released in eight cities throughout Victoria. Six months after release, recoveries were made in three sites in where 64, 256 and 1024 mummies were released. After one year, mummies were found even at a place where only 16 mummies were released.

Over a period of two years after initial release, the changes in numbers of host and parasitoid were monitored in a rose field. Before the release of *A. rosae*, spring was

the season when *M. rosae* was most abundant as have been recorded previously. After the release, *M. rosae* did not reach the same high infestations levels as have been recorded previously in spring. High rates of parasitism of nearly 80 % in spring suggested that *A. rosae* may have been the main natural enemy in regulating aphid numbers.

During summer *M. rosae* and *A. rosae* virtually disappeared from roses in Adelaide. During autumn aphids reached high numbers and *A. rosae* did not have any significant impact on its host. This ineffectiveness of *A. rosae* in autumn was also demonstrated in an experiment using insecticide-treated exclusion cages. No significant change in aphid numbers was found when natural enemies were excluded from roses.

Aphid infestations sampled in different rose plots varied considerably when wider surveys were undertaken.

Introduction to thesis

The rose aphid *Macrosiphum rosae* (L.) is the most serious pest of roses in South Australia. Up to 1993, nurseries and home gardeners depended exclusively on insecticides to control this introduced pest, since native predators did not regulate numbers of rose aphids. In Europe, America and Eurasia, populations of *M. rosae* are attacked by a number of parasitoid species. The most common species is the Aphidiinae wasp *Aphidius rosae* Haliday. This thesis deals with the introduction of *A. rosae* as a classical biological control agent into South-Eastern Australia.

Aims. The ultimate aim of this project was to establish *A. rosae* in South-Eastern Australia to improve biological control of the target pest *M. rosae*. Appropriate host specificity tests had to be carried out to ensure environmental safety of the control agent and to achieve clearance for release into the field from the Australian Quarantine and Inspection Service and the National Park and Wildlife Service. Except for host references, little was known on the biology of *A. rosae*. Laboratory experiments were carried out to gain a better understanding of the interactions between host and parasitoid in the field. In the field, I wanted to work on some fundamental questions in biological control regarding establishment and spread of released insects to provide a contribution in understanding broader aspects of biological control. Furthermore, it was aimed to monitor the performance of the control agent in the Adelaide environment for the first two years after initial release. Finally it was planned to invent an experimental field technique to estimate the impact of the control agent on rose aphid populations.

The framework of this study was partly outlined by financial realities. Commercial funding bodies were not willing to invest in this project. Over a period of four years this project was undertaken with estimated total running costs of $A 6000,-. On the other

hand this created academic freedom without concerns about immediate control of rose aphids.

Overview. Since this thesis deals with a wide range of topics, the relevant literature is reviewed in the appropriate chapter to improve the continuity of the text. All theoretical investigations of the target pest, resulting prospects for classical biological control (CBC) and the choice of the control agent are considered in Chapter 2. All further theory regarding the actual practical procedures of CBC (collection, rearing, host specificity tests, release, monitoring and evaluation of impact) are dealt with in subsequent chapters. Chapter 3 describes the collection of *A. rosae* and general rearing methods. In Chapter 4 the influences of different host instars and adults of *M. rosae* on its parasitoid *A. rosae* are investigated. The additional stress of superparasitism and low host-plant quality are also tested. Comparisons are made between the intrinsic rate of increase of *M. rosae* and its parasitoid *A. rosae* in Chapter 5. The influence of parasitoid size on the life-time fecundity and intrinsic rate of increase of *A. rosae* is investigated. Additionally the impact of aphid cornicle wax on parasitoids is described. Chapter 6 deals with host specificity tests and the host selection process of *A. rosae*. Chapter 7 deals with quantitative aspects of foraging. Field observations of the foraging behaviour of *A. rosae* in a patch are described and results are discussed in regard to biological control of *M. rosae*. The relation of insect numbers released to success in establishment is investigated in Chapter 8. The spread of *A. rosae* in Adelaide following colonisation is described in Chapter 9. Chapter 10 is a descriptive survey of changes in numbers of *M. rosae* and *A. rosae* in the field for the first two years following the initial release of the control agent. Together with Chapter 9 it provides an indication of the initial performance of *A. rosae* in South Australia. Chapter 11 deals with the development of an exclusion method to estimate the net impact of natural enemies on rose aphids in the field. Different field techniques to assess the effectiveness of natural enemies are introduced, and the choice of the most appropriate method is discussed. The outcome of

the experiment is discussed in relation to the aphid infestation in the field at the time of the experiment. An integrating general discussion is presented as the final Chapter 12.

This thesis was written with the intention that experimental chapters basically could stand alone and serve as the basis of separate papers for future publications. At time of submission of this thesis, Chapter 6, 8 and 9 were submitted to journals and were in review.

Preliminary consideration of *Aphidius rosae* as control agent of *Macrosiphum rosae*

2.1 Classical biological control

Classical biological control (CBC) can be described as the attempt to regulate pest populations (e.g. mites, insects, mammals, weed, pathogens) by natural enemies (e.g. parasites, predators, pathogens) that are imported and released into a new environment for this purpose (Caltagirone, 1981).

The modern history of CBC began in 1888 with the outstanding success of the introduction of the predatory Vedalia beetle, *Rodolia cardinalis* (Mulsant), from Adelaide, South Australia (S.A.), into California to control the exotic cottony cushion scale, *Icerya purchasi* Maskell (Clausen, 1978). This success played a major part in stimulating world-wide interest in CBC. The first successful introduction of a parasitic wasp occurred in 1906 when *Encarsia berlesei* (Howard) of North American origin was imported into Italy to control the mulberry scale *Pseudaulacaspis pentagona* Targioni-Tozetti (Greathead, 1986). The most spectacular CBC success by an insect in Australia was the introduction of the pyralid moth *Cactoblastis cactorum* (Berg) in 1925 to control the exotic prickly pear cactus, *Opuntia stricta* Haw. (Wilson, 1960).

The underlying basis of CBC is that some populations can be regulated effectively by natural enemies. Exotic pest species often escape this regulation by geographic separation from their natural enemies (Van den Bosch & Messenger, 1973). In contrast to these coevolved old pest/natural enemy associations, which are basically transferred to a new geographic location, Hokkanen & Pimental (1984) advocated the use of 'New Associations in CBC' in which exotic natural enemies of related pest species are used for control. They hypothesised that the long coexistence of a pest and natural enemy

might lead to loss of 'virulence' and that a new natural enemy might have a greater impact on the target pest, which might be exotic or native. However, other authors disagree with this controversial hypothesis (see below).

Successful CBC combines permanency, selectivity, environmental safety and economy in pest management (DeBach, 1974) and can be considered a cornerstone in Integrated Pest Management (IPM). Data from Huffaker et al. (1976) showed a return of $US 30 for each Dollar spent in biological control (BC) of agricultural pests in California. For Australia alone, the economic benefits of BC was estimated to exceed more than A$ 1 billion (Tisdell, 1990). Despite these benefits, CBC is a minor method in terms of usage compared to the use of pesticides. The primary reason for this relative neglect is that CBC is widely perceived as unreliable (Beirne, 1985). Hall & Ehler (1979) showed that the overall rate of establishment of natural enemies of insects and arachnids worldwide was 0.34 and only 60 % of these have resulted in some kind of control. Slightly more optimistic data from Waage (1990) suggested a rate of establishment of 0.39 but only 44 % of these produced some success in reducing target pests. Particularly disturbing is that the rate of establishment of natural enemies in CBC is declining (Hall & Ehler, 1979). The second major reason to feel restrained in the development of CBC is the concern of unwanted impact of the control agent on non target species and the environment (e.g. Howarth, 1991).

Unfortunately many CBC attempts are poorly described in the literature. Only a minority have been comprehensively documented, so it is difficult to evaluate the characteristics that lead to either success or failure (Hughes, 1989). To overcome this obstacle, scientists have tried to identify trends in success or failure of CBC by broad statistical analysis of existing data (e.g. Hall & Ehler, 1979; Hall et al., 1980; Greathead, 1986; Kfir, 1993; Hopper & Roush, 1993). Many theoretical ecologists suggest that models of population dynamics can provide guidance on how to best implement BC since BC can be considered as an application of the principles of population ecology (e.g. Kareiva, 1990). In contrast, many practical BC workers share the view that theoretical approaches often have little to offer for applied entomology.

2.2 Biology of target species in CBC, with respect to *Macrosiphum rosae*

General. Gathering as much information on the pest as possible is an essential starting point. This process begins with correct species identification. Misclassification of California red scale, *Aonidiella aurantii* (Maskell), as a species of *Chrysomphalus*, a genus that originated in South Africa, misdirected the collection of natural enemies towards this continent during 1934/35 (Delucchi et al., 1976). It was not until correct identification in 1937 that researchers were able to look for effective control agents in its real area of origin, the Far East (Compere, 1969). A knowledge of basic biology and ecology of the pest in the target area is essential to proceed further in a CBC program. Information on target pests might be obtained by an extensive literature review and/or by experimental approaches and field investigations.

Host plant of Macrosiphum rosae. Species of *Rosa* have been modified through selection and hybridisation in cultivation in many cultures, giving rise to some 20 000 cultivars (Bailey & Bailey, 1976). However, the number of species is relatively small, probably comprising no more than 150 species (Bean, 1980). Essentially hybridisation has brought together the genomes of diploid eastern 'Tea' roses and tetraploid western 'Cabbage' roses, resulting in modern roses which are used for display and cutting. As hybridisation has advanced, the system of cultivar classification has become complicated and inexact. It is now nearly impossible to classify roses accurately. Rose aphids feed on virtually all cultivars, even though suitability might varies.

Macrosiphum rosae. The rose aphid *Macrosiphum rosae* (L.) belongs to the tribe Macrosiphini in the sub-family Aphidinae (Homoptera). The evolutionary radiation of this aphid line is linked closely with that of Rosaceae (Blackman & Eastop, 1985). The rose aphid is a rather large aphid species and sizes can vary between 1.7 and 3.6 mm for apterae and 2.2 to 3.4 mm in alatae. Colours range from dark green to pink, red brown

or magenta. The green and brown morphs are the most prominent forms and one may change to the other over at least two generations in the field. Pink is often the intermediate colour during this changeover (Maelzer, 1977). In the field, adults of *M. rosae* are easily distinguished from other species on roses by their general appearance combined with long and deep shiny black siphunculi and black knees.

In temperate regions the aphid displays a heteroecious holocyclic life-cycle. In spring and autumn the rose aphid reproduces on its primary host, wild and cultivated *Rosa spp.*, but during summer its alternates to various species from Dipsacaceae (*Dipsacus*, *Succisa*) and Valerianaceae (*Centranthus*, *Valeriana*) (Borner & Heinze, 1957). Occasionally the species can be found on other species of Rosaceae (*Fragaria, Geum, Pyrus, Malus, Rubus*) and Onagraceae (*Chamaenerion, Epilobium*) during summer (Blackman & Eastop, 1985). However, this life-cycle is not obligatory and depends upon environmental conditions (Borner & Heinze, 1957). Some parts of the population may remain on roses throughout summer, but still produce sexuales in autumn. In regions with mild winters, reproduction of *M. rosae* is mainly completely anholocyclic on roses, e.g. in S. A. (Maelzer, 1977) . In these cases the aphids reproduce parthenogenetically and viviparously all the year round on roses. Sexuals and eggs are not produced (Maelzer, 1977). The possibility of sexual reproduction by the rose aphid in colder parts of Australia can not be completely excluded, even though no evidence has been found so far (Mary Carver, pers. communication). By using an Australian strain from Cooma, at the foothills of the Snowy Mountains, Wöhrmann et al. (1991) found only very weak response of the rose aphid to form sexuals when exposed to strong inducing stimuli.

In Australia, small numbers of *M. rosae* might be found on *Centranthus ruber* (L.) and *Scabiosa spp.* but this is probably insignificant (Maelzer, 1977; Wöhrmann et al., 1991).

Roses are the most popular garden plant in the world and rose aphids can be considered cosmopolitan, except in Eastern Asia (Blackman & Eastop, 1985). *M. rosae* is probably native to Eurasia (Borner & Heinze, 1957) and was introduced into

Australia as a result of European colonisation (Maelzer, 1977). In S.A., *M. rosae* is the most serious insect pest of roses.

M. rosae feeds mainly on the young shoots and buds of roses, up to a stage when the sepals start to fold back (Maelzer, 1976, 1977). The chemistry in the bud then becomes unfavourable for the aphids because the balance between the antioxidant ascorbic acid (Vitamin C) and catechin changes in the phloem sap (Miles, 1985; Miles & Peng, 1991; Miles & Oertli, 1993). The aphids can no longer sufficiently oxidise the harmful catechin.

The growth of a colony of *M. rosae* on a rose bud and its potential for damage depends upon a complex interaction between seasonal rose growth, temperature, rainfall, predation and density-dependent dispersal (Maelzer, 1977; Tomiuk & Wöhrmann, 1980). The population dynamic of *M. rosae* coincide mainly with the growth of the host-plant (Maelzer, 1977; Tomiuk & Wöhrmann, 1980). There are two peaks in spring and one in autumn in S.A.. Up to 1993, *M. rosae* had practically no parasitoids in S.A. Very occasionally, mummies of *Aphidius ervi* Haliday are found on roses in Adelaide. *Aphelinus gossypii* Timberlake occurs periodically on roses but in Maelzer's study (1977) only 0.04 % of aphids were parasitized. However, a number of native species including ladybirds, syrphids and lacewings prey upon the pest and Maelzer (1977) stated that they were able to keep numbers of aphids down in late spring and late autumn.

2.3 The nature of the pest problem, with respect to *Macrosiphum rosae*

General. Lloyd (1960) contended that indirect pests (those that do not damage the product, e.g. aphids sucking on the leaves or stems of a fruit bearing plant) are more amenable to BC than direct pests (those that damage the product, e.g. aphids feeding on lettuce) because the former can be tolerated at higher densities than the latter. If the damage threshold is too low, CBC is not likely to succeed because even a small amount of damage is economically not tolerable (e.g. cosmetic damage of flowers in the

horticultural cut flower industry). As an example for the other end of the range might be taken the damage of the alfalfa aphid, *Therioaphis trifolii f. maculata* (Buckton), on relatively poor quality lucerne in SA. On crops with such low profit margins insecticide sprays are expensive and BC might be the only possible control (Wilson et al., 1982).

Damage by Macrosiphum rosae. By sucking and removing sap from the vascular system *M. rosae* reduces the amount of nutrients available for the bud and the plant as a whole. Visible damage to the bud is rare and only heavily infested young shoots tend to deform or even dry up. Maelzer (pers. communication) describes the economic damage threshold as 50 aphids per infested bud. Above this, the plants respond with reduced growth and inhibited shooting of new buds. However, roses which are grown for cutting or display have a much lower damage threshold, since the presence of aphids is disturbing to the customer for cosmetic reasons. An indirect damage caused by rose aphids is the accumulation of excreted honeydew, favouring the growth of sooty mould which causes not only cosmetic damage but also reduces photosynthetic activity (Williams, 1986).

According to Blackman & Eastop (1985) the rose aphid is able to transmit at least 12 viruses, including the persistent virus strawberry mild yellow edge but not the important rose mosaic or rose streak. However, compared to the main vectors of transmission which are vegetative propagation with virus infected buds, pruning with contaminated equipment, natural pollen transmission and transmission by free-living soil inhabiting Nematodes, e.g. *Xiphinema spp.*, the role of *M. rosae* as vector is minor (Horst, 1983).

Feasibility of CBC of Macrosiphum rosae. The target clientele of CBC of *M. rosae* was assumed to consist of commercial nurseries, communities and home gardeners.

In 1992, the rose industry in Australia was valued at around $A 100 million per annum (Rose Society of S.A., pers. communication). Approximately 70 % was apportioned to the cut flower industry. Pot and bare root growers for garden supply

shared the other 30 % of the market. Nurseries relied exclusively on insecticides for aphid control. Historically, at least two applications in spring and one in autumn are necessary to suppress *M. rosae* in most years.

CBC of *M. rosae* was assumed to be most beneficial for commercial pot and bare root growers, since many of their plants are pruned before sale and light infestations of aphids are tolerable during their main growth period. Many Australian councils and citizens were assumed to benefit from CBC on roses as well, since roses are common in urban areas. The benefit of supplanting sprays with BC agents was considered very high, not only in terms of costs but also in reduction of environmental pollution and maintenance of gardeners' health.

The introduction of an effective control agent of *M. rosae* was considered to be a potential cornerstone in a future IPM program on roses and an invaluable investment in the future. The use of an effective natural enemy was thought to help avoid the breeding of insecticide-resistant strains of rose aphids, a problem alarmingly increasing in many arthropods pests since the 1960's (e.g. DeBach, 1974; Roush & Tabashnik, 1990). Additionally, the use of control agents for rose aphids was thought to complement the use of predatory mites which are often essentially employed in the pest management of mites.

In Australia, an IPM program for the now established Western flower thrips, *Frankliniella occidentalis* (Pergande) is being developed. On roses, it is unlikely to work if no complementary attention is paid towards the aphid problem as well. Only combined applications of control agents can help to overcome the combined attack of various pests.

Damage by aphids occurs in proportion to their abundance (Dixon, 1985). Therefore, any decrease in numbers resulting from the introduction of a CBC agent has to be seen as beneficial (Hughes, 1989).

2.4 Choice of control agents in CBC, with respect to *Aphidius rosae*

General. Effective control agents of the pest might not be known and preliminary investigation of the natural enemy guild has to be undertaken in the presumed area of origin of the pest (Van den Bosch et al., 1982).

Even though advocated by Hokkanen & Pimentel (1984), the selection of 'new pest/natural enemy associations' is considered to be less successful and therefore should be avoided (Greathead, 1986; Hughes, 1989; Waage, 1990). Collecting enemies under low-density situations in the field, e.g. by exposing pests in the field, may be preferable than to sample enemies from outbreaks since former may be better adapted to maintain pest levels at endemic levels (Waage, 1990). Several species of control agents might be found, identified and their impact on the pest evaluated in pre-introductory studies. These studies are preferably done in the country of origin, or, if unavoidable, under quarantine conditions (Hughes, 1989). Waage (1990) gave an overview of criteria that may be applied to agent selection. Perhaps the most prominent factor in choice of natural enemies has been ease of handling and, if necessary, the ease of rearing (Greathead, 1986).

For many pests, the literature will be comprehensive enough to get an overview of natural enemies without conducting extensive exploration in the field. In CBC of aphids, the selection of potentially suitable agents is generally possible by using available knowledge (Carver, 1989).

Often the release of more than one natural enemy is recommended (e.g. Huffaker et al., 1976). The underlaying rationale here is that different species might exploit different niches of the pest and that their actions complement each other. In contrast, other authors, e.g. Turnbull and Chant (1961) have recommended the release of only one most promising species. Their argument was based on potential negative interactions and the resulting reduction in effectiveness of CBC agents. Ehler & Hall (1982) analysed data from Clausen (1978) and suggested that competitive exclusion was likely when more than one species was released. Keller (1984) reconsidered the same

data set and did not find empirical evidence for such a conclusion. In fact, a number of detailed case studies which would prove this theory as a regular event are still missing. Even though competitive displacement of parasitoids in CBC of aphids was observed (Mackauer & Kambhampati, 1986), a reduction in levels of control was not evident. Most BC workers currently consider the risk of competitive exclusion of CBC agents as low and prefer multiple species releases to a single species release whenever possible (David Rosen, pers. communication, 1995).

In California, populations of the olive scale *Parlatoria oleae* (Colvee) were significantly reduced by the introduction of *Aphytis maculicornis* (Masi) in the 1950's but commercial control was not achieved. This changed in 1957 with the introduction of a second parasitoid, *Coccophagoides utilis* Doutt. Coexistence because of different niche use resulted in suppression of pest density under the economic threshold (in Van den Bosch et al., 1982).

Broad statistical analysis of existing data has revealed the likelihood of success in pest/control agent associations. If resources are limited this can give guidelines on which control agents to concentrate research. For example, specific parasitoids of homopteran pests are most likely to succeed in controlling the target pest, especially when the pest occurs in permanent, stable environments on woody plants (Kfir, 1993). Empirical records seem to favour parasitoids over predators (Greathead, 1986). In general, parasitoids are more host specific and the less mobile immature stages do not need to search for food. Parasitoids are generally better adapted and synchronised in interrelationships and have lower food requirements per individual, resulting in the potential ability to maintain a balance with their host at a lower density (Doutt & DeBach, 1964). However, it would be quite wrong to deprecate the value of predators since there are many examples of outstanding control by predators (Huffaker et al., 1976).

Finally, if one or more species are chosen for introduction, it is crucial to decide which strains to use.

The search for the best preadapted strain might be done by matching the climate of the target area with the area of distribution, resulting in the introduction of a climatically adapted strain, or by the introduction of strains from many areas of natural occurrence with the hope that the fittest will establish and yield best results (Van den Bosch et al., 1982). For example, *Aphidius sonchi* Marshall was released in Australia as a control agent of the sow thistle aphid, *Hyperomyzus lactucae* (L.), and established widely (Carver & Woolcock, 1986). An important aspect for failure or success of establishment was apparently which strain of parasitoids was used. In most attempts a mixture of a French and a Japanese strain was successfully released. Establishment was also observed when only the French strain was released, but never when only the Japanese strain was used. In a much published case, the French strain of *Trioxys pallidus* (Haliday), a parasitoid of the walnut aphid, *Chromaphis juglandicola* (Kalt.), established with limited success along the milder coastal valleys of Southern California (Van den Bosch & Schlinger, 1962). The release of a climatically better adapted Iranian strain led to prompt establishment and total commercial control (Frazer & Van den Bosch, 1973; Van den Bosch et al., 1979).

Selection of Aphidius rosae. In seeking a control agent of *M. rosae*, it seemed most promising to look for a parasitoid. The existing parasitoid guild for rose aphids in Australia was negligible and native predators did not provide sufficient control. It was hoped that a specific parasitoid would fill the missing niche of parasitic control and, if not being effective by itself, at least would add to the impact of existing predators. In the majority of successful introductions of parasitic control agents against aphids, the parasitoids belonged to the group of Aphidiinae (Carver, 1989).

Attention was drawn to *Aphidius rosae* Haliday by D. Maelzer and M. Carver. A second potential parasitoid, *Praon volucre* (Haliday), was considered to be less effective since this wasp is not a specific parasitoid of rose aphids and it failed to establish in an

earlier introduction into Australia (Carver & Woolcock, 1986). Alternatively, polyphagous parasitoids like *P. volucre* sometimes become established more easily, and can utilise and contribute to the control of alternative pests in the ecosystem (Stary, 1967).

There is little evidence that multiple introductions of CBC agents is detrimental. Instead they can sometimes provide the key for success. If success in BC of *M. rosae* would have been the most pressuring factor, the simultaneous introduction of both species would have been chosen. But restricted finances and labour limited the scope of the project. The balance between rearing and the scientific outcome of this project would have shifted disproportionately towards rearing if two species would have been introduced. Furthermore, it was desirable to keep the aphid-parasitoid system as simple as possible for future field investigations. Therefore, only the more promising control agent *A. rosae* was finally chosen. *A. rosae* was collected in Italy where the climate is similar to South Australia (Chapter 3).

Specificity of Aphidius rosae. Aphidiinae are strictly solitary endophagous parasitoids. The host range of these parasitoids is restricted to the Aphidoidea. *A. rosae* was considered as host specific in the Australian environment. Wherever it has been recorded, it has been recorded only from *M. rosae* (Stary 1966a; 1976, 1987; Raychaudhuri et al., 1979; Pennachio, 1989; Halima-Kamel & Hamouda, 1993) or from other species of the genus *Macrosiphum* which do not occur in Australia, e.g. *Macrosiphum (Sitobion) rosaeiformis* (Das) (Agarwala, 1983; Agarwala & Raychaudhuri, 1981) and *Macrosiphum funestum* (Macchiati) (Stary, 1966b; Mackauer & Stary, 1967).

Biology of Aphidiinae. Except for some studies on the population dynamics of *M. rosae* in Germany, in which the rate of parasitism by *A. rosae* reached 70 % (Tomiuk & Wöhrmann, 1980), hardly any experimental work has been done on the biology of this parasitoid. Nevertheless, since the biology of taxa in the Aphidiinae can be considered

as homogeneous (Stary, 1970), a general introduction to the bionomics and life histories of these parasitoids will be sufficient to give a framework for what to expect.

Comprehensive reviews were given by Stary (1970, 1988). Much of the following is extracted from these summaries. More than 400 species in approximately 60 genera and subgenera of Aphidiinae are known from all over the world (Stary, 1988). The distribution of Aphidiinae follows closely that of their hosts, and most species occur in the temperate and subtropical regions of the northern hemisphere. Because of the limited aphid fauna in Australia, only about 15 species of Aphidiinae occur on this continent, the majority imported as BC agents (Carver & Stary, 1974; Hughes, 1989; Carver, 1992).

Adult Aphidiinae range from about one to several mm in size. Their colouration range from yellowish to brown and mainly black. Colours in a species can be variable and might depend upon abiotic factors, such as temperature during ontogenesis (Liu & Carver, 1985).

After oviposition of the alecithal egg it takes several days until the larva emerges. For example, *A. ervi* needs approximately four days to eclose to the first larval instar and another four days to kill the aphid at 20 °C (Sequeira & Mackauer, 1992a). Egg and embryonic stages have hardly any distinct effects on the host. Aphidiinae are koinobionts, and parasitized aphids continue to growth until the parasitoid reaches its destructive feeding phase. In general, four larval instars are distinguished (Tremblay, 1964). First, second and third larval stages feed on the haemolymph of the aphid, whereas the fourth instar is mandibulate and starts to feed on the tissues of the host. During the initial phase of parasitism the mass gain of the parasitoid is small, but e.g. the mass of *Aphidius smithi* Sharma and Subba Rao increases more than 30-fold during the third and fourth larval instar (Mackauer, 1986).

After complete consumption of the aphid tissue, the larvae split open the empty aphid skin on the ventral side. In the majority of species the larva spins a cocoon inside the host's integument, e.g. the genus *Aphidius,* but in other species, the larva leaves the host and pupates underneath, using the aphid exoskeleton as a kind of tent, e.g. the

genus *Praon*. In those cases in which the larva remains inside the aphid, the aphid skin becomes indurated and an enlarged shiny aphid form called a mummy appears. Mummies containing diapausing cocoons are thicker and appear much darker than mummies containing non-diapausing larvae. Prepupal, pupal and adult stages develop within the mummy. About a fortnight is required for the life-cycle at 20 °C. The emerging parasitoid bites a round emergence hole out of the mummy skin which is easily broken off.

In general males of Aphidiinae emerge earlier than females. The newly-emerged wasp needs a short time to mature, and mating may occur soon afterwards. Females mate only once whereas males might mate several times. The longevity is variable, depending mainly upon temperature and availability of food (mainly honeydew), but can be longer than three weeks. Aphidiinae are synovigenic. The reproductive capacity varies between species but can be greater than 1000 eggs. Reproduction is mostly biparental. Fertilised (diploid) eggs result in females whereas unfertilised (haploid) eggs produce males. The sex-ratio in the field is typically female-biased.

Oviposition is similar among different species. After encountering a host, the female bends its abdomen forward between the legs towards the potential host (Fig. 7.2). The time required for oviposition is variable among species, and can range from less than a second to about a minute. Host discrimination of parasitized aphids is not perfect and superparasitism occurs both in the laboratory and in the field. Preference for smaller host instars is typical but not fixed.

Dispersal of Aphidiinae occurs actively by the adult wasp or passively as developmental stage inside an aphid. Mummies get distributed by human activities or attached to leaves dispersed by wind.

The seasonal history of Aphidiinae is closely linked to the abundance of their hosts. They occur soon after the first aphids appear and enter diapause somewhat earlier than the host. The parasitoids never follow holocyclic aphid species in another habitat and instead enter diapause or switch temporarily to different aphid species. The number of generations per season is greatly dependent upon environmental factors.

Parasitism can substantially reduce aphid populations but can not prevent spread of aphids nor virus transmission. Parasitoid action is normally seasonally delayed compared to aphid abundance, and therefore might not prevent damage to plants earlier in the season.

To keep the aphid-parasitoid system as simple as possible for future field investigations, only one natural enemy of *M. rosae* was introduced into Australia. The success-rate of specific parasitoids of homopteran pests occurring in permanent, stable environments on woody plants, made *A. rosae* the most promising natural enemy to be released for BC of *M. rosae* in South-Eastern Australia. The parasitoids were collected in Italy where the climate is similar to South Australia.

Field collection and rearing of *Aphidius rosae*

3.1 Introduction

Insect rearing is a fundamental component of most biological control programs. Only very rarely are field-collected insects released directly into a target ecosystem. Typically the control agent is bred for a few generations in captivity because of quarantine restrictions or to build up numbers. The collection process and subsequent rearing influence the genetic variability of the control agent, so it is important to conduct these activities in a manner that maintains the fitness of the laboratory culture (Mackauer, 1976a).

Collection. The main aim of the collection process is to obtain as much genetic variability of the field population as possible. Success or failure of the whole program may depend on the amount of genetic variation among the insects that are collected. Typically, as many individuals as possible are collected. Mackauer (1976a) suggested that a founder sample of 500 individuals from a sufficiently large area would yield a level of genetic diversity adequate for most biological control programs. He also emphasised the importance of sampling in more than one site. Alleles which may be important in the intended area of release may be common at least somewhere in the home range of the insect, even though they may be rare overall. Theoretically, approximately 50 % of existing alleles will be sampled with the collection of 100 individuals, while remaining alleles are relatively rare. Expansion of the sample size to 1000 individuals increases the efficiency only by 15 %. Therefore, these rare alleles are more likely to be sampled in environments where they are important and more common (reviewed in Roush, 1990). Another way to increase the diversity of allele frequencies is

to sample at different times of the year (Mackauer, 1976a). Theoretically, 20 individuals per strain might be the minimum required to minimise inbreeding effects (Nei et al., 1975).

It is common practice in biological control programs to exclude hyperparasitoids from importation (Sullivan, 1987), even though facultative hyperparasitoids may sometimes be judged as beneficial for biological control (Ehler, 1979).

Rearing. Compared to the complexity of the field, an insectary represents a highly uniform and artificial environment for insects (Finney & Fisher, 1964). Abiotic factors such as temperature, day length and humidity are kept favourable and constant, food and water are provided and natural enemies excluded. Under such circumstances, insect populations can undergo rapid changes. These can be divided into non-permanent conditioning and permanent changes in genetic variability through losses of alleles.

Genetic drift, selection and inbreeding are the main causes of loss of genetic variability in rearing programs (Roush, 1990). Genetic drift in insect rearing can occur as a consequence of an initially small sample size (Founder effect) and/or random sampling of individuals forming the next generation. Artificial selection can be caused by conditions that unintentionally favour some genotypes over others. Selection pressure caused by artificial rearing conditions can result in populations that are optimally adapted to their cage environment but not to the environment in the field. Inbreeding depression of a population is the loss of vigour resulting from interbreeding among relatives.

Conditioning of control agents to their rearing environment may not permanently change the genetic structure of the population, but can effect the outcome of biological control programs (e.g. Milne, 1986b).

Roush (1990) recommended that the mean number of progeny per parent should be maximised to avoid genetic drift and inbreeding. For very small colonies, he suggested pedigree records to avoid matings of closely related individuals. To minimise laboratory selection, the separation of the population into several lines is suggested.

Boller (1972) advised the use of stressful and varying rearing conditions (e.g. fluctuating temperatures, no permanent supply of food or water resources) to maintain a higher degree of adaptability and flexibility in the insect population.

The implementation of these recommendations in a rearing program depends upon the ease with which the insect species is reared and the availability of materials, space and labour, which are ultimately limited by available finance.

This chapter deals briefly with the collection of *Aphidius rosae* Haliday in Italy and describes rearing procedures used in quarantine as well as general rearing methods referred to in later experiments. The sex-ratio and the rate of diapausing mummies were noted in different generations.

3.2 Methods

Collection of A. rosae. South and Central Italy have a Mediterranean climate that is similar to Adelaide (Papadakis, 1975). It was assumed that parasitoids from this zone would be preadapted to the climate that prevails in the area of initial release. Cooperative arrangements were made with the Dipartimento di Entomologia e Zoologia agraria, Universita degli Studi Di Napoli, to secure a source of insects over the period of the project.

In June 1992, *A. rosae* was collected by myself as mummies at two places in Lazio and three places in Tuscany, Italy. All mummies found were collected and stored separately. Additionally, Maria Cristina Digilio at the Dipartimento di Entomologia e Zoologia agraria collected mummies in Naples and Potenza. These insects were the founder of a colony which was reared for 18 generations before permission was given by the Australian National Parks and Wildlife Service and Australian Quarantine Service to release *A. rosae* into the field. To increase the genetic diversity and to counteract possible genetic drift and inbreeding depression, a second shipment of mummies was

obtained from Naples in June 1993. Finally, a third collection took place in Naples and Potenza in June 1994.

Identification. Following the initial importation of *A. rosae* into Australia in 1992, specimens of the F1 generation were sent to C. Digilio, Dipartimento di Entomologia e Zoologia agraria, Naples, for identification, as well as to the Australian National Insect Collection in Canberra. Hyperparasitoids were sent to Mary Carver, CSIRO, Canberra.

Preservation of genetic variability. In the first parental generation each female was mated in a gelatine capsule (1 ml volume) containing a small rose leaf. Some males had to be used twice (Table 3.1). Each female was held separately in a cylindrical gauze cage (see below) with approximately 50 *Macrosiphum rosae* (L.) of mixed age. The resulting mummies were collected and stored separately in gelatine capsules. For the F2 generation, four randomly chosen females from each of the original female lines were mated with unrelated males and reared together in cylindrical gauze cages.

This procedure was labour intensive and so was not continued for the F3 generation. From there on, the colony was divided in only two lines. From each of the original 12 lines, three females were randomly collected to establish each of the two breeding lines.

In each generation, females were collected as founders of the new generation after approximately 24 days. At this time most non-diapausing individuals had already emerged and virtually all females were fertilised. For each of the two lines, 30 females were randomly collected (F4 to F18 generation). Only small or slow individuals were eliminated. Even though fertilisation was likely to have occurred, a few males were added to the cage as well.

Each generation produced changing numbers of diapausing mummies which were partly stored at 18°C and partly in the refrigerator at 4°C (Polgár, 1986). They represented an allele pool remained from earlier generations and when these individuals emerged, they were regularly introduced into the rearing cages to maintain the genetic

base of the culture. Up to five females that emerged from diapause or females that mated with males emerged from diapause, were added to a line in each generation.

The individuals from the collection in 1993 were bred to increase numbers and then combined with the existing colony for release.

As soon as sufficient numbers of mummies were available in the field, the permanent laboratory culture was regularly mixed with field-collected individuals.

From the collection in 1994, only four females and seven males of *A. rosae* emerged. These insects were crossed with wasps from the laboratory culture and the resulting colony was kept separately and treated as already described. Around 1500 parasitoids of the F3 to F5 generation were released into the field from August to September 1994. To avoid interference with the census at the Waite Campus (Chapter 10) they were released in a commercial rose garden in Willunga, 50 km south of the Waite Campus.

Plant material. Initially, aphid and parasitoid cultures were maintained on potted roses, but at that time the quarantine facilities at the Waite Campus did not provide any plant production facilities and only limited space. Roses need high light intensities and plant quality declined rapidly when plants were brought indoors. Furthermore, plants had to be destroyed after use and could not be recycled in a rearing system outside the quarantine facilities. To provide a permanent and sufficient supply of high quality potted plants, substantial rearing space with cool temperatures and high light intensities would have been required, but these were not available.

The use of rose cuttings collected in the field offered a solution. Rose shoots placed in water with cut flower fertiliser (Flower Fresh ™, Flower Fresh Products, Glengowrie, South Australia) provided sufficient nutrition for aphids, were available all year round, required much less space than a potted plant and costed nothing. The disposal of this bio-hazardous waste from quarantine was minimised by the small volume. The only effort invested was the systematic pruning of plants in the field to

guarantee a continuous supply of rose shoots even at unfavourable times of the year, and fastidious cleaning of the shoots to eliminate aphid predators and their eggs.

Shoots were taken from the rose variety Tea hybrid 'McGredy's sunset' (Appendix 1). For rearing, suitable shoot buds for aphid growth from stage 1 to 5 were used (Maelzer, 1977)(Fig. 10.1). Occasionally wild shoots produced by the root-stock, which periodically had to be removed from rose plants anyway, were used in rearing.

Aphids. M. rosae was collected in the field and cultured for several generations before they were replaced by new insects. Many older instars and adult aphids dropped off the plant and were easily caught in a bowl placed underneath the shoot when a person breathed on an infested bud. During most of the year, aphids were collected around the Waite Campus. During summer, when aphids virtually disappeared, collections were undertaken in Hahndorf in the Adelaide Hills which has a cooler climate. Before the release of *A. rosae*, field-collected aphids were used directly for rearing, whereas after the release had taken place, only their offspring were used. This precaution became necessary because of hyperparasitism in the field (Chapter 10).

Abiotic factors. All insects were mainly reared at 18 ± 2°C, 65% relative humidity. This temperature was relatively low for rearing of insects, but it was close to the average temperature in the field when aphids are abundant (15-17°C, Maelzer, 1981). The room temperature was regulated by an air conditioner which had to be preset for cooling only or heating only to 18°C. Temperature varied slightly because of this inflexibility. All cages were illuminated with fluorescent True Light® at 16L:8D. Additional day light was allowed to enter through the windows. Especially during the hot summer (when no experiments were carried out), day light was able to heat cages close to the window 4°C above the room temperature.

Cages. Three types of cages were used during the project. Throughout this thesis they are referred to as 1) cylindrical shoot cage, 2) gauze shoot cage and 3) plexiglass cage.

1) The cylindrical shoot cage was mainly used in the initial phase of rearing and for experiments in which easy access to the insects was essential. One rose shoot was held in a plastic cup (350 ml). The shoot was passed through a hole in the lid which was sealed with a foam stopper (Fig. 3.1a). The cage section consisted of a plastic cylinder (3.25 cm x 27 cm [radius x height]) with a gauze top and three gauze strips along the side (Fig. 3.1b). The edges between the top cylinder and the lid of the cup were sealed with plasticine.

2) The gauze shoot cage was constructed from a short gauze sleeve (10 cm x 15 cm), open at one end and sewn together at the other (Fig. 3.1c). The open end was sealed by a rubber band which squeezed together the gauze and an inner piece of foam. A rose shoot was placed through the middle of the foam. Three wooden sticks inside the foam prevented the collapse of the gauze and created space for the rose bud and insects. The basal part of the shoot was placed in water with cut flower fertiliser. This kind of cage occupied much less space than the previously described shoot cage since many shoots could be held in the same cup of water. It was easy and cheap to produce this cage in large quantities. A major disadvantage was the limited access to insects inside because of the fragile construction. Therefore, it was mainly used in experiments where large numbers of separated aphids had to kept for only a limited time.

3) The twin-plexiglass cage was the main cage for the maintenance of the parasitoid culture (Fig. 3.2). Each chamber measured 52 cm x 56 cm x 45 cm (length x width x height). The bottom and side walls were plexiglass, whereas the top consisted of fine gauze. One door in each chamber allowed access to add or remove shoots from the cage. An additional gauze sleeve allowed access for maintenance of cultures and minimised the risk of insect escape. Each chamber had the capacity to hold up to 25 plastic cups with shoots.

Rearing of A. rosae. The actual set up of cages, plants, aphids and parasitoids was specific for each experiment and is described in the appropriate chapters. Here, only the

set up of the general breeding culture of *A. rosae* from the F3 to the F18 generation is described.

Chambers of the twin-plexiglass cage normally contained 15 shoots. Declining shoots were regularly replaced. After aphids were collected they were released onto shoots in the plexiglass cage. They were allowed to acclimatise and settle on the plants for one day. Approximately 500 adults and older aphid instars were released into each chamber. The following day 30 females and a few males of *A. rosae* were released into each chamber. No food was supplied since honeydew of aphids was thought to be a sufficient resource for parasitoids (Stary, 1970). Water was given once each day, by spraying it with a syringe on the top gauze, or a vial of water with a cotton wick was placed in the cage.

The first progeny emerged after 14 or 15 days, and from then on, wasps emerged continuously until the cages were cleaned. Normally, after 24 days all parasitoids were collected with an aspirator and kept overnight in plexiglass cages (20 cm x 15 cm x 15 cm) supplied with honey and water. Old shoots were removed and the cages were washed with soapy water. New shoots and aphids were then set up for a new cycle of breeding.

Old shoots, cups and the walls of the cage were searched for diapause-form mummies. These were stored separately in the refrigerator at 4°C, 75% r.h., or in gelatine capsules (1 ml volume) in the rearing room.

The number of offspring, their sex-ratio and the fraction of diapause-form mummies were noted at arbitrary times.

Fig. 3.1 a) Rose shoot in plastic cup (350 ml), filled with water supplemented with fertiliser. Foam was used to close the hole in the lid. This unit was used in bigger cages or as base for **b)** the cylindrical shoot cage. The top and windows on the side of these cages were gauze. The base was sealed with plasticine. **c)** The gauze shoot cage was a short gauze sleeve, sewn together at the top and open at the bottom. The bottom was sealed by a rubber band which squeezed together the gauze and an inner piece of foam. Three wooden sticks created space inside the cage.

a
b
c

Fig. 3.2 The twin-plexiglass cage, the principle rearing unit for the culture of *Aphidius rosae* in quarantine. For details see text (size of each chamber 52 cm x 56 cm x 45 cm [length x width x height]).

Fig. 3.3 Diapausing mummy form (dark brown, thick inner lining of aphid shell) of *Aphidius rosae* parasitizing *Macrosiphum rosae*. For comparison with a non-diapause form see Appendix 4.

Fig. 3.4 Aggregation of diapause mummies of *Aphidius rosae* in one corner of the rearing chamber. A short time before death by parasitism, many parasitized aphids (*Macrosiphum rosae*) left the rose shoots they were reared on (see text for explanation).


3.2


3.3
1 mm


3.4

3.3 Results and discussion

Due to heavy hyperparasitism of the field-collected material in all three collections only 21 females and 12 males emerged in 1992, 8 females and 8 males in 1993, and only 4 females and 7 males in 1994. In the latter year, an extremely hot spring had created very unfavourable conditions for aphids in Naples and consequently parasitoids were not abundant. Relatively few aphids and mummies were found and many of them hyperparasitized (Table 3.1).

The guild of hyperparasitoids consisted of *Asaphes vulgaris* Walker, *Pachyneuron aphidis* (Bouché), *Coruna clavata* Walker, all Pteromalidae, and *Alloxysta victrix* (Westwood) and *Alloxysta sp.*, both Charipidae (specimens were kindly identified by Mary Carver).

Table 3.1 The emergence of *Aphidius rosae* and hyperparasitoids from field-collected mummies from Italy. All values are numbers of individuals.

Location	*A. rosae*, females	*A. rosae*, males	Hyper-parasitoids	Other dead mummies
Tuscany, June 1992	5	1	179	4
Lazio, June 1992	7	8	118	6
Naples, Potenza, June 1992	9	3	94	21
Naples, Potenza, June 1993	8	8	165	5
Naples, Potenza, June 1994	4	7	80	6

A key factor for the successful rearing of *A. rosae* was the relatively low temperature of 18°C. Roses senesce more slowly at cooler temperatures and aphids feeding on them grow better (Maelzer, pers. communication). *M. rosae* were significantly smaller when grown at 23 to 25°C on potted plants and cuttings. Dixon (1985) pointed out that size in aphids is a consequence of the relative effect of food

quality and temperature on the growth and development rates. Aphids reared at high temperatures are smaller than those reared at lower temperatures because increasing temperature disproportionately decreases the time to reach maturity, compared to the increasing growth rate. The indirect effect of declining plant quality and the direct effect of high temperatures on insects (Maelzer, 1977) were considered as possible reasons why a first lot of seven females and three males of *A. rosae* died without reproducing. Occasionally very small mummies formed that contained only dead parasitoids. This situation changed when rearing temperatures were lowered to 18°C. Fox et al. (1967b) showed that the mortality of *Aphidius smithi* Sharma and Subba Rao parasitizing *Acyrthosiphon pisum* (Harris) increased at higher temperatures .

A further advantage of lower temperature was that it lengthened the generation time and therefore lowered the risk of genetic decay in the culture.

Table 3.2 First generation of *Aphidius rosae* reared on *Macrosiphum rosae* in the laboratory. Parasitoids emerged from mummies collected in Italy. Insects were reared on rose cuttings at 18 ± 2°C, 65% r.h., 16L:8D.

Parents	F 1		
Female # (Male)	Females	Males	Sex-ratio
1 (A)	11	4	0.27
2 (B)	29	4	0.12
3 (C)	39	12	0.24
4 (D)	69	45	0.39
5 (E)	5	2	0.29
6 (F)	10	10	0.5
7 (G)	43	26	0.38
8 (H)	25	15	0.38
9 (I)	0	0	-
10 (F)	21	16	0.43
11 (A)	0	0	-
12 (B)	154	57	0.27
13 (C)	7	7	0.5
14 (D)	20	18	0.47
Total	433	216	0.33

From the parasitoids which were held at 18°C, two females and one male did not reproduce. Therefore only 12 females and eight males collected in Italy contributed to the first generation of *A. rosae* reared in quarantine (Table 3.2). Insects increased rapidly in numbers. A colony size of 649 insects was already achieved in the F1 generation.

The sex-ratio in all generations was female-biased, ranging between 0.29 and 0.45. Such ratios were also observed in the field (Chapter 10). Results from Chapter 4 as well as investigation of other parasitoid species (e.g. Godfray, 1994) show that male-biased sex-ratios can be observed on low quality hosts. Therefore, it may be assumed that the rearing method provided good host quality.

Diapausing mummies (dark brown, with thick shell)(Fig. 3.3) were found in most generations. The numbers increased at times when diapausing mummies would be expected to increase in the field, i.e. at the end of spring in November and at the end of autumn in May (Table 3.3). Facultative diapause has also been recorded in other Aphidiinae. For example, in laboratory rearing of *Aphidius sonchi* Marshall, diapausing individuals occurred all year round in the stock culture. The proportion remained fairly constant, being usually about 6 % (Liu & Carver, 1986). In contrast, *A. rosae* showed variation in the production of diapausing mummies. The influence of natural daylight through the windows of the rearing room may have influenced the incidence of diapause, despite artificial illumination.

Many of the diapausing mummies were found away from the shoots. Accumulations were common in the darkest corner of the cage (Fig. 3.4). Parasitized aphids often leave the plant shortly before being killed by the growing parasitoid (Behrendt, 1968). Some parasitized aphids display a negative phototaxis and prefer a darker rather than a lighter substrate to mummify (Brodeur & McNeil, 1990). This enables the parasitoid to form mummies in concealed places, increases the probability of escaping hyperparasitism, and can also result in a change of microclimatic conditions. Such changes in aphid behaviour are more frequently observed when the aphids contain parasitoids that will enter diapause and might be exposed to adverse climatic conditions and hyperparasitism for prolonged periods (Brodeur & McNeil, 1989a).

Table 3.3 The sex-ratio (proportion males) and numbers of diapause-form mummies in separate generations of *Aphidius rosae* reared on *Macrosiphum rosae* in the laboratory. Frequency of diapausing mummies was determined after approximately 24 days when cultures were renewed. The considerable number of parasitized, but living aphids after 24 days, and the number of mummies still containing parasitoids but not being the diapause-form, are not shown. Cultures were maintained at 18°C ± 2°C, 65% r.h., 16L:8D.

Generation	Time	*A. rosae*, females	*A. rosae*, males	Sex-ratio	Diapause-form mummies
F3	October 1992	448	241	0.35	51
F5	Nov./Dec. 1992	260	189	0.42	192
F7	January 1993	273	111	0.29	54
F9	March 1993	287	141	0.33	17
F11	April/May 1993	395	324	0.45	243
F14	June 1993	520	318	0.38	205

The cold storage of diapausing mummies at 4°C was successful. The storage of those mummies represented a last reserve in case the cultures would crash. Additionally, they were used to preserve genetic diversity in the culture.

In summary, using rose-cuttings for aphid rearing turned out to be a reliable food source for culturing *M. rosae*. The collection and importation procedures probably did not result in loss of vital genetic diversity as the successful performance and survival of *A. rosae* showed in the field (Chapter 8, 9 and 10).

Influence of host quality on *Aphidius rosae*

4.1 Introduction

Salt (1941) reviewed the effects of hosts on their parasitoids and reported that the size of parasitoids is greatly influenced by host size. He also listed additional effects on morphology, sex-ratio, fecundity and rate of development of the parasitoid. Since this classic review, host size is generally considered as an index of host quality, assuming that large hosts contain more nutritional resources than small ones, enabling the larvae to achieve improved growth and therefore better fitness (e.g. Waage, 1986).

Parasitoids can be divided in two host-exploitation categories. Parasitoids which develop in non-growing and paralysed hosts are called idiobionts. In contrast, parasitoids in hosts which continue to grow and metamorphose during at least the initial phase of parasitism are called koinobionts (Askew & Shaw, 1986). The fundamental difference between these two host-exploitation strategies is that hosts of idiobionts contain a fixed amount of resources for the parasitoid larvae, whereas hosts of koinobionts continue to feed and gain additional nutrition. This gives koinobionts the theoretical possibility to alter their own growth and development as well as that of their host in order to maximise nutritional suitability for parasitoid development (Vinson & Iwantsch, 1980). In idiobiont parasitoid-host systems it is assumed that host quality is a linear function of size whereas for the koinobiont *Aphidius ervi* Haliday (Sequeira & Mackauer, 1992b) and *Venturia canescens* (Gravenhorst) (Harvey et al., 1994), host quality was shown to be not necessarily a linear function of host size at time of parasitization.

Aphid instar at time of parasitization, host-plant quality and superparasitism are considered as key determinants of host quality for koinobiont Aphidiinae. Substantial research has been undertaken to evaluate the influence of these factors on Aphidiinae. The following is an overview.

4.1.1 Aphid instar and morph. Also most Aphidiinae can successfully develop in all instars of their host (Stary, 1988), the different nutritional quality of host instars can substantially influence parasitoid ontogeny. E.g. the first and fourth instar of the pea aphid differ in their dry weight by more than one order of magnitude (Mackauer & Sequeira, 1993). Since aphid colonies consist of a range of different instars much attention has been given to this aspect of host-parasitoid interaction and its consequences for parasitoid's oviposition choices.

Size. In general, the size of Aphidiinae is considered to increase with aphid size. This increase may or may not be linear. Male and female drymass of *Ephedrus californicus* Baker increased steadily with larger host instars of *Acyrthosiphon pisum* (Harris) at parasitization (L1<L2<L3<L4) (Sequeira & Mackauer, 1993). The same pattern was observed for the adult size of *Diaretiella rapae* MacIntosh, parasitizing *Brevicoryne brassicae* (L.) (Hafez, 1961) and *Aphidius ervi* Haliday parasitizing *A. pisum* (Sequeira & Mackauer, 1994). In contrast, the adult drymass of *A. ervi* did not increase linearly (L1<L2<L4<L3) with larger host size in an earlier experiment of Sequeira & Mackauer (1992a). When the parasitoid *Aphidius sonchi* Marshall was reared in the first instar of *Hyperomyzus lactucae* (L.), emerging wasps were smaller than those reared from the third instar (Liu, 1985). The increase of parasitoid size, resulting from growth in different host instars, is in general much smaller than the large differences in aphid size at parasitization. This is indicative of the passive character of the koinobiont parasitoid during the first days after parasitization. Liu (1985) argued that the suitability of the aphid to the development of the parasitoid varies as the aphid develops, but that the size of the parasitoid, irrespective of host stage at reception of the parasite egg, is largely determined by the size of the aphid when the parasitoid is in its

destructive feeding phase. *Aphidius smithi* Sharma and Subba Rao even successfully parasitized embryos of *A. pisum* still inside the mother but resulting offspring were much smaller than their counterparts reared on aphids already born (Mackauer & Kambhampati, 1988). Qualitative differences between alatae and apterae had no measurable effect on the outcome of male *A. smithi* (Mackauer, 1986) but have been observed for *D. rapae* (Hafez, 1961).

Sex-ratio. Much attention has been paid to the influence of aphid instars on the sex-ratio of parasitoids (defined as the proportion of males in the progeny) since offspring and sex allocation by parasitoids is a central problem in evolutionary models (Godfray, 1994). The majority of parasitic wasps have a haplo-diploid sex determination with fertilised eggs resulting in females and unfertilised eggs resulting in males. This mechanism involves the theoretical possibility for the mother to manipulate the sex-ratio of offspring (Charnov, 1982). Among solitary parasitoids, a significantly greater proportion of idiobionts than koinobionts show some evidence for host size dependent sex-ratios (85 % vs 42%) (King, 1989).

In most aphid/Aphidiinae interactions, smaller parasitized hosts tend to produce male-biased sex-ratios of emerging wasps whereas the sex-ratio of parasitoids emerging from larger hosts shifts towards female-biased. For example, Wellings et al. (1986) found an emergence sex-ratio of 0.65 for both two and four days old hosts at time of parasitization by *A. ervi* whereas the sex-ratio of parasitoids emerging from six days old hosts was 0.37. Supporting results for the same species were found by Sequeira & Mackauer (1992b) who found more females of *A. ervi* emerging from the fourth instar aphid than from any of the earlier ones. The sex-ratio of *Aphidius nigripes* Ashmead emerged from L1 hosts was 0.83 compared to 0.38 for parasitoids emerged from L3 and 0.40 for wasps emerged from adults (Cloutier et al., 1981). Mated females of *E. californicus* fertilised nearly 50 % of their eggs laid in large host aphids (*A. pisum*) but only about 20 % of those laid in small hosts (Cloutier et al., 1991). In contrast, the sex-ratio of *A. sonchi* reared from aphids attacked in instar one and three were not significantly different (Liu, 1985).

Evolutionary models of offspring and sex allocation by parasitoids predict that females can achieve better fitness by selectively allocating male eggs to lower quality hosts and female eggs to high quality hosts (e.g. Waage & Godfray, 1985). This prediction is mainly based on 1) parasitoid size is a positive function of host size 2) a wasp's reproductive success is determined by its size and 3) small size is of less importance for fitness in males than in females. In contrast to this active decision making role of females in sex allocation Wellings et al. (1986) interpreted the shift in sex-ratio of *A. ervi* as attributable to differences in the pre-emergence survival rates of male and female progenies. They argued that it seems unlikely that koinobionts have involved some mechanism to evaluate future resource acquisition rates of the host, a hypothesis which was also postulated by Waage (1982).

Development rate. Aphid instars can also have strong influence on the time a parasitoid needs to mature. Depending upon the species and/or experimental method, results between Aphidiinae can vary and may not necessarily display an decrease in developmental time with larger host size at time of parasitization. Both the larval period and prepupal period of *A. smithi* were longer in smaller hosts of *A. pisum* (Fox et al., 1967a). The total duration of development of *D. rapae* was found to be slightly longer in L1 and L2 hosts compared to older instars and adults (Hafez, 1961). Time from oviposition to 50 % adult eclosion of *A. ervi* varied between host instars, with parasitoids from L2 eclosing on average one day earlier than their counterparts in other aphid instars (Sequeira & Mackauer, 1992a). However, in 1992b and 1994 the same authors found that parasitoids of *A. ervi* that developed from L3 required one day longer to emerge than wasps from other instars. As possible explanation for irregular patterns of suitability they refer to disproportionate growth of aphid gonads and associated nutritional shortcomings in the haemocoel of different instars (Sequeira & Mackauer, 1992b). Development time of *E. californicus* varied from oviposition to adult eclosion with the host instar at parasitization, and was shortest in first and fourth nymphal instars (Sequeira & Mackauer, 1993).

In contrast, development times and survival of *A. sonchi* were almost identical in the first and third instars of *H. lactuae* (Liu, 1985). Additionally, no significant differences could be seen in developmental time of *A. smithi* males which developed in alatae and apterae of *A. pisum*, but females of *D. rapae* increased developmental time by 60 % when larvae grew in alatae instead of apterae (Hafez, 1961).

Fecundity. On average, females of *A. sonchi* reared from the first instar hosts contained fewer eggs than those reared from the third (Liu, 1985) and females of *D. rapae* emerging from L1 and L2 had less eggs in their ovaries than females emerging from older instars (Hafez, 1961). Since Aphidiinae are synovigenic, investigations were also undertaken to determine the influence of host instars on life-time fecundity. Smaller parasitoids of *A. smithi* displayed a smaller life-time fecundity than their larger counterparts (Mackauer & Kambhampti, 1988). Sequeira & Mackauer (1994) showed that life-time fecundity of *A. ervi* increased over a limited range of sizes only, and declined with further increases in size. Fecundity was not a linear function of parasitoid size or size of aphid at time of parasitization.

Summary. In general, the instar of an aphid at time of parasitization can influence parasitoid development. Parasitoids emerging from smaller hosts are generally smaller, develop slower and display lower fecundity and a male-biased sex-ratio. However, some species react very sensitively to different host instars whereas others are not affected.

4.1.2 Host plant quality. The suitability and quality of the plant available to an aphid is important in determining its size, survival and reproductive rate (Dixon, 1985), and therefore is assumed to have also a strong influence on parasitoid growth (Stary, 1970). *E. californicus* developing in normally feeding pea aphids achieved a greater adult dry mass and shorter developmental time than their counterparts which fed on lower food quality, simulated by aphids starved four to six hours daily (Koumé & Mackauer, 1991). Polyphagous aphids can be of different quality or even suitability for aphidophaga when feeding on different plant species (e.g. Blackman, 1967). Additionally, even different varieties of a host plant can vary significantly in food quality for aphids and influence

the tri-trophic system (Van Emden, 1995). Aphids feed mainly on parts of the plant which offer high-quality food, e.g. actively growing parts (Dixon, 1985). Aphids growing on unfavourable parts of the plant become smaller (Dixon, 1987).

Aphids themselves alter the nutritional quality of their food plants by injecting growth-inhibiting saliva and by simply removing phloem sap (Miles, 1989a,b). If crowding occurs and too many aphids remove too much sap, food quality declines, resulting in smaller aphids. Declining food quality can also lead to the production of alate offspring, as shown e.g. for the pea aphid *A. pisum* (Sutherland, 1969).

4.1.3 Superparasitism. For the endoparasitic solitary Aphidiinae, only one individual per host can develop to maturity, so the supernumeraries must be eliminated. According to Tremblay (1966), the mechanism of competition among the various stages of Aphidiinae inside an aphid is a combination of physiological and accidental injury, in which the older larva normally survives. In some Aphidiinae, superparasitism due to poor discrimination of parasitized and unparasitized aphids seems to be a common phenomenon. It occurs without any obvious pressure from the environment, whereas in other species of Aphidiinae it has been shown that superparasitism occures in overpopulated patches (Stary, 1970). Superparasitism is common, especially when parasitoid density is high and the wasps lack the opportunity to disperse, e.g. forced by cold temperatures in the field or artificial cage conditions during rearing programs. However, compared to the behavioural aspects of superparasitism, its effects on the development of surviving larvae are not well documented. Since the host represents a finite pool of nutritional resources, common opinion is, that as the number of competitors for these resources increases, the amount of resources available to each individual decreases (Vinson & Iwantsch, 1980). Hofsvang & Hågvar (1983) found that larvae of *E. cerascicola* developed slower in superparasitized aphids than single larvae. The same tendency was demonstrated for *Praon palitans* Muesebeck (Force & Messenger, 1965). Wylie (1965) showed that superparasitism reduced survival and size, and affected the sex-ratio of the gregarious parasitoid *Nasonia vitripennis* (Walk.). He

suggested that effects were due to food shortage. Cloutier & Mackauer (1980) reported an increased food consumption of superparasitized L2 pea aphids, compared to singly parasitized aphids and unparasitized aphids. They concluded that the potential for parasitoid growth will probably rise with higher food consumption of the host. According to them, superparasitism in second instars of pea aphids might be adaptive for *A. smithi* because it enables wasps to exploit small hosts more efficiently.

The influence of host quality can have profound effects on most life-history parameters of Aphidiinae. To interpret findings and discuss life-time strategies it is necessary to determine the interelationships of these parameters. In the field none of these factors can be seen isolated and host quality for the parasitoid depends upon all the interactions in its tri-trophic system, which is influenced further by environmental conditions.

The aims of this study were to improve rearing in quarantine and to gain information about the biology of the wasp, and thereby to enhance understanding of its performance in the field after release. The influence of different instars and adults of *Macrosiphum rosae* (L.) on its parasitoid *Aphidius rosae* Haliday were investigated. To put additional realistic stress on the aphid-parasitoid system the influence of superparasitism and low host-plant quality were examined as well.

4.2 Methods

Plant material. All plant material consisted of 30 cm long bud shoots of *Rosa sp.*, var. Tea hybrid 'McGredy's sunset', 2nd and 3rd stage (Maelzer, 1977). During the experiment they were kept fresh by immersion in water supplemented with fertiliser for cut flowers (Chapter 3). Differences between 'normal' plant quality and 'poor' plant quality were simulated by renewing shoots after four days for aphids reared on 'normal' plant quality to compensate for shoot maturation, whereas aphids reared on 'poor' plant quality had to feed for eight days on the same shoot.

Insects. Apterae from uncrowded colonies of *M. rosae* were collected from the field and kept for 24 h on rose shoots. Adults were removed and their offspring were used in the experiment. They were reared on rose shoots until they reached the desired age. Special care was taken when handling younger instars. Aphids were transferred with a fine brush and only handled when their rostrum was not inserted into the plant.

Parasitoids were three to five days old when used in experiments. All wasps were females that had access to food, aphids and males throughout their lives. Every parasitoid was used only for one oviposition.

Abiotic factors. The experiment was undertaken at 18 + 2°C, 65% relative humidity. All cages were illuminated with fluorescent True Light®at 16L:8D. Before oviposition, aphids were reared on rose shoots in a 52 x 56 x 45 cm cast acrylic cage with a gauze top. After oviposition, they were transferred to cylindrical gauze cages (Chapter 3).

Experimental design. Five age classes of aphids were offered to *A. rosae* for oviposition : L1 = 1-2 days old, L2 = 3-4 days old, L3 = 5-6 days old, L4 = 7-8 days old and adults = 10-11 days old. Aphids used for L3, L4 and adults consisted of apterous forms only, whereas aphids in classes L1 and L2 consisted of apterous and alate forms since differentiation was not possible. Each class was divided in three treatments, consisting of singly and superparasitized aphids reared on plants of 'normal' quality, and singly parasitized aphids reared on plants of 'poor' quality. Each treatment had two replicates. Aphids belonging to the same age class, treatment and replicate were reared on the same shoot.

Aphids were exposed to parasitoid females for oviposition one by one in 1.0 ml gelatine capsules and were observed under a dissecting microscope. Oviposition was considered to have taken place only after an obvious prolonged contact of the ovipositor with the aphid and a following slight jerk. Previous experiments showed that these observations were a reliable indication that oviposition had taken place. Aphids were

excluded from the experiment in cases where oviposition was uncertain (43 % of all attempts). An attempt was made to use equal numbers in each treatment but this was not possible. Older aphids, especially adults, showed strong defensive behaviour, so a greater number indistinct oviposition attempts was observed in older hosts. Consequently a greater proportion of older aphids were excluded from the experiment.

Younger instars were vulnerable to injury. Any aphid that sustained an injury was excluded. Sample sizes were not equalised among replicates due to separation of coloured aphid morphs in the experimental set up (see below). Sometimes oviposition occurred in the legs, antennae or heads of aphids. These aphids were disregarded, but reared separately to see if development of the wasps would be possible.

Control aphids in each replicate were reared on the same shoot as their experimental counterparts. This was possible because aphids in each replicate consisted solely of the same colour morph. Control aphids had a different colour and were easy to distinguish. In each replicate two sets of differently coloured control aphids were used.

The first set of 10 control aphids was the same instar as their experimental counterparts. They were used to evaluate the mortality of larvae of *A. rosae* in parasitized aphids by using the mortality of control aphids as a standard. For each cage, the number of aphids that formed mummies, the number of parasitized aphids that survived parasitism or had died, and the number of surviving control aphids were recorded. Surviving aphids were dissected to check for developing larvae of *A. rosae*. The corrected mortality of *A. rosae* (M_C) was then calculated as

$$M_c = 1 - \frac{PC}{A_p C_s}$$

where P = number of emerging parasitoids, A_p = number of parasitized aphids, C_s = number of surviving control aphids, C = number of control aphids.

The second set of control aphids always consisted of 10 second instar aphids. Their adult body length was measured after 12 days and was used to evaluate the impact of nutritional variation of rose shoots among treatments and replicates.

Cages were checked for mummies approximately every 12 hours from day eight up to day 15 after parasitization. Surviving aphids were then dissected. Only three obviously parasitized aphids were reared further than day 15. Newly formed mummies were removed and kept separately in gelatine capsules. Mummies were checked for emergence of parasitoids approximately every 12 hours. Upon emergence parasitoids were killed in the freezer and measurements were taken in the next 48 hours. The size and form of mummy, sex and size of parasitoid, size of ovaries and number and size of eggs, developmental time and mortality of the parasitized aphids and time to emergence were recorded. The number of observations varied slightly for various reasons, e.g. the number of measured ovaries varied from the number of females because some ovaries were destroyed during dissection. In another case, the number of individuals did not correspond to the number of observations for 'time to mummification', because a few mummies were hidden in the cage (Chapter 3) and not found until day 15. Consequently their age was unknown and they were not used for the parameter 'time to mummification'.

Measurement of parasitoid size. Parameters often used to determine the relative size of parasitoids are the length of hind tibia, length of forewing and head width. Measurement of the body length is unreliable since the soft abdomen is subject to extension. Most accurate measurements can be achieved by taking the dry weight of the insect, but suitably precise scales were not available.

In a separate experiment body length (the distance from the front of the head to the tips of the ovipositor sheaths, b) length of hind tibia, c) length of forewing (the distance between wing base and the furthest marginal end of the wing), and d) width of head (the furthest distance between the outer edges of the compound eyes) were measured in 66 females of *A. rosae*. It was not known which of these would best represent the size of the parasitoid, but it was assumed that the measurement which exhibited the highest mean correlation coefficient with all others would represent the best linear index of size.

Measurement of ovary size and numbers of eggs. The ovaries of females were carefully removed from the abdomen and placed on a slide in a drop of water before a coverslip was placed over the specimen. A drop of methylene blue was applied to stain the ovaries and eggs. By measuring the length and width of the ovaries the two dimensional face of each ovary was determined and the average taken. It was assumed that the pressure of the coverslip would equally flatten the ovaries, resulting in a constant depth. Gentle pressure on the coverslip forced the ovary walls to burst and freed the eggs. Since Aphidiinae are synovigenic, the contents of ovaries consisted of a mix of fully developed eggs concentrated towards the posterior region and undeveloped eggs concentrated towards the anterior region of the ovary. Only those eggs which had the typical elongated, lemon-shaped form of mature *Aphidius* eggs were counted (Stary, 1970).

Analyses. Most data were analysed with SAS (SAS Institute, 1985) by multiway analysis of variance using the General Linear Models Procedure (GLM) for unbalanced data. Normally the raw data set was used, but an arcsine transformation of %-mortality data was undertaken to stabilise variances (Zar, 1984).

All Pearson correlation coefficients were calculated by SAS, using the CORR procedure. Data on the sex-ratio were analysed by χ^2-analysis.

4.3 Results

Parameter for parasitoid size. In a correlation analysis of different parameters for female size, Pearson correlation coefficients ranged from 0.79 to 0.92 (Table 4.1). Length of forewing and hind tibia were equally suitable for use as an index of size with a mean of 0.88, but since it was easier to measure the length of hind tibia, this was chosen as the indicator of size for *A. rosae*. Data exhibited homoscedasticity when residuals were plotted over the range of sizes.

Table 4.1 Comparison between Pearson correlation coefficients of measurements of size of female *Aphidius rosae* (n = 66).

Parameter of size	Hind tibia length	Forewing length	Head width	Body length
Hind tibia length	-	0.92	0.84	0.87
Forewing length	0.92	-	0.86	0.85
Head width	0.84	0.86	-	0.79
Body length	0.87	0.85	0.79	-
Mean	0.88	0.88	0.83	0.84

Size of parasitoids. In a General Linear Models Procedure (GLM) the dependence of parasitoid size, measured by the length of the hind tibia, was tested against the influence of aphid instar at time of parasitization, nutritional treatment, gender of parasitoid, replicate and interactions of factors (Table 4.2). It can be concluded that the size of the parasitoid was dependent upon interactions between the instar of the parasitized aphids and the treatment (plant nutrition and superparasitism) and between host instar and the gender of the parasitoid offspring. Females were larger than males in all cases (Fig. 4.1).

Table 4.2 Regression analysis at the effects of treatment, aphid instar of *Macrosiphum rosae* and wasp gender on **length of parasitoid hind tibia** of *Aphidius rosae*. Test of hypotheses using the type III MS for Inst x Gen x Treat as an error term in the SAS GLM procedure. Treatments were singly parasitized aphids reared on 'normal' rose quality, superparasitized aphids on 'normal' rose quality and singly parasitized aphids on 'poor' rose quality. Aphid instars consisted of five host classes : L1, L2, L3, L4 and adults. Two replicates were carried out.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
a) complete analysis					
Aphid instar (Inst)	4	0.30431	0.07601	96.90	0.0001*
Gender (Gen)	1	0.20747	0.20747	264.25	0.0001*
Treatment (Treat)	2	0.13505	0.06753	86.01	0.0001*
Inst x Treat	8	0.04471	0.00559	7.12	0.0059*
Inst x Gen	4	0.01818	0.00455	5.79	0.0173*
Gen x Treat	2	0.00405	0.00202	2.58	0.1367
Replicate	1	0.00002	0.00002	0.03	0.8772
Inst x Gen x Treat (error)	8	0.00628	0.00079	0.61	0.7723
b) reanalysis with non-significant terms omitted					
Inst	4	0.30981	0.07745	74.22	0.0001*
Gender	1	0.20758	0.20758	198.91	0.0001*
Treatment	2	0.13508	0.06754	64.72	0.0001*
Inst x Gen	4	0.01838	0.00460	4.4	0.0261*
Inst x Treat	8	0.04496	0.00562	5.39	0.008*
Inst x Gen x Treat (error)	10	0.01044	0.00104	0.81	0.62

Corrected total DF = 242
* = $p < 0.05$

The size of males increased steadily from host classes L1 to L4 with a continuous pattern : hosts reared on 'poor' plant quality < superparasitized hosts < singly parasitized hosts on 'normal' plant quality. Parasitoid size from hosts reared on 'poor' plant quality increased the least (Fig. 4.1a). In contrast, size did not differ between females emerged from hosts reared on 'poor' plant quality and females emerged from superparasitized hosts (Fig. 4.1b). Females displayed a steady increase in size from host class L1 to L4. Between L1 and L3, females emerged from hosts reared from 'normal' plant quality were larger than their counterparts reared from hosts from 'poor' plant quality or superparasitized hosts, but no differences were observed between treatments in the L4

class. Females reared in adult hosts became smaller than females reared from L4 hosts in all three treatments. The same was observed for males emerged from hosts reared on 'normal' plant quality but males from both adults reared on 'poor' plant quality and superparasitized adults were larger than their counterparts emerged from L4. The largest females and males from adult hosts emerged from superparasitized aphids.

To give an impression of size differences of *A. rosae* in this experiment, Fig. 4.2 shows small and large males and females.

Time to mummification. The time to mummification depended significantly upon the interaction between aphid instar and nutritional treatment (Table 4.3). *A. rosae* reared from singly parasitized aphids on 'normal' plant quality tended to reach mummification earlier than their counterparts emerged from superparasitized aphids and hosts reared on 'poor' plant quality, except those reared in adult aphids (Fig. 4.3). Parasitoids developing in adult hosts needed longer to reach mummification than parasitoids from L2, L3, and L4. The slowest development was observed for parasitoids developing in superparasitized L1 hosts and parasitoids reared from the L1 class growing on 'poor' plant quality.

There was a negative correlation between the time to mummification of males and their size ($n = 139$, $r = -0.507$, $p < 0.0001$) (Fig. 4.4a). Over the whole range of data, a hind tibia length of approximately 0.5 mm appeared to be a threshold for successful development. Smallest individuals which needed the longest for their development were concentrated between superparasitized L1 hosts and L1 aphids reared on 'poor' plant quality. The same pattern was detected for females (Fig. 4.4b). The threshold for minimal size was here just under 0.6 mm hind tibia length. A negative correlation between size and developmental time was less evident than for males, $r = -0.247$, $p = 0.012$, $n = 104$.

Table 4.3 Regression analysis at the effects of treatment, aphid instar of *Macrosiphum rosae* and wasp gender on **time to mummification** of *Aphidius rosae*. Test of hypotheses using the type III MS for Inst x Gen x Treat as an error term in the SAS GLM procedure. Treatments were singly parasitized aphids reared on 'normal' rose quality, superparasitized aphids on 'normal' rose quality and singly parasitized aphids on 'poor' rose quality. Aphid instars consisted of five host classes : L1, L2, L3, L4 and adults. Two replicates were carried out.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
a) complete analysis					
Aphid instar (Inst)	4	61.5028	15.3757	12.06	0.0018*
Gender (Gen)	1	0.38553	0.38553	0.30	0.5974
Treatment (Treat)	2	34.4364	17.2182	13.51	0.0027*
Inst x Treat	8	72.8554	9.10692	7.14	0.0058*
Inst x Gen	4	2.31822	0.57955	0.49	0.7672
Gen x Treat	2	0.07792	0.03896	0.03	0.97
Replicate	1	2.22751	2.22751	1.75	0.2227
Inst x Gen x Treat (error)	8	10.1970	1.27453	1.17	0.3156
b) reanalysis with non-significant terms omitted					
Inst	4	61.4695	15.3674	17.34	0.0001*
Treat	2	33.1297	16.5648	18.69	0.0001*
Inst x Treat	8	71.6921	8.9615	10.11	0.0001*
Inst x Gen x Treat (error)	15	13.291	0.88607	0.81	0.6647

Corrected total DF = 243
* = $p < 0.05$

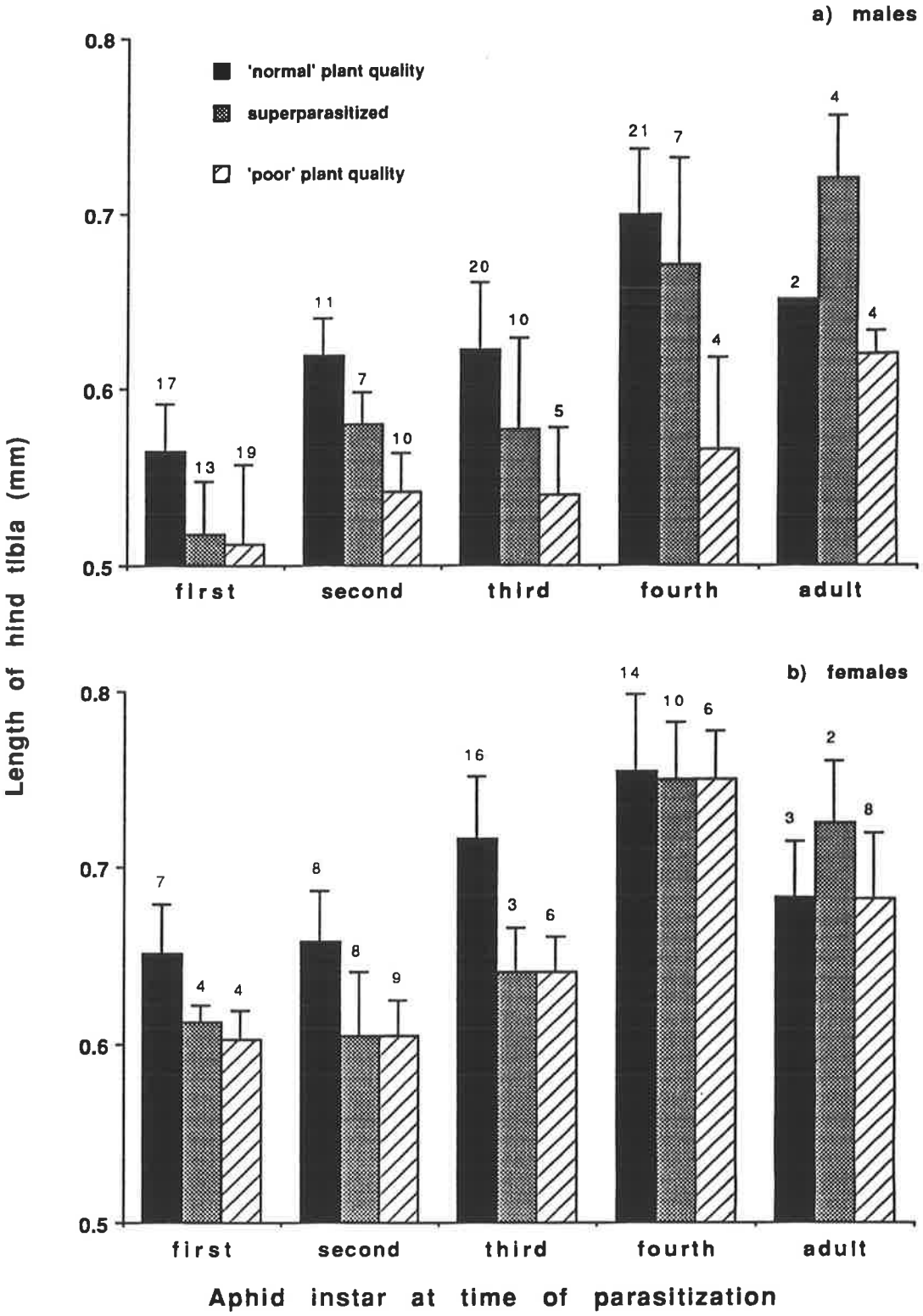


Fig. 4.1 The influence of different instars of *Macrosiphum rosae* and additional nutritional stress factors (superparasitism and 'poor' plant quality) on size of *Aphidius rosae*, measured by hind tibia length. N is given as numbers over columns. Error bars represent SD.

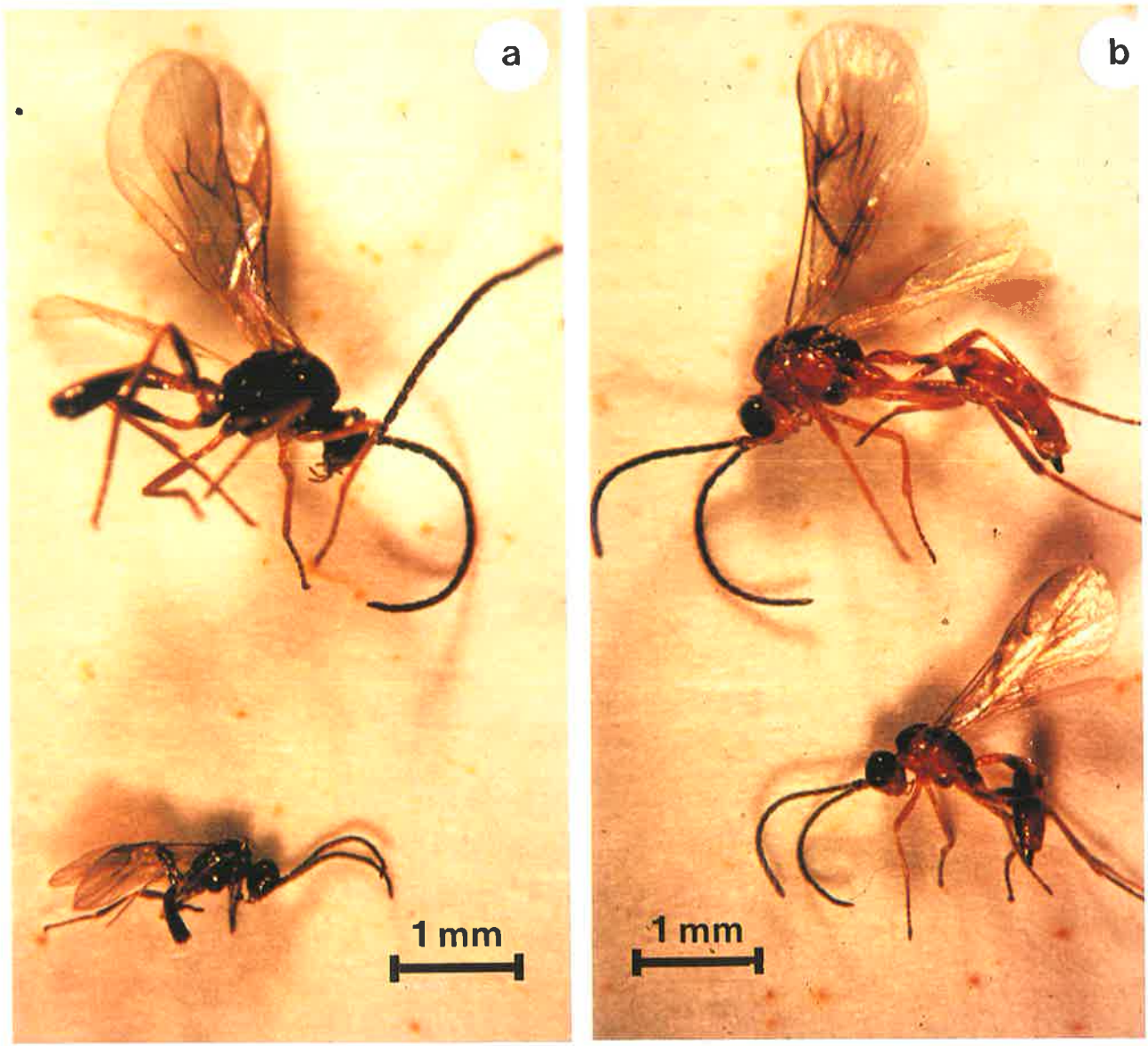


Fig. 4.2 Size differences between individuals of *Aphidius rosae*, reared on *Macrosiphum rosae*. Large individuals were reared from fourth instar aphids at time of parasitization on 'normal' plant quality whereas small parasitoids were reared from first instar aphids at time of parasitization on 'poor' plant quality, **a)** males **b)** females.

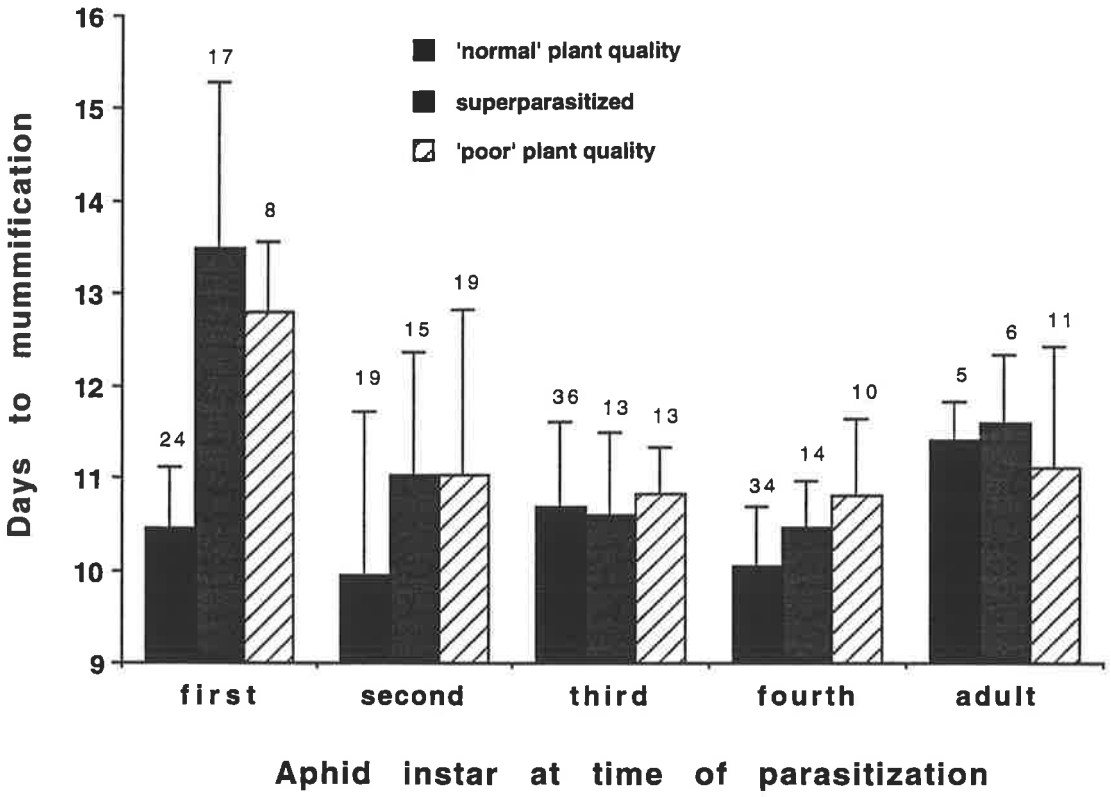


Fig. 4.3 The influence of different instars of *Macrosiphum rosae* at time of parasitization and additional nutritional stress factors (superparasitism and 'poor' plant quality) on larval developmental time of *Aphidius rosae*, measured by time from oviposition to mummification. N is given as numbers over columns. Error bars represent SD.

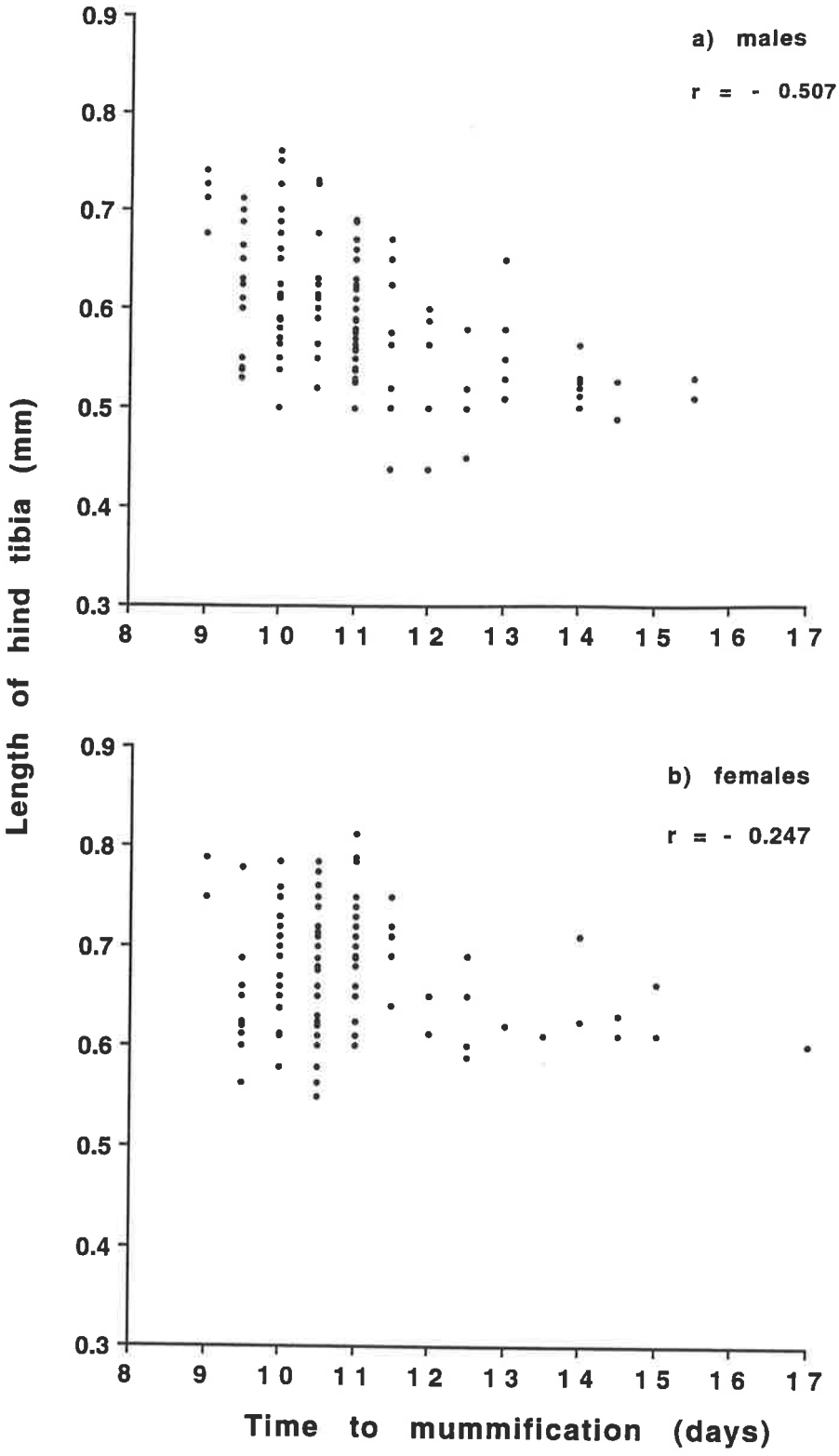


Fig. 4.4 Correlation between individual size of *Aphidius rosae*, measured by hind tibia length, and larval developmental time, measured by time from oviposition to mummification, **a)** males, n = 135, $p < 0.0001$, **b)** females, n = 104, $p = 0.012$.

Time to emergence. Time to emergence would have been a more complete measurement for pre-adult development but only 67 individuals (25 %) underwent a spontaneous development in this experiment, considered as emergence in less than 24 days. The remainder entered diapause. Because of such a small sample size, statistical analysis was not undertaken.

Size of ovaries and eggs, and number of eggs. The size of ovaries at time of emergence depended upon the interactions between aphid instars and nutritional treatment (Table 4.4). A positive correlation was found between the size of females and the size of their ovaries, $r = 0.708$, $p < 0.0001$, $n = 101$ (Fig. 4.5). Additionally, a correlation was observed between female size and the number of eggs at time of emergence, $r = 0.506$, $p < 0.0001$, $n = 100$ (Fig. 4.6). The GLM procedure revealed no significant influence of aphid instar, nutritional treatment or replicate on the number of eggs at time of emergence (Table 4.5). Since larger females contained larger ovaries and more eggs, the size of ovaries and number of eggs was also positively correlated, $r = 0.614$, $p < 0.0001$, $n = 101$. The number of eggs in emerging females was negatively correlated with time to mummification, $r = 0.235$, $p = 0.0206$, $n = 97$ (Fig. 4.7). The mean egg size was not significantly influenced by aphid instar or nutritional treatment (Table 4.6) and showed no significant correlation to parasitoid size, $r = 0.154$, $p < 0.128$, $n = 99$. The mean egg size was 0.1462 ± 0.0092 mm (SD) with a minimum of 0.12 mm and maximum of 0.158 mm.

Table 4.4 Regression analysis at the effects of treatment and aphid instar of *Macrosiphum rosae* on **the size of ovaries at time of emergence** of *Aphidius rosae*. Test of hypotheses using the type III MS for Inst x Treat as an error term in the SAS GLM procedure. Treatments were singly parasitized aphids reared on 'normal' rose quality, superparasitized aphids on 'normal' rose quality and singly parasitized aphids on 'poor' rose quality. Aphid instars consisted of five host classes : L1, L2, L3, L4 and adults. Two replicates were carried out.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
a) complete analysis					
Aphid instar (Inst)	4	0.46603	0.11651	6.97	0.0102*
Treatment (Treat)	2	0.12713	0.06357	3.8	0.0691
Replicate (Rep)	1	0.01413	0.01413	0.84	0.3849
Inst x Treat (error)	8	0.13377	0.01672	1.41	0.2039
b) reanalysis with non-significant terms omitted					
Aphid instar	4	0.59961	0.1499	4.42	0.0259*
Inst x Treat (error)	10	0.33949	0.03395	2.86	0.0041*

Corrected total DF = 100
* = $p < 0.05$

Table 4.5 Regression analysis at the effects of treatment and aphid instar of *Macrosiphum rosae* on **number of eggs at time of emergence** of *Aphidius rosae*. Test of hypotheses using the type III MS for Inst x Treat as an error term in the SAS GLM procedure. Treatments were singly parasitized aphids reared on 'normal' rose quality, superparasitized aphids on 'normal' rose quality and singly parasitized aphids on 'poor' rose quality. Aphid instars consisted of five host classes : L1, L2, L3, L4 and adults. Two replicates were carried out.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
Aphid instar (Inst)	4	23869.00529	5967.25132	3.06	0.0834
Treatment (Treat)	2	2933.45966	1466.72983	0.75	0.5020
Replicate	1	88.59709	88.59709	0.05	0.8178
Inst x Treat (error)	8	15600.76845	1950.09606	1.18	0.3235

Corrected total DF = 99.

Table 4.6 Regression analysis at the effects of treatment and aphid instar of *Macrosiphum rosae* on **size of eggs** of *Aphidius rosae*. Test of hypotheses using the type III MS for Inst x Treat as an error term in the SAS GLM procedure. Treatments were singly parasitized aphids reared on 'normal' rose quality, superparasitized aphids on 'normal' rose quality and singly parasitized aphids on 'poor' rose quality. Aphid instars consisted of five host classes : L1, L2, L3, L4 and adults. Two replicates were carried out.

Source	DF	Type III SS	Mean Square	F-value	PR > F
Aphid instar (Inst)	4	0.00041	0.0001	3.54	0.0605
Treatment (Treat)	2	0.0001	0.00005	1.89	0.2130
Replicate	1	0.00001	0.00001	0.24	0.6355
Inst x Treat (error)	8	0.00023	0.00003	0.34	0.9481

Corrected total DF = 99.

Sex-ratio. The sex-ratio of wasps emerging from the first instars was homogeneous between treatments, heterogeneity $\chi^2 = 0.892$, DF 5, $0.95 < p < 0.975$ and was male-biased (sex-ratio of 0.77, n = 64)(Fig. 4.8). This ratio varied significantly from a theoretical 1:1 ratio, $\chi^2 = 18.06$, DF 1, $p < 0.001$. Sex-ratio data from parasitoids emerging from second, third, fourth and adult classes were pooled since a heterogeneity chi-square analysis indicated homogeneity, heterogeneity $\chi^2 = 15.5826$, DF 21, $0.75 < p < 0.90$, n = 198. Pooled data did not vary significantly from a 1:1 sex-ratio, sex-ratio = 0.54, $\chi^2 = 0.6141$, DF 1, $0.25 < p < 0.5$.

Pooled data from first instars were then tested in a fourfold table against all other pooled data and differed significantly, $\chi^2 = 9.992$, DF 1, $p < 0.005$.

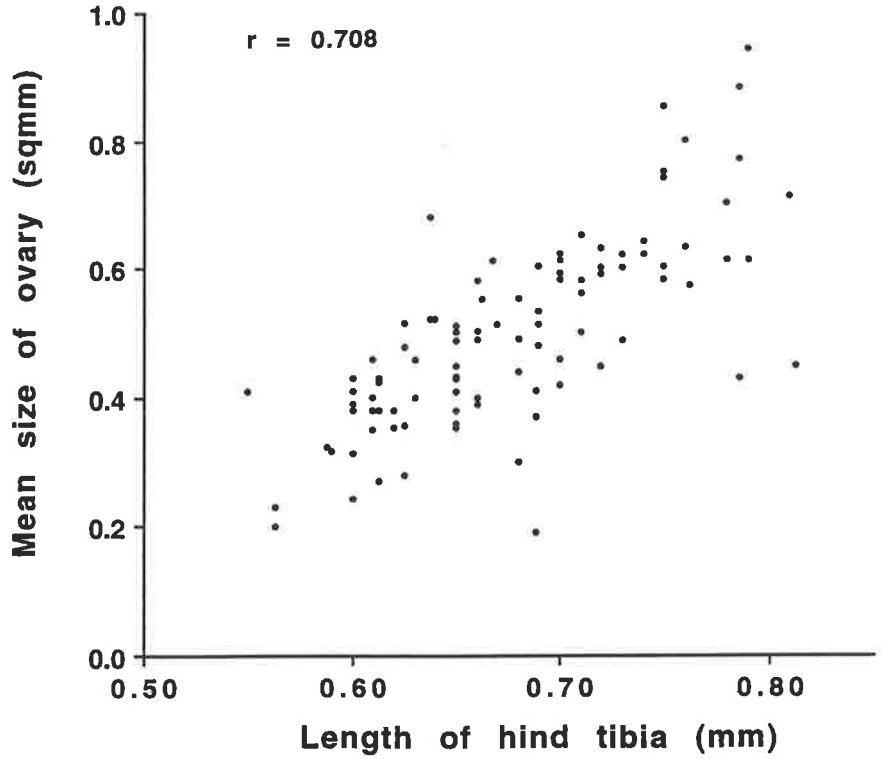


Fig. 4.5 Correlation between size of female *Aphidius rosae,* measured by hind tibia length, and mean size of ovaries at time of emergence, measured as two dimensional face under a coverslip; host = *Macrosiphum rosae*, n = 101, $p < 0.0001$.

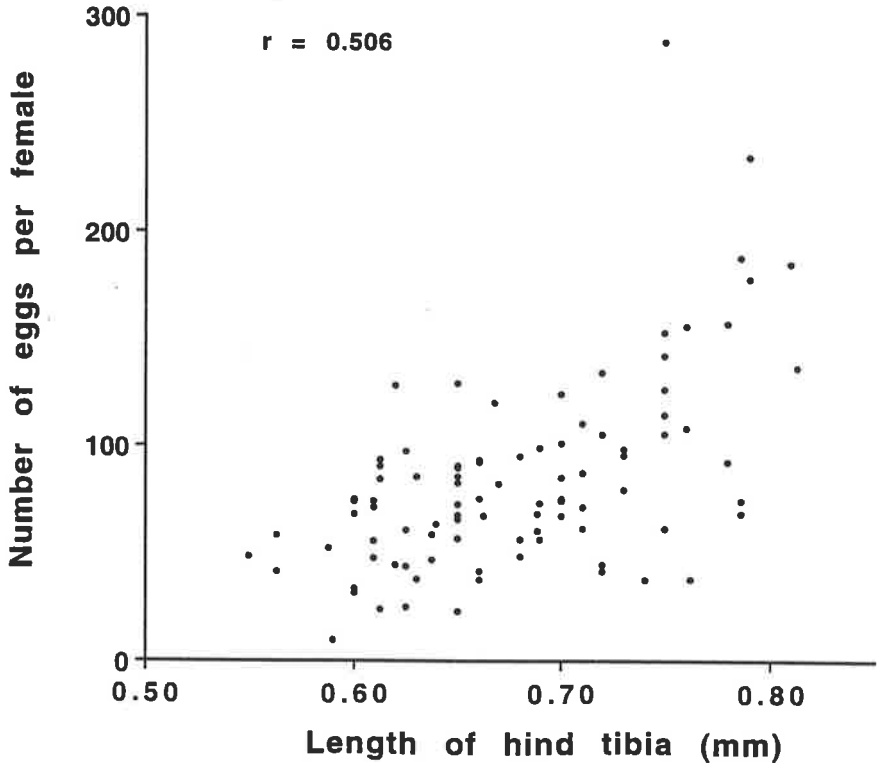


Fig. 4.6 Correlation between number of eggs per female at time of emergence and size of *Aphidius rosae* ; host = *Macrosiphum rosae*, n = 101, $p < 0.0001$.

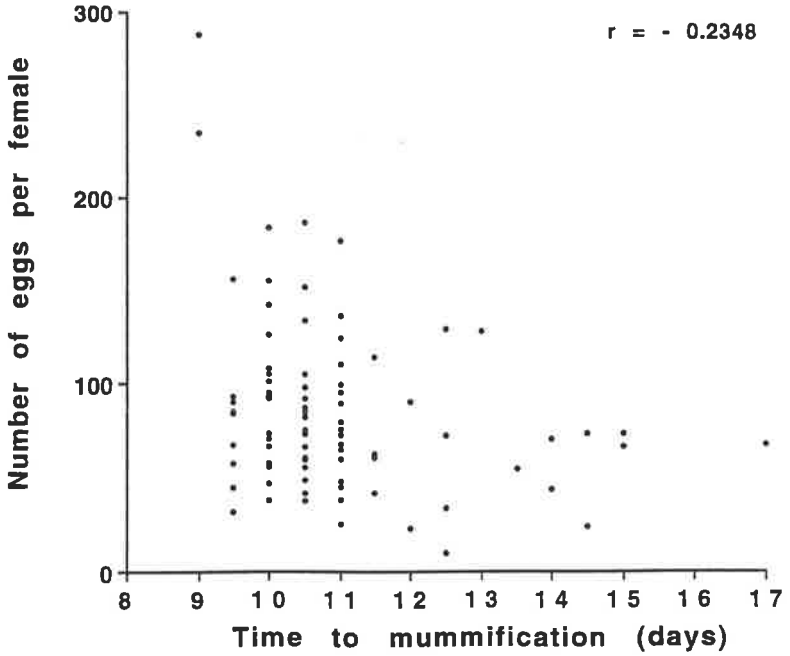


Fig. 4.7 Correlation between number of eggs per female of *Aphidius rosae* at time of emergence and larval developmental time, measured by time from oviposition to mummification; host = Macrosiphum *rosae*, n = 97, p = 0.0206.

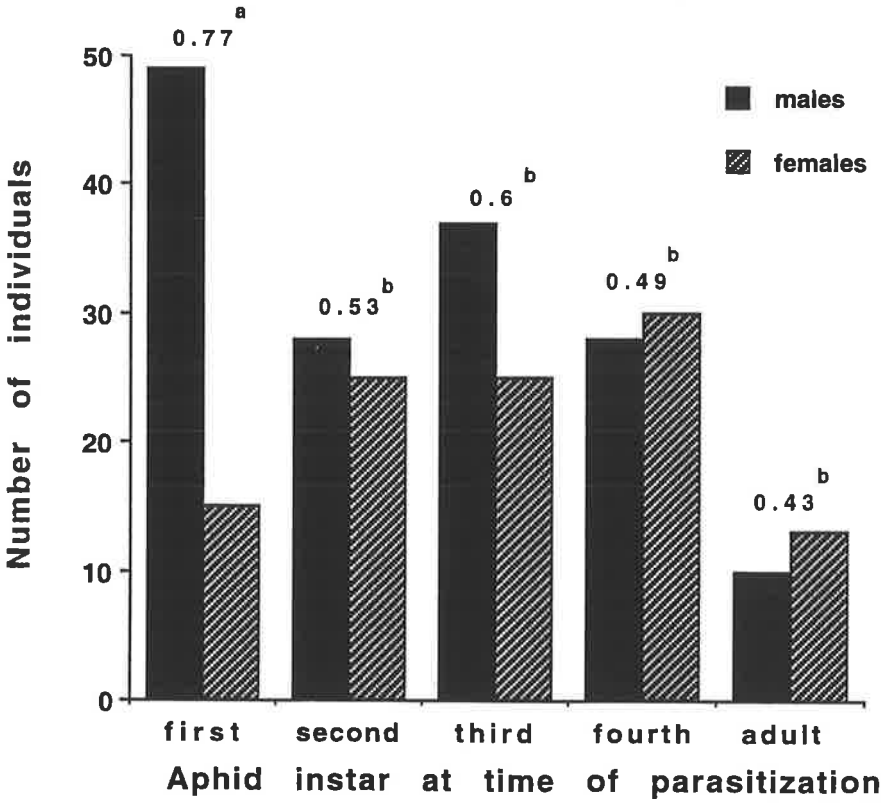


Fig. 4.8 Realised sex-ratio of emerging parasitoids of *Aphidius rosae* from different instars of *Macrosiphum rosae*. The sex-ratio is expressed as number over columns and defined as the proportion of males. Columns with same letters show no significant differences. For homogeneity of b : heterogeneity χ^2 =15.583, DF =21, 0.75 < p< 0.90, n = 198; b vs a, χ^2 = 9.992, DF 1, p< 0.005, n for a = 64.

Form and size of aphid mummies. Parasitized L1 hosts did not complete development to adulthood (Fig. 4.9). Most of them were killed by the growing parasitoid as fourth instars. 22 % did not even moult to the fourth instar and died as third instars. Parasitoids growing from second instars, also killed their host mainly as fourth instars. Only a few moulted to adults. More than half of the aphids parasitized as third instars reached adulthood, the rest died as fourth instars. Parasitized fourth instars moulted always to the adult stage. The width of a mummy was correlated with the size of the emerging parasitoid, $r = 0.788$, $p < 0.0001$, $n = 258$ (Fig. 4.10).

Mortality. At the end of the experiment, most parasitized aphids were mummified (Table 4.7). Aphids not forming mummies were mainly individuals which had died before the end of the experiment. The reason for death of these aphids was unknown. To obtain an indication how many of these deaths may have been caused by parasitism, e.g. oviposition trauma or nutritional exhaustion, a set of 10 unparasitized control aphids were reared on each shoot.

When aphids in the classes L1 to L4 were reared without nutritional stress factors, i.e. on 'normal' plant quality, 17 aphids from 134 parasitized aphids died (12.7 %) from unknown reasons, compared to nine dead aphids in 79 control aphids (11.4 %) (Table 4.7). This result demonstrated that under favourable rearing conditions premature mortality of *M. rosae* caused by parasitism can be neglected.

In addition to the number of aphids which died for reasons other than mummification, the number of surviving aphids without traces of parasitoid larvae were counted to estimate the overall mortality of *A. rosae*. 15 days after parasitization the 21 remaining aphids were dissected but no larvae of *A. rosae* were found. No significant differences in mortality of *A. rosae* were found between nutritional rearing factors but a GLM procedure revealed significant differences in levels of mortality between aphid instars and adults (Table 4.8). Mortality of parasitoid larvae was highest in the adult categories.

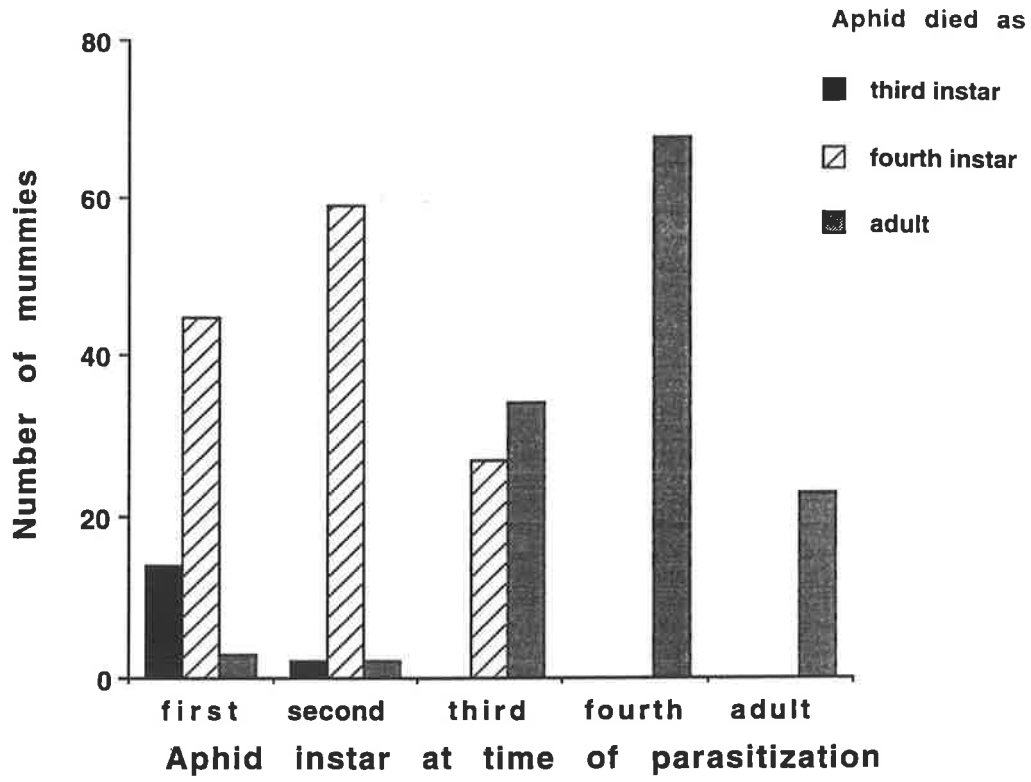


Fig. 4.9 Influence of *Aphidius rosae* on morphological growth capacity of its host *Macrosiphum rosae*, when parasitizing different instars, n = 262.

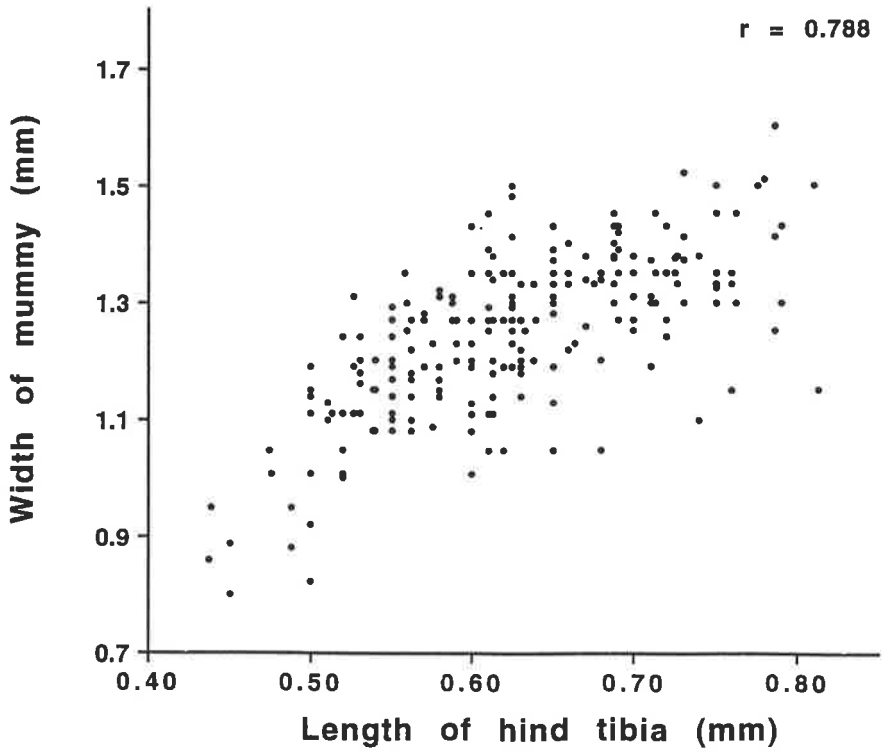


Fig 4.10 Correlation between width of mummies of *Macrosiphum rosae* and corresponding size of *Aphidius rosae*, measured by hind tibia length, n =258, p < 0.0001.

Table 4.7 Mortality of larvae of *Aphidius rosae* caused by death of the host or the parasitoid larva in different instars and adults of its host *Macrosiphum rosae*. Nutritional stress factors for the parasitoid were simulated by rearing hosts on 'normal' and on 'poor' plant quality, as well as using superparasitized aphids. The corrected mortality rate was obtained by using control aphids as standard (see text for details). Control aphids had the same age as parasitized aphids and were reared on the same shoot. They differed in their colour morph. Two replicates were carried out. The number of parasitized aphids varied between treatments and replicates. Mortality was determined 15 days after oviposition took place.

Aphid instar at time of parasitization	Nutritional variations of aphid rearing	Total number of parasitized aphids	Total number of mummies formed	Total number of surviving aphids	Total number of dead aphids	Total number of dead control aphids (no. of controls)	Corrected mortality, mean (rep 1/rep 2)
L1	'normal' plant quality (N)	27	24	1	2	2 (20)	0.13 (-0.11/0.37)
	N but superparasitized (S)	30	23	1	6	5 (20)	-0.03 (-0.02/-0.04)
	'poor' plant quality (P)	28	17	2	9	4 (20)	0.28 (0.37/0.19)
L2	N	28	19	2	7	4 (19)	0.21 (0.22/0.20)
	S	27	19	1	7	5 (20)	0.22 (0.06/0.37)
	P	19	14	1	4	3 (20)	0.13 (0.12/0.14)
L3	N	40	36	0	4	2 (20)	0 (-0.11/0.11)
	S	30	13	0	17	5 (20)	0.35 (0.58/0.11)
	P	23	13	2	8	4 (20)	0.31 (0.25/0.37)
L4	N	39	35	0	4	1 (20)	0.09 (0.05/0.12)
	S	19	10	0	9	1 (20)	0.35 (0.26/0.43)
	P	19	14	1	4	4 (20)	0.22 (0.22/0.22)
Adults	N	14	5	6	3	2 (15)	0.79 (1.0/0.58)
	S	13	6	3	4	3 (15)	0.79 (0.58/1.0)
	P	15	12	1	2	2 (15)	0.47 (0.19/0.75)

Table 4.8 Regression analysis at the effects of treatment and aphid instar of *Macrosiphum rosae* on **fraction of mortality** of *Aphidius rosae*, measured as death of host or non development of parasitoid 15 days after oviposition. Test of hypotheses using the type III MS for Inst x Treat as an error term in the SAS GLM procedure. Treatments were singly parasitized aphids reared on 'normal' rose quality, superparasitized aphids on 'normal' rose quality and singly parasitized aphids on 'poor' rose quality. Aphid instars consisted of five host classes : L1, L2, L3 L4 and adults. Two replicates were carried out. Data on fraction of mortality were corrected by using control aphids as standard (see text for details) and transformed to their arcsine.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
a) complete analysis					
Aphid instar (Inst)	4	2.469	0.61725	6.95	0.01*
Treatment (Treat)	2	0.0681	0.034	0.38	0.6936
Replicate (Rep)	1	0.0605	0.0605	0.68	0.4333
Inst x Treat (error)	8	0.71086	0.0889	0.87	0.5632
b) reanalysis with non-significant terms omitted					
Aphid instar	4	2.469	0.61725	6.21	0.0037*
Inst x Treat (error)	10	0.779	0.0779	0.78	0.6449

Corrected total DF = 29
* = $p < 0.05$

Size of control aphids. 12 days after the parasitization of experimental aphids, the body sizes of control aphids, reared on the same shoots as parasitized aphids, were measured and data were used as an indication of differences in plant quality (Table 4.9). A GLM procedure revealed significant differences between nutritional treatments (Table 4.10) but not between instars or replicates. Control aphids reared on 'normal' plant quality were bigger than their counterparts reared on 'poor' plant quality (mean of 2.69 cm compared to a mean of 2.32 cm). The largest aphid size was 3.05 cm.

Successful oviposition in appendages. Oviposition in head and femur of aphids was successful. 10 mummies formed out of 16 parasitized aphids.

Table 4.9. Size of control aphids. As control, 10 second instars of *Macrosiphum rosae* were placed together with aphids parasitized by *Aphidius rosae* (different colour morph) on shoots of *Rosa sp.*, var. Tea hybrid 'McGredy's sunset'. Aphids from the same replicate were reared on the same shoot. Two replicates were used per experimental variation. After 12 days the body length of surviving control aphids were measured. Under the assumption that plant quality would be reflected in aphid growth, control aphids were meant to detect irregularities in plant quality.

Aphid instar at time of parasitization	Nutritional variations of aphid rearing	Number of surviving control aphids	Mean size in cm (SD)	Size Min./Max. in cm
	'normal' plant quality (N)	15	2.65 (0.15)	2.46/3.05
L1	N but superparasitized (S)	14	2.66 (0.16)	2.29/3.00
	'poor' plant quality (P)	15	2.35 (0.10)	2.22/2.51
	N	13	2.75 (0.15)	2.49/3.00
L2	S	14	2.66 (0.10)	2.45/2.81
	P	11	2.28 (0.12)	2.11/2.50
	N	13	2.69 (0.13)	2.51/2.90
L3	S	11	2.68 (0.12)	2.51/2.91
	P	12	2.30 (0.13)	2.11/2.56
	N	11	2.70 (0.13)	2.56/3.00
L4	S	14	2.77 (0.11)	2.64/3.00
	P	16	2.34 (0.09)	2.18/2.46
	N	15	2.68 (0.15)	2.46/3.00
Adults	S	12	2.70 (0.10)	2.56/2.90
	P	13	2.32 (0.10)	2.20/2.50

Table 4.10 Regression analysis at the effects of treatment and aphid instar of *Macrosiphum rosae* on body size of control aphids. Test of hypotheses using the type III MS for Inst x Treat as an error term in the SAS GLM procedure. Treatments were singly parasitized aphids reared on 'normal' rose quality, superparasitized aphids on 'normal' rose quality and singly parasitized aphids on 'poor' rose quality. Aphid instars consisted of five host classes : L1, L2, L3, L4 and adults. Two replicates were carried out.

Source	DF	Type III SS	Mean Square	F-value	Pr > F
a) complete analysis					
Aphid instar (Inst)	4	0.069878	0.014747	0.79	0.5619
Treatment (Treat)	2	6.186955	2.062318	93.54	0.0001*
Replicate (Rep)	1	0.047891	0.047891	2.17	0.1787
Inst x Treat (error)	8	0.176374	0.022047	1.46	0.173
b) reanalysis with non-significant terms omitted					
Treatment	2	6.198318	2.066106	106.55	0.0001*
Inst x Treat (error)	12	0.232692	0.019391	1.27	0.2378

Corrected total DF = 199
* = $p < 0.05$

4.4 Discussion

Size, fecundity and developmental time. Experiments confirmed that host quality was dependent on aphid instar at time of parasitization. With increasing instar at time of parasitization, aphids became more suitable for parasitoid growth, with an overall increase in parasitoid size and fecundity from L1 to L4 and a decrease in developmental time. Development was also affected by nutritional stress factors after oviposition took place. It can be concluded that growth of *A. rosae* was susceptible to changes in nutritional quality.

Parasitoids growing in aphids feeding on 'normal' plant quality grew larger and faster than their counterparts in superparasitized aphids and aphids feeding on 'poor' quality plants. Except of in the first instar, 'poor' plant quality put more developmental stress on to the developing parasitoid larvae than superparasitism. Two developing and competing parasitoid larvae inside the tiny first aphid instar might over exploit the growing host and restrict its growing capacity disproportionately compared to other instars. However, in the field, the effects of superparasitism may be less pronounced. E.g. in this experiment, superparasitism occurred in less than 10 minutes so both larvae developed and could have competed for nutrition. Under field conditions superparasitism might occur as much as 24 hours or more apart and therefore might have little or no influence, since growth of the second larva could be greatly restricted, as shown e.g. for *A. ervi* (Micha et al., 1992).

Fecundity, as indexed by ovary size and number of eggs at eclosion was positively correlated with parasitoid size. In contrast, Sequeira & Mackauer (1994) found a steady increase in size of *A. ervi* when parasitizing different instars of the pea aphid (L1<L2<L3<L4), but this increase in size did not reflect its life-time fecundity (L1<L3<L4<L2) or its fecundity after day three (L1<L4<L3<L2). Females of *A. ervi* emerging from L4 showed the lowest number of mature eggs at eclosion.

A. rosae was able to develop in the whole range of aphid developmental stages under different nutritional conditions, an ability which certainly increases flexibility of host exploitation and enhances the potential of biological control in the field.

Host exploitation must be considered in light of the life-history strategy of a species (e.g. Godfray, 1994). Superparasitized first instar aphids and first instar aphids growing on 'poor' host plants appeared to be less suitable for parasitization, presumably because they did not provide sufficient nutrition for the growing parasitoid larvae. This suggests that the larvae of *A. rosae* have some minimal nutritional requirements for development. If the host can not provide these, development of *A. rosae* is inhibited, therefore allowing the aphid to feed for a longer time and gain additional nutrition. This seemed to overcome a threshold of minimal size. As soon as this was achieved, a trade off from developmental time against size was not evident. A negative correlation between size and time to mummification suggested that parasitoid larvae which grew in hosts that provided enough resources to grow large also developed faster. This was also supported by the corresponding negative correlation between the number of eggs at emergence, plotted against time to mummification. The same life-history strategy was observed for *A. ervi* (Sequeira & Mackauer, 1992b) and *E. californicus* (Sequeira and Mackauer, 1993). In both studies, the optimisation of body mass took precedence over the minimisation of development time below a size threshold, but above this threshold, additional nutritional host resources were used to decrease development time as well as to increase body mass.

Developing faster is not only an advantage in terms of improved rate of population increase (Lewontin, 1965; Mackauer, 1986)(Chapter 5), but also helps to escape hyperparasitism. A female resting underneath a leaf, and thereby maturing her eggs, might be safer than a counterpart displaying a strategy of prolonged larval development in order to utilise more host nutrition while at the same time being exposed longer to hyperparasitism or predation.

It would have been desirable to analyse additional data on the time to emergence of *A. rosae*, but only 67 individuals underwent spontaneous development. The

experiment was performed in May, towards the end of autumn, a time of the year when a large percentage of parasitoids enters diapause in the field (Chapter 10). At this time many individuals of the laboratory culture of *A. rosae* entered diapause as well (Chapter 3). The room in which the experiment was undertaken was partially illuminated by sunlight through bright windows so the external photoperiod could have influenced the incidence of diapause. Since the time to mummification was positively correlated with the time to emergence (Chapter 5), time to mummification should be considered as a reliable indicator of developmental time.

Adult hosts. Adult size, unsettled behaviour of hosts under experimental conditions in the gelatine capsule, and the associated strong defence and escape behaviour of the host made it difficult to obtain sufficient numbers of parasitized adults. Therefore, the interpretation of adult suitability is limited by the availability of data. However, there was a higher proportion of adult aphids without any traces of larva parasitoid growth. For parasitoids growing alone in adult aphids on 'normal' and 'poor' plant quality, it appeared that adults were less suitable than fourth instars, as indicated by prolonged developmental times and decreased sizes. Similarly, Mackauer & Kambhampati (1988) found that only 40 % of parasitized viviparous pea aphids became mummified when parasitized by *A. smithi* and parasitoids eclosed from only 60 % of them. These results confirmed findings from Mackauer (1973) and Fox et al. (1967a), who showed that reproductive pea aphids were less suitable for parasitism of *A. smithi*. Only six parasitoids emerged from superparasitized adults but they developed faster and became larger than their counterparts reared in singly parasitized adult aphids. It may be speculated that superparasitism helps *A. rosae* to overcome the obviously enhanced host defence of adults *M. rosae*.

Sex-ratio. The sex-ratio of parasitoids emerging from first instar aphids was 0.77 and differed significantly from a sex-ratio of 0.54, displayed by wasps emerging from the other instars. These results conform to the often observed pattern for Aphidiinae producing more males in less suitable instars. This male-biased sex-ratio was not related to higher mortality of larvae during their development. Therefore females of *A. rosae* may be able to recognise host quality and can make decisions to oviposit fertilised or unfertilised eggs. Cloutier et al. (1991) found that differential preadult mortality of *E. californicus* had no effect on the primary sex-ratio under given experimental conditions. This experiment indicated that a growing host can represent a reliable resource that is predictable from its initial size. Mackauer & Kamphambati (1988) supported this view by allowing *A. smithi* to parasitize embryos of *A. pisum* still inside the mother. Assuming the females did not perceive the embryos as distinct hosts, and that the female was able to make a choice to lay a fertilised or unfertilised egg, the sex-ratio was not significantly different from the sex-ratio that was observed in adult viviparous aphids. The extremely small host size would have influenced the sex-ratio strongly if any differential pre-emergence survival rate would have operated between the sexes. However, if females can actively determine the sex of their offspring, then the quality of an aphid at time of parasitization may be crucial for the decision and results may vary greatly with rearing conditions. Additionally, females with different experience or different egg-loads might produce different sex-ratios in the same quality of hosts. Therefore, results should be seen as qualitative only, not absolute. E.g. in a pilot experiment, the sex-ratio of *A. rosae* emerging from the first instar was 0.5, whereas the sex-ratios of parasitoids emerging from other instars were female-biased.

Form of mummies. The form of mummies can indicate the aphid life stage at time of parasitization. When *A. rosae* parasitized younger instars, most of them did not reach adulthood. Furthermore, the width of a mummy was correlated with the size of the emerging parasitoid. Both measurements can be a useful tools in the field, e.g. to obtain

data about the most commonly parasitized host instars (Cloutier et al., 1981) (Chapter 10).

This experiment revealed aspects of the nutritional interaction between parasitoid, host and host plant. *A. rosae* was significantly influenced by declining host quality, as indicated by decreasing body and ovary size, decreasing egg load and an increase in larval developmental time. Therefore, host quality is a crucial factor in experimental design and experimental results depend upon host quality. E.g. in experiments on *A. ervi* and its host *A. pisum*, Sequeira & Mackauer (1986) measured mean dry weights between 238 and 369 µg for *A. ervi* when emerging from aphids which were parasitized as L1 to L4. The same authors, using the same species and same methods, measured in comparable experiments mean dry weights between 154 to 212 µg for *A. ervi* (Sequeira & Mackauer, 1994) and another time they obtained a mean dry weight range of 155 to 247 µg (Sequeira & Mackauer, 1992b). This variation was also reflected in the developmental times. Detailed interpretation might be difficult if standardisation is troublesome. Aphids, which feed on plant sap, are sensitive to variation in the quality of the host plants. Because of these constraints, discussion of the influence of host quality on *A. rosae* should be restricted to qualitative interpretation.

The use of rose shoots kept fresh in water mixed with Flower Fresh™ for a limited number of days proved to be a sufficient method for obtaining rose aphids with sizes comparable to those in the field. The results reported in this chapter provided an essential foundation for future experimental work with *A. rosae*, and also helped to develop a practical rearing program for *A. rosae* used in biological control. This was especially important for rearing insects to be released into the field. Insufficient host quality might not necessarily result in the death of the parasitoid but can drastically decrease individual fitness.

Fecundity and intrinsic rate of increase of *Aphidius rosae* and *Macrosiphum rosae*

5.1 Introduction

Among other attributes, high fecundity is commonly considered an important attribute of an effective biological control agent (e.g. Stary, 1970). High reproductive rates have been one of the major reasons why Aphidiinae are considered to be relatively successful as biological control agents. Aphidiinae are synovigenic (Stary, 1988) and eggs mature in the ovaries each day. Therefore, simple dissection and counting of eggs in the ovaries after the emergence of females may greatly underestimate their total fecundity. E.g. females of *Ephedrus californicus* Baker emerged with 250 eggs in their ovaries but realised a life-time fecundity nearly five times higher (Cohen & Mackauer, 1987).

Considerable variation in reproductive rates have been found both among species and within species of Aphidiinae (Table 5.1). The realised fecundity of Aphidiinae depends very much on the experimental methods used and Cohen & Mackauer (1987) considered low fecundities only as a consequence of unsuitable methods. E.g. Collins & Dixon (1986) demonstrated that the oviposition rate of *Monoctonus pseudoplatani* Marshall decreased rapidly over time when parasitoids were searching in only one experimental arena but returned immediately to initial rates when they were allowed to enter a new arena with new aphids. Additionally, Mackauer (1983a) achieved different mean life-time fecundities between 192 and 870 eggs/female by offering different host densities to *A. smithi*. In general, the life-time fecundity of Aphidiinae can range from several hundred to more than 1000. Fecundity may vary with several factors, such as female size (e.g. Liu, 1985)(Chapter 4), temperature (e.g. Force & Messenger, 1964) and host species (Powell & Wright, 1988).

Table 5.1 Experimental realised life-time fecundity of Aphidiinae. Results are expressed in gross numbers of laid eggs per female including super numerous eggs, in net numbers of laid eggs excluding super numerous eggs, or in the number of realised offspring.

Species	Life-time fecundity	Gross/Net	Reference
Aphidius colemani (Viereck)	316 eggs	net	Prinsloo et al., 1993
Aphidius smithi Sharma & Subba Rao	30-60 offspring	net	Wiackowski, 1962
A. smithi	870 eggs	gross	Mackauer, 1983a
Aphidius nigripes Ashmead	100-600 offspring	net	Cloutier et al., 1981
Ephedrus californicus Baker	1193 eggs	gross	Cohen & Mackauer, 1987
Ephedrus cerasicola Stary	961 eggs	gross	Hågvar & Hofsvang, 1990
Praon exsoletum Nee (= *palitans* Muesebeck)	578 eggs	gross	Force & Messenger, 1964
Trioxys complanatus Quilis (= *utilis* Muesebeck)	845 eggs	gross	Force & Messenger, 1964

Most species of Aphidiinae reach their maximum daily fecundity in the firsts days after emergence and then their daily reproduction rate declines rapidly (Stary, 1988; Hofsvang, 1991).

The fecundity of a biological control agent gives an indirect indication of its potential population increase in the field. However, without additional information on the death rate, or survival rate, and speed of development, the total life-time fecundity is not very informative. The construction of age-specific life tables are commonly used to estimate predictive statistics on population increase (e.g. Andrewartha & Birch, 1954;

Southwood, 1978a). From such tables, the intrinsic rate of increase r (also called the innate capacity for increase r_m or instantaneous rate of natural increase) can be calculated which allows comparison of potential growth rates among species.

An approximate method for the determination of the intrinsic rate of increase was detailed by Andrewartha & Birch (1954)

$$r = \log_e R_0 / T$$

where R_0 is the net reproductive rate per female (fraction of original cohort alive at day_x of pivotal age [l_x] * progeny produced at day_x [m_x])

$$R_0 = \sum l_x m_x$$

and where T represents the mean duration of a generation.

$$T = \sum x\, l_x m_x / \sum l_x m_x$$

This formula can led to considerable error in short life cycles and Birch (1948) used an alternative form of the exponential growth model to estimate r.

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

Using a computer, trial r values are substituted into the expression until the left hand-side is (arbitrarily) close to one. This formula is commonly used for computing r (Laughlin, 1965).

The intrinsic rates of increase for several aphidiine species are given in Table 5.2.

It is very unlikely that parasitoids are able to display their full reproductive potential in the field (Gilbert & Gutierrez, 1973). Mortality of adults in the laboratory is low but is expected to be considerably higher under field conditions. However, since Aphidiinae lay most of their eggs during the early days of their life, death will not lower r substantially, provided that at least some successful reproductive period takes place. Mackauer (1983a) showed that r for *A. smithi* was mainly determined by fecundity in the early days of reproductive activity. It decreased from r = 0.358 for a mean

experimental longevity of seven days, to $r = 0.355$ for females with only four reproductive days to 0.334 for females with a longevity of only three days.

Table 5.2 Intrinsic rate of increase (r) in Aphidiinae. r is expressed as females/female/day.

Species	r †	°C	Reference
Aphidius ervi Haliday	0.316 - 0.326	18.3°C	Botto et al., 1993
Aphidius smithi Sharma & Subba Rao	0.358	20.5 °C	Mackauer, 1983
Ephedrus californicus Baker	0.371	23°C	Cohen & Mackauer, 1987
Ephedrus cerasicola Stary	0.29	21°C	Hågvar & Hofsvang, 1990
Praon exsoletum Nees (= *palitans* Muesebeck)	0.12	18°C	Force & Messenger, 1964
Trioxys complanatus Quilis (= *utilis* Muesebeck)	0.27	18°C	Force & Messenger, 1964
T. complanatus	0.38	21.1°C	Force & Messenger, 1964

† The sex-ratio for *T. complanatus* is 0.59 whereas sex-ratios in other studies are 0.5.

The experimental study of r of an aphid parasitoid can exclude most mortality factors but it can not exclude aphid defence. Aphids can release a cornicle wax through their siphunculi, which is both the carrier of alarm pheromones (Dixon, 1985) and a defensive weapon (Edwards, 1966). The release of this cornicle wax is under reflex control. Touching an aphid elicits the release of a drop of fluid that is composed of lipid droplets dissolved in water (Strong, 1967). Drops may not simply appear on top of the siphunculi but sometimes are ejected under pressure. These drops are very sticky and

rapidly crystallise due to supercooling in seconds (Edwards, 1966). Parasitoids or even small predators e.g. first instar syrphid larvae can be stuck to the plant or their locomotion can be severely impaired. Edwards (1966) hypothesised the potential effectiveness of this defensive mechanism. In contrast, Goff & Naut (1974) considered aphid defence by cornicle excretion as ineffective against species of the genus *Aphidius*, since oviposition in this genus takes place in less than a second. By the time the cornicle wax appears, the oviposition attack of the wasp may already be finished. Overall, the defensive capacity of cornicle wax has received less attention than other means of aphid defence, such as kicking or dropping, e.g. Gross (1993).

In this chapter comparisons are made between the intrinsic rates of increase of *Macrosiphum rosae* L. and its parasitoid *Aphidius rosae* Haliday. The influence of parasitoid size on the life-time fecundity and *r* of *A. rosae* was investigated. Additionally the impact of cornicle wax on parasitoids was noted.

5.2 Methods

The experiment was undertaken in the laboratory at 18 ± 2°C, 65 r.h., 16L:8D.

5.2.1 Methods for *M. rosae*

Adults of *M. rosae* were collected from the field from *Rosa sp.*, Tea hybrid 'McGredy's sunset', on which aphids were reared in the experiment. Aphids were allowed to give birth to offspring for 12 hours before they were removed. These offspring were reared until day 12, when all of them had moulted to adults. Freshly moulted adults were selected for the experimental determination of age-specific survival and fecundity on day 10 (the expected average time aphids needed to moult to adulthood)

Their offspring were counted and removed at 24 hr intervals. Shoots were replaced every four days. This procedure continued until the death of the aphid.

5.2.2 Methods for *A. rosae*

Ten mated females of *A. rosae* were randomly collected from the laboratory culture which, by this time, was already regularly supplemented with individuals from the field (Chapter 3). The parasitoids were given access to approximately 700 first to fourth instar hosts on rose shoots in cylindrical gauze cages for eight hours. Parasitoids were then removed and aphids were reared for 16 days. Newly formed mummies were collected daily from day 10 onwards, when the first mummy appeared. They were stored in gelatine capsules (1ml) until emergence which was recorded daily.

At the morning of day 18, 20 newly-emerged females were collected. As expected from previous results (Chapter 4), the rearing method had produced a range of female sizes. The whole range of sizes was presented in the experiment. Females were supplied with water and honey throughout their life-time. They were observed to mate once in gelatine capsules (Chapter 3).

To provide females with aphids, adults of *M. rosae* were collected from the field and reared in cylindrical gauze cages (Chapter 3). They were set up on a rose shoot for 24 hours and then transferred to a new cage. All offspring had a defined age of 0-24 hours when their parents were transferred. These offspring were reared until they reached the third instar (~ five days). Only third instars of *M. rosae* were used in the experiment because they represented a compromise between age (parasitoids prefer younger instars for oviposition [Chapter 10]) and relatively easy handling (first and second instars are impossible to handle in large quantities without causing considerable mortality). Third instars were settled for 12 hours on rose shoots before parasitoids were released into the cylindrical gauze cages.

Parasitoids were provided with 80 hosts per day, divided into two equal groups. 40 hosts were exposed to parasitoids for 10 hours during a 'day' period and the same

number of aphids was used during a 14 hours 'night' period. Since the laboratory conditions provided light for 16 hours, the 'night' period included four hours light in the evening and additional two hours light in the morning. This procedure was chosen because Mackauer (1983a) demonstrated that less than 80 aphids / day resulted in significant decrease of daily fecundity of *E. californicus* and Collins & Dixon (1986) showed the importance of the regularly replacement of exposed hosts. After parasitoids were removed, combined 'day' and 'night' aphid colonies were reared on rose shoots in gauze sleeve cages (Chapter 3) for five days when first instar larvae of *A. rosae* were easily detected. Aphids were killed and stored in the freezer before dissection took place.

These procedures started at the second day of the experiment and were repeated daily throughout the life-time of parasitoids. During the first day females were exposed to one group of 80 aphids for 18 hours until 8 o'clock in the morning of the following day.

Aphids were dissected under a stereo microscope. 40 randomly chosen aphids were dissected for each parasitoid and day. Random selection was achieved with a pie-counting grid and random number table (Cavalli-Sforza, 1980). The number of parasitized aphids and the number of larvae were noted.

After parasitoids died, the lengths of their hind tibias were measured and the presence of aphid cornicle wax was noted.

In the experiment present, a range of sizes of *A. rosae* was used to find any correlation between size and life-time fecundity and *r*, as indicated in the results from Chapter 4. Since the formula for *r* takes average fecundity, survival rate and developmental time into account, there seemed to be no reason why this parameter can not be used for calculating individual *r* values. Rather than obtaining a distribution around the mean a distribution around a slope was expected. In this case, the survival rate l_x can only be 1 or 0 for individuals and the formula for *r* can therefore be reduced.

$$\sum e^{-r\,x}\, m_x = 1$$

The estimation of *r* was undertaken with EDPOP (Roger Laughlin, University of Adelaide, Department of Crop Protection), a population growth program. Only the gross reproductive rate of females was used in the statistics. For the calculation of *r*, the number of eggs was adjusted to the sex-ratio observed in the field and in the laboratory (Chapter 3 and 10). Numbers of eggs were multiplied by 0.6 to obtain values for female offspring/female/day. For a better standardised comparison of different intrinsic rates of increase it is common practise to set the age-specific survival rate to 1.0 until individuals reach reproductive age.

The size of the hind tibia of 194 females of *A. rosae*, captured at irregular intervals from the field during 1993/94, were measured and used to estimate the average size of *A. rosae* in the field.

Statistical analysis. To determine the Pearson correlation coefficient (r) for data, the SAS CORR procedure was used (SAS, 1985). An arcsine transformation of data was undertaken in one case, where the fraction of super numerous eggs was correlated with the total number of eggs oviposited (Zar, 1984).

The differences between the median of male and female times to emergence were analysed with a two-sample t-test after an F-test revealed unequal variances.

5.3 Results

M. rosae. The aphid needed an average of 9.98 days ± 0.79 (SD) to moult to adulthood. 20 out of 30 females, started to give birth in low numbers on the same day that they moulted. The number of births peaked on day 3 (day 13 of the pivotal age) with an average of 6.57 births per female, followed by a rapid decline to around four offspring/day. For the next seven days the daily fecundity was relatively stable and then declined steadily (Fig. 5.1a). Long-lived aphids did not reproduce during the last days of their life. Dissections of the dead individuals revealed that no developed embryos were present, except in two aphids that carried one each. The mean life-time fecundity of *M.*

rosae was 81.59 ± 19.69 (SD), the net reproductive rate (R_0) was 78.85 and the mean duration of generation (T) was 18.48 days. Aphids lived up to 31 days (41 days of pivotal age) at 18°C and mortality was nil until day 15 (25 days of pivotal age) (Fig. 5.1b). The intrinsic rate of increase (r) was 0.319. The generation doubling time (DT) was calculated as $DT = \ln 2 / r = 2.22$ days (Andrewartha & Birch, 1954).

A. rosae. Daily dissections of small proportions of parasitized aphids were undertaken and under given rearing conditions, the larvae of *A. rosae* were detected for the first time five days after oviposition took place. *A. rosae* needed on average 11.7 ± 1.6 (SD) days to mummify, n = 279. 27 mummies did not display spontaneous development and were disregarded. The individual time to emergence correlated with the time for mummification, r = 0.89 for males, n = 116, and r = 0.91 for females, n = 136. The first male emerged 15 days after oviposition, whereas the first females did not emerge before 16 days (Fig. 5.2). Males emerged on average after 18.1 days (variance 2.97) whereas females emerged in average after 18.6 days (variance 1.94). A two-sample t-test for unequal variances revealed that the means were significantly different, t = 2.32, p = 0.02. Both sexes had their peak of emergence on day 18.

Aphid defence by cornicle wax complicated the experiment . Two females were found dead after seven and 10 days. They stuck to the plant. Another female stopped laying eggs after five days (as dissections of aphids revealed later on) but kept living up to day 16. An examination under the stereo microscope revealed that her ovipositor sheaths were stuck together by aphid secretions and the ovaries were filled with eggs. Because of their relatively short reproductive period, these three females were not considered for the correlation analysis of life-time fecundity. Interestingly, these were the largest females.

After death, all females were checked for any impact of aphid defence by cornicle wax. Only eight parasitoids had no sign of aphid wax on them (Table 5.3). All together five females died directly under the influence of the waxy secretion which glued them on to the plant or stuck over their whole body and which crippled them

completely (Fig. 5.3.a). Two females had blocked mouthparts (Fig. 5.3.a) and three further parasitoids had covers over their eyes or on the base of the head (Fig. 5.3.b). 10 females had wax on their antennae (Fig. 5.3.a). Three females had non functional ovipositor sheaths, sealed by wax (Fig. 5.3.a).

The interaction with the host is a normal part of the life of female *A. rosae*. Most incidents happened towards the end of the parasitoid's life-time without having considerable impact on the results, so the mortality caused by aphid defence was considered an unavoidable natural event (except in the three cases of early impact) and was not taken into account in calculations.

Table 5.3 The presence of cornicle wax from *Macrosiphum rosae* on various body parts of its parasitoid *Aphidius rosae* after death of the wasp. During their whole life-time (average of 18.6 days after emergence ± 3.2 (SD) 20 females of *A. rosae* were offered daily 80 aphids for oviposition at 18 ± 2 °C. During their life-time the 20 females laid 7715 eggs = successful attack on aphids. Eight wasps were not affected by cornicle wax.

	Antennae	Mouthparts	Head	Wings	Legs & Thorax	Ovipositor
Female 1	x			x	x	
Female 2	x					
Female 3	x		xx			
Female 4	x	x				
Female 5	x	x	x	xx	xx	x
Female 6			x			x
Female 7	x					
Female 8	x	x		x	xx	x
Female 9		x				
Female 10	x					
Female 11	x			x	xx	
Female 12	x			x	x	
Frequency, total n = 20	0.5	0.2	0.15	0.25	0.25	0.15

x = cornicle wax on body part, xx = body part heavily contaminated.

A. rosae displayed individual life-time fecundities between 144 and 790 eggs/female (Fig. 5.4). The individual life-time fecundity was correlated with the size of females, $r = 0.908$, $p = 0.001$, $n = 17$.

The intrinsic rate of increase ranged from r = 0.258 to 0.322 (Fig. 5.5). The correlation between r and parasitoid size was positively correlated, r = 0.895, p = 0.001 n = 17. Only one individual showed an intrinsic rate of increase bigger than the mean r of *M. rosae*.

The intrinsic rate of increase for total life-time was not much higher than intrinsic rates of increase already achieved after three and six days (Appendix 2). Half of all eggs were laid by most individuals during the first six days of adult life.

The doubling time for individuals ranged between 2.15 days and 2.69 days.

Since individuals displayed significant differences in their daily fecundity the age-specific fecundity curve can not be shown as average number of eggs/female/day but is instead expressed as the proportion of average daily fecundity of individuals compared to their own individual life-time fecundity (Fig. 5.6a).

No correlation was found between the size of *A. rosae* and longevity , r = 0.005, p = 0.98, n = 17 (Fig. 5.7). Females of *A. rosae* lived up to 24 days (42 days of pivotal age) at 18°C and mortality was nil until day 13 (31 days of pivotal age)(Fig. 5.6b). The average longevity of female parasitoids was 17.2 days ± 3.4 (SD)(35.2 days of pivotal age).

Superparasitism was common and for individuals reached daily rates up to 47 % of the eggs oviposited (Fig. 5.8). No or low rates of superparasitsm were only observed when the daily number of eggs laid was low. For the first six days of the reproductive period of females, the rate of super numerous eggs was positively correlated with numbers of eggs laid, r = 0.67, p = 0.0001, n = 118 (Fig. 5.8) (the correlation analysis was undertaken with arcsine transformed data). Since highest numbers of eggs were laid during the first six days , superparasitism was only significant during this time.

The average size of the hind tibia of females, collected from the field was 0.68 mm ± 0.005 (SD).

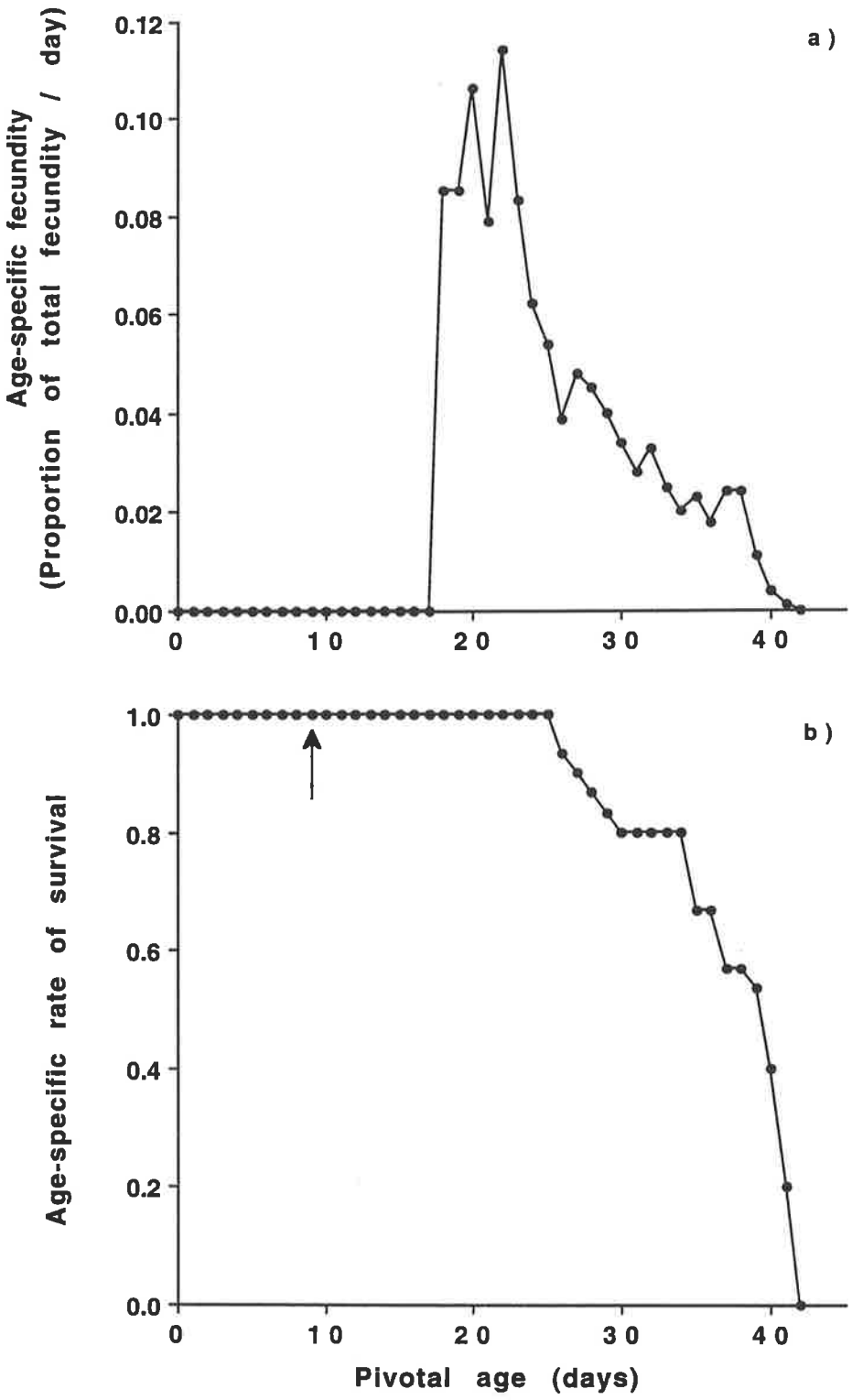


Fig. 5.1 a) Age-specific fecundity (m_x) and **b)** age-specific rate of survival (l_x) of *Macrosiphum rosae* reared on rose shoots in the laboratory at 18 ± 2°C, n = 30, r = 0.319. The arrow indicates the moult to adulthood at day ten.

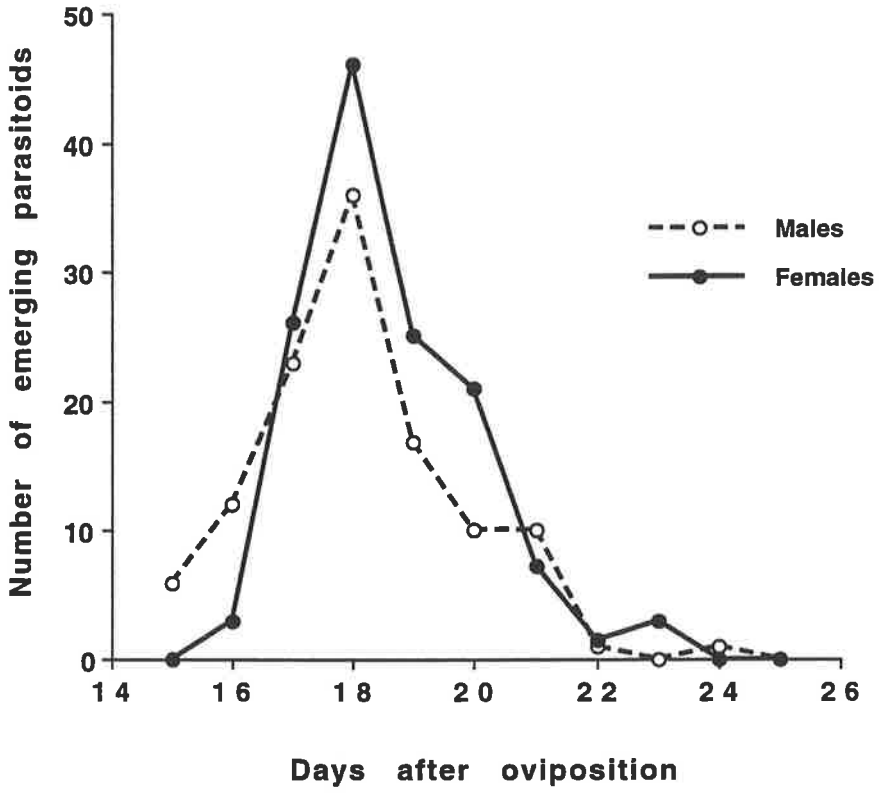


Fig. 5.2 Time from oviposition to emergence of females and males of *Aphidius rosae*, reared on mixed instars of its host *Macrosiphum rosae* at 18°C ± 2°C, n = 252.

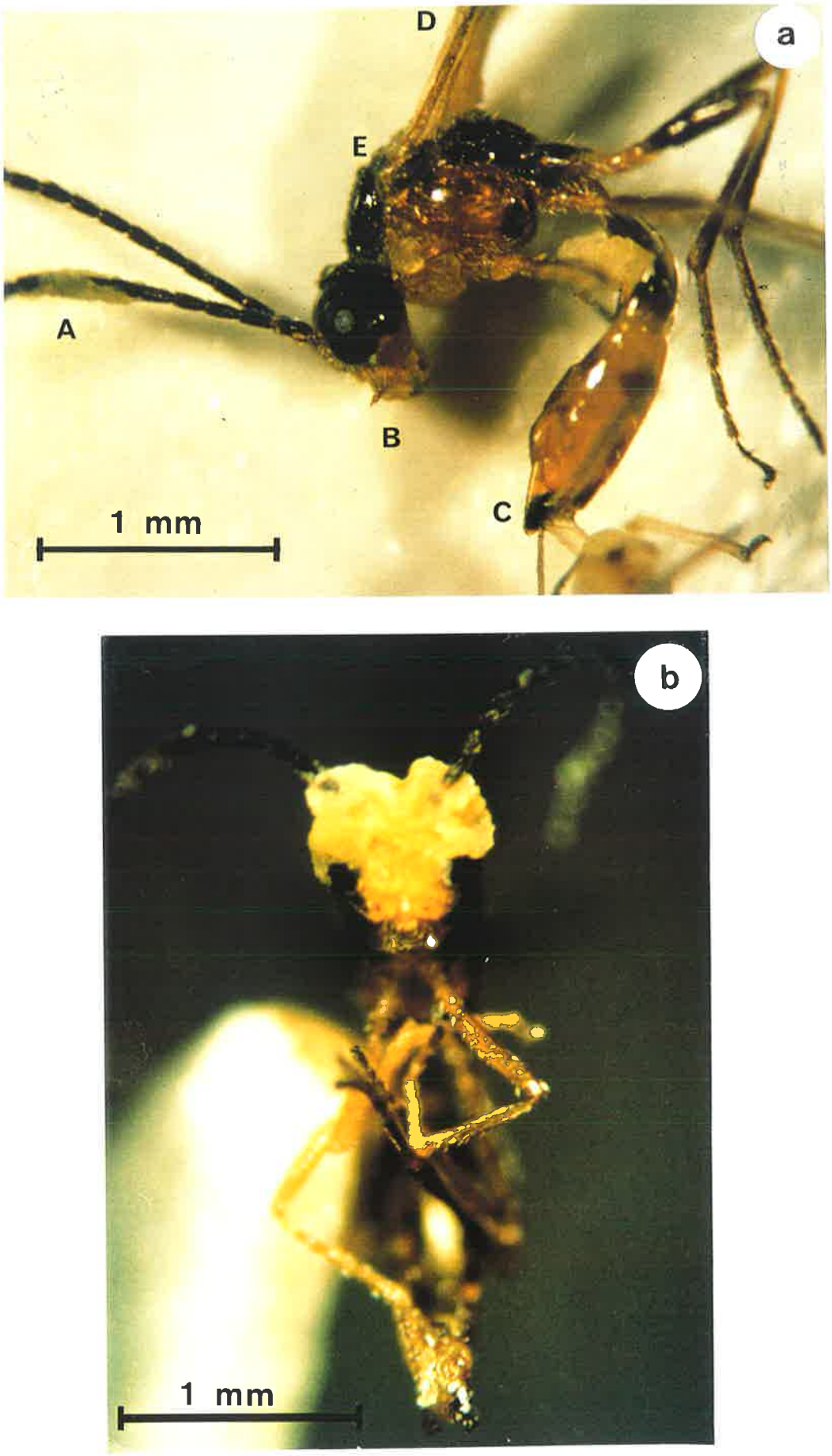


Fig. 5.3 Cornicle wax of *Macrosiphum rosae* on various body parts of *Aphidius rosae*. **a)** wax on antennae (A), on mouthparts (B), around ovipositor sheaths (C), on wings (D) on thorax (E) and **b)** on forehead.

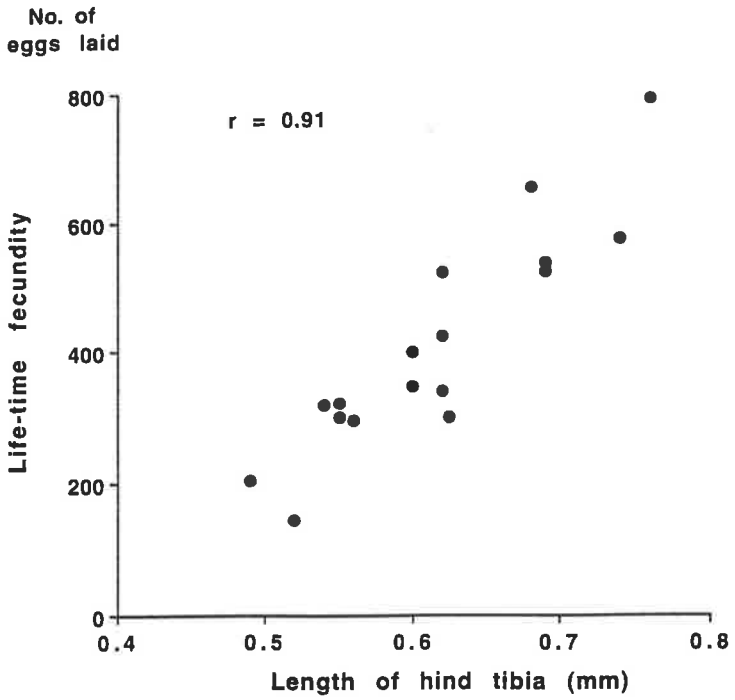


Fig. 5.4 Correlation between length of hind tibia and gross life-time fecundity of *Aphidius rosae* reared at 18 ± 2°C. The parasitoid was supplied with 80 third instars of its hosts *Macrosiphum rosae* daily and had access to honey and water at all times (r = 0.91, p = 0.0001, n = 17).

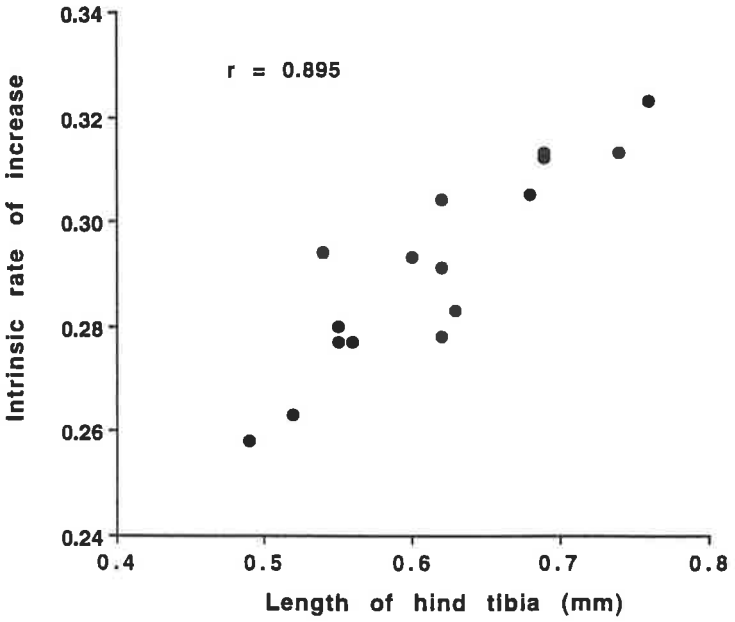


Fig. 5.5 Correlation between length of hind tibia and intrinsic rate of increase (females/female/day, based on gross reproductive rate, sex-ratio = 0.4) of *Aphidius rosae* reared at 18 ± 2°C. The parasitoid was supplied daily with 80 third instars of its hosts *Macrosiphum rosae* and had access to honey and water at all times (r = 0.895, p = 0.0001, n = 17).

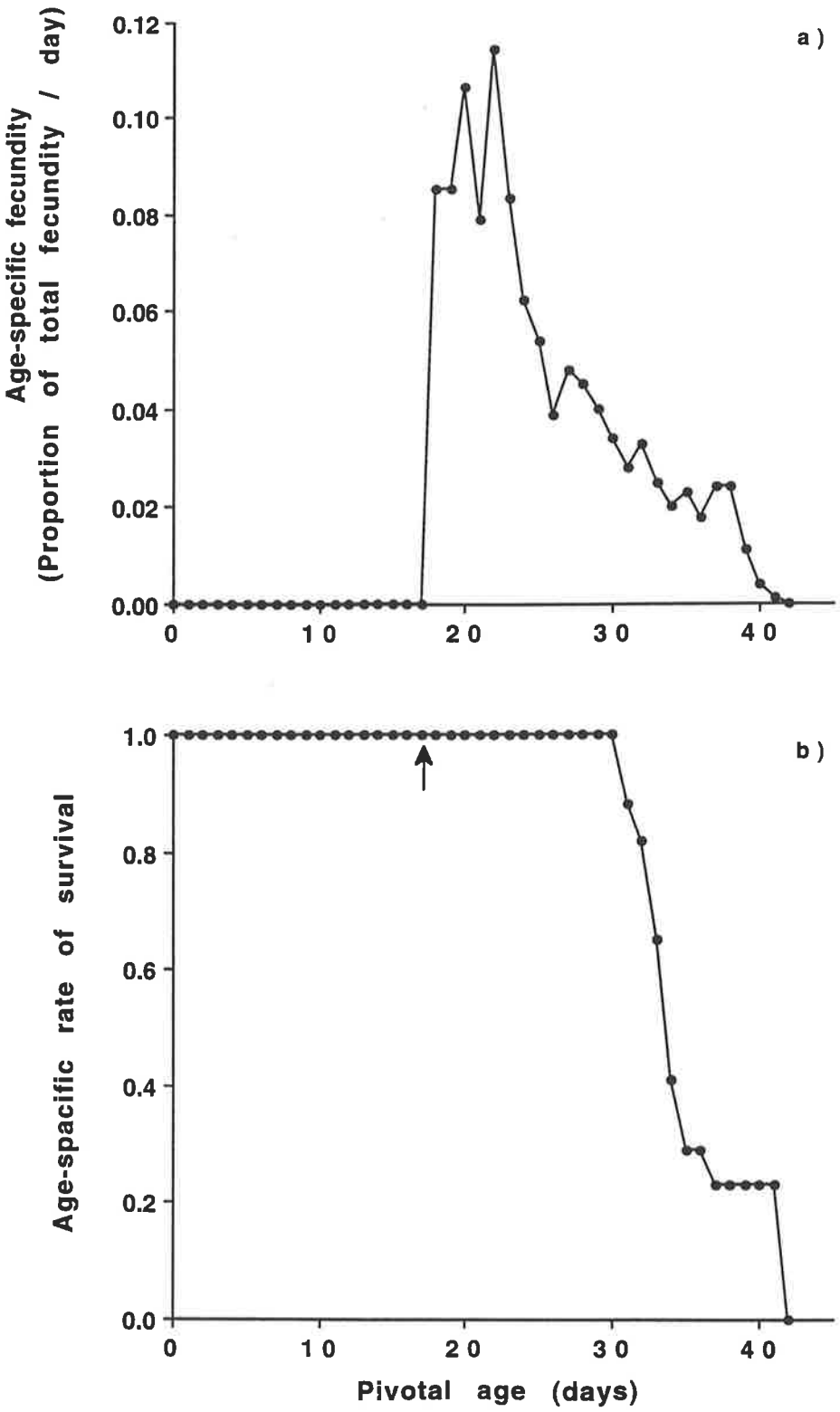


Fig. 5.6 a) Age-specific gross fecundity and **b)** age-specific rate of survival (lx) of *Aphidius rosae*, reared on third instars of *Macrosiphum rosae* (80 hosts /day) at 18 ± 2°C. The age-specific rate of fecundity rate is expressed as average daily fraction of each individual compared to its own life-time fecundity, n =17. The arrow indicates the emergence of adult parasitoids.

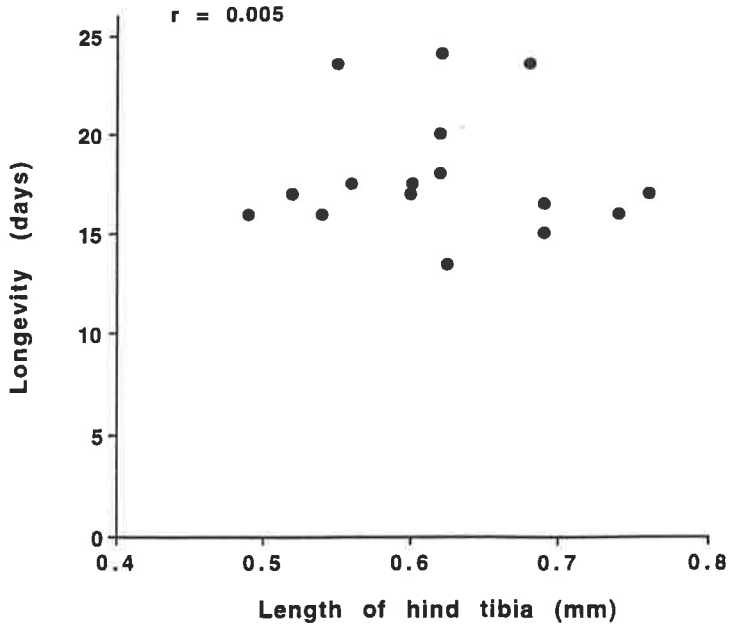


Fig. 5.7 Correlation between length of hind tibia and longevity of *Aphidius rosae* reared at 18 ± 2°C. Parasitoids had continuos access to honey, water and their host *Macrosiphum rosae* (r = 0.005, p = 0.98, n = 17).

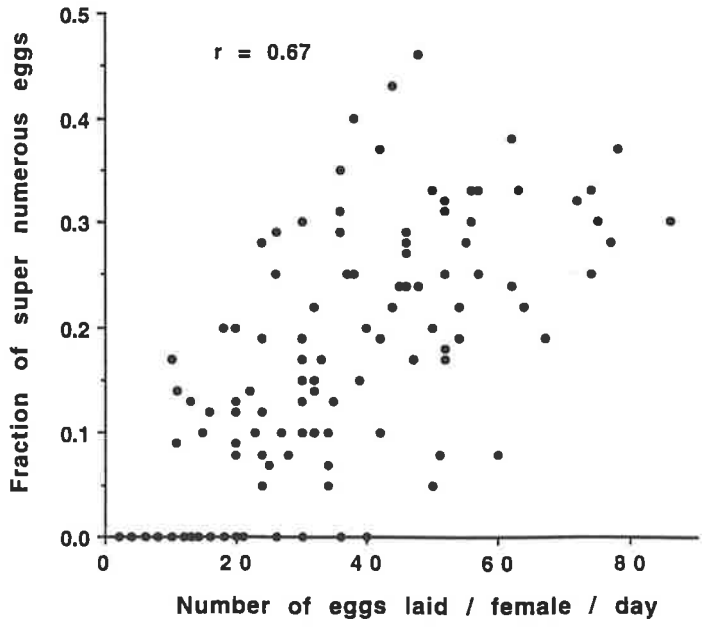


Fig. 5.8 Correlation between daily number of eggs laid and the rate of super numerous eggs oviposited. In the laboratory, *Aphidius rosae* was supplied daily with 80 third instars of its host *Macrosiphum rosae* settled on rose shoots at 18 ± 2°C, n = 20. Graphs show daily results up to day six (r = 0.67, p = 0.0001, n = 119). One female reproduced only for five days.

5.4 Discussion

M. rosae. The net reproductive rate (R_0) of *M. rosae* was with 78.85 around 30 % higher than the R_0 of 61.46 found by Maelzer (1977) but in the present study *M. rosae* needed ten days to reach adulthood at 18°C compared to a calculated developmental time of only 8.5 days in Maelzer's (1977) study. He calculated an intrinsic rate of increase of 0.344 for *M. rosae* at 20.5°C, compared to 0.319 at 18°C calculated in the present study. Differences in developmental time and life-time fecundity in both studies could have been a result of different experimental methods, e.g. plant quality and temperature. The shape of the curve for the age-specific fecundity of *M. rosae* was similar in both studies.

A. rosae. Earlier emerging males than females were not surprising since it was shown for many species of Aphidiinae that male parasitoids emerge somewhat earlier than females (Stary, 1970, 1988). Time to mummification took roughly one day longer than results in Chapter 4 revealed. Slight variation of life-time parameters were noted throughout the years of rearing in quarantine and again, may be attributed to the not precise temperature regulation in the laboratory (Chapter 3).

The life-time fecundity of *A. rosae* ranged between 144 and 790 eggs/female. This demonstrated clearly that data obtained by dissection of emerging females severely underestimated the egg-production of the parasitoid (compare Fig. 4.5 and Fig. 5.4).

During the experiment considerable superparasitism occurred which reached daily values for individuals of up to 47 % of all eggs laid (Fig. 5.8a). Superparasitism is commonly observed under unfavourable laboratory conditions, and for *A. smithi* resulted in up to 85 % of all eggs being wasted (Mackauer, 1983a). Force & Messenger (1964) found up to 40 % of eggs wasted by *P. exsoletum*. The recognition of parasitized hosts was shown to be imperfect in Aphidiinae (e.g. Micha et al., 1992). However, even in cases where parasitized hosts were recognised, superparasitism occurred under circumstances which forced the wasp to stay together with a high fraction of parasitized

aphids. Eventually, oviposition tendencies became too strong and oviposition took place (Hofsvang & Hågvar, 1983; Collins & Dixon, 1986). In the field, searching conditions are entirely different from the laboratory and parasitoids are likely to disperse when parasitoid/host rates are high (Stary, 1970). The high rates of superparasitism observed in the present experiment were considered artefacts. Superparasitism was ignored and gross reproductive rates were used in analysis rather than net reproductive rates.

Aphid defence by ejection of supercooling wax was shown to be an important aspect in the interaction between *M. rosae* and *A. rosae*. 12 out of 20 tested females were affected by wax during their life-times (Table 5.3). In five instances the contact with the aphid wax was directly related to mortality. Wax-sealed ovipositor sheaths effectively stopped reproduction of some females. Contamination of parts of the antennae may have had an effect on searching efficiency. In one case the whole front of the head of a female was so severely covered with cornicle wax that no movement of the basal part of the antennae was possible and effective attennation was impossible (Fig. 5.3b). Sealed mouthparts stopped parasitoids from feeding and even more importantly, intake of water or grooming. Since the antennae and head are directed towards the aphid during the first contact with the host, it seemed logical that these body parts were most affected. During the years of the project, the antennae of foraging females were regularly observed to be contaminated with wax. Females reacted with panic-stricken immediate retreat when they came in contact with the wax and wiped their antennae against the surface of the plant. This was followed by intense grooming.

Compared to the total number of aphids attacked (7715 numbers of ovipositions) the number of incidences was low. This reflected also observations during the rearing process in the laboratory and in the fields, where affected wasps were regularly observed but only in very smal numbers. However, the significance of this aphid behaviour may not be reflected in the successful contamination of parasitoids but in the resulting careful handling of aphids by the parasitoid. Parasitoids obviously can avoid the impact of cornicle wax because their behaviour seemed to be adapted to the potential danger when they forage for hosts (Chapter 7). The theoretical consequences are discussed in Chapter

7 and 10. For the results of the present experiment the quantitative impact of aphid defence was neglected, except for the three females which suffered unusual early mortality or loss of reproduction capacity. Most accidents happened obviously towards the end of the parasitoids life, when the reproductive rate was slowed down and did not contribute effectively to the life-time fecundity and especially not to *r* (Appendix 2)

The longevity of *A. rosae* was not correlated with size (Fig. 5.7). Data on the genus *Aphidius* suggest that longevity in this genus is independent from size. E.g. the longevity of *A. smithi* was not correlated with size (Mackauer & Kambhampati, 1988). The same pattern was observed for *A. ervi* (Sequeira & Mackauer, 1994) and *A. nigripes* (Cloutier et al., 1981).

Individuals of *A. rosae* had intrinsic rates of increase between 0.258 and 0.323. The size of females was correlated with *r* (Fig. 5.5). Results from Chapter 4 revealed that the number of eggs in emerging females was correlated with size as well. Those eggs present in the ovaries at emergence are likely to be the main resource of eggs laid during the first days of the reproductive period and therefore will contribute essentially to the value of *r*. Hence, the number of eggs at emergence can give a good indication of the individual intrinsic rate of increase.

A. ervi displayed intrinsic rates of increase of 0.326 and 0.316 females/female/day in two different populations from California at 18.3°C (Botto et al., 1993)(Table 5.2). The total life-time fecundity of those individuals was only 225 and 160 eggs, respectively, but the parasitoids commenced reproduction after 15 days, compared to 18 days in this study. Comparison of intrinsic rates of increase of other species of Aphidiinae with *A. rosae* is difficult since most experiments were carried out between 20 and 25°C. Force & Messenger (1964) showed that *r* depends on temperature. From their results in which *r* values were plotted against temperature, it can be estimated that *T. complanatus* and *P. exsoletum* displayed intrinsic rates of increase of 0.27 and 0.12, respectively, at 18°C.

For biological control, comparison of the intrinsic rates of increase between a pest and its control agent is an important consideration in predicting potential control capacity (e.g. Ehler, 1990). Only one individual of *A. rosae* displayed a higher intrinsic rate of increase than the cohort of *M. rosae* tested. Nevertheless, the intrinsic rate of increase of larger parasitoids was very similar to that of the host (Fig. 5.5). The average size of females of *A. rosae*, collected from the field showed that their potential intrinsic rate of increase would represent a similar *r* to that of the host under the tested conditions. The tested parasitoid cohort contained a range of individual sizes to clarify the influence of size on reproductive success whereas the cohort of the aphids did not. An unbiased comparison of *r* between the species may favour slightly the aphid, but a more realistic analysis should take into consideration that any increase in parasitoid numbers must lower the reproductive success of the host, especially since most parasitized aphids in the field do not reach reproductive age (Chapter 10).

The results demonstrated that *A. rosae* has the potential to react quickly to an increase of aphid numbers. How much this potential is realised under field conditions depends upon the complex influences of the environment, e.g. weather, seasonal synchronisation with the host, inter- and intra specific competition, density-dependent parasitism, predation and hyperparasitism. These are discussed in Chapter 10.

Host selection of *Aphidius rosae* with respect to assessment of host specificity in biological control

6.1 Introduction

Ecological problems caused by the introduction of exotic organisms in the past have led most countries to establish strict quarantine legislation that demands assessment of ecological risks associated with release of biological control agents. Criteria such as the host range of the control agent, the range of habitats it inhabits, its genetic plasticity, its behaviour and mutualism with other species have to be considered to evaluate the degree of risk to non-target organisms (Howarth, 1991). To minimise risks, it is essential to conduct ecologically meaningful tests of host specificity. Official guidelines are needed which provide clear procedures to both the experimenter and statutory authorities (Field, 1993). However, in practise, it is very difficult for the experimenter to assess with limited resources in the laboratory the theoretical performance of the control agent in the open field. Divergence between predictions and performance can result because of artificial laboratory conditions that may affect host selection behaviour, or because all potential hosts cannot be tested (Nechols *et al.*, 1992). Quarantine officers may have difficulty in their efforts to ensure very high ecological safety without overstating potential negative impacts of the control agent, which might prevent excellent biological control opportunities with all their environmental benefits.

This chapter deals with the host selection of *Aphidius rosae* Haliday and the assessment of its host specificity in the Australian environment. It discusses the use of a wind tunnel as a meaningful tool in quarantine standards to assess environmental risks.

Host selection by parasitoids is a complex process, with its own behavioural hierarchy (Weseloh, 1981). For many species of parasitoids it can be divided into three

steps : 1) habitat location, 2) host location and 3) host acceptance (Doutt, 1959), to which 4) host suitability can be added (Vinson, 1976). Comprehensive reviews on this topic have summarised the progress in understanding of parasitic hymenopteran behaviour (Arthur, 1981; Van Alphen & Vet, 1986; Vinson, 1976, 1981; Vinson & Iwantsch, 1980; Weseloh, 1981). Field collections of Aphidiinae showed that certain species can be associated with certain habitats (Stary, 1966a) but because of difficulties in experimental design, it is largely unknown if abiotic factors such as humidity, light or spatial pattern influence the response of parasitoids towards major habitat types (Wharton, 1993). Chemicals appear to play the major role in the orientation of parasitoids and plant cues are often considered to be an important source of information to searching individuals (Vinson, 1981).

A. rosae was considered to be specific to *Macrosiphum rosae* (L.) in the Australian environment. Wherever it has been recorded, it has been recorded only from *M. rosae* (Pennachio, 1989; Raychaudhuri *et al.*, 1979; Stary, 1966b; 1976, 1987) or additional from other species of the genus *Macrosiphum* which do not occur in Australia, e.g. *Macrosiphum (Sitobion) rosaeiformis* (Das) (Agarwala, 1983; Agarwala & Raychaudhuri, 1981) and *Macrosiphum funestum* (Macchiati) (Stary, 1966a; Mackauer & Stary, 1967). Völkl (1994) studied *A. rosae* in the field, parasitizing *Sitobion fragariae* (Walker). He cited Mackauer & Stary (1967) and Stary (1973) to show that this aphid species is one of the main hosts of *A. rosae*. However, in neither of these taxonomic studies *S. fragariae* was mentioned as host for *A. rosae*. Therefore this record may be questionable and needs confirmation. In any case, *S. fragariae* does not occur in Australia (Mary Carver, personal communication, CSIRO, Canberra). Dependent upon the geographic location, *A. rosae* is considered specific to *M. rosae* or considered as restricted to closely related species of the genus *Macrosiphum*.

To demonstrate the specificity of *A. rosae* in relation to the fauna of potential hosts in Australia, I tested its attraction to hosts and host plants in a wind tunnel, exposed host and non-host species to the wasps in choice/no-choice tests and investigated to what extent the closely related and most likely non-target host, the potato

aphid, *Macrosiphum euphorbiae* (Thomas), would be suitable for successful development of the parasitoid. The main criterion for the selection of the other aphid species used in tests was their abundance in the same park and garden habitat utilised by *M. rosae*. *M. euphorbiae* and *Rhodobium porosum* (Sanderson) occur periodically on roses in South Australia.

6.2. Methods

Insects. Aphids were obtained from various glasshouse cultures (*M. rosae, M. euphorbiae*, *Acyrthosiphon kondoi* Shinji, *Myzus persicae* (Sulzer) and *Aphis gossyppii* Glover) or collected from the field (*Hyperomyzus lactucae* (L.) and *R. porosum*). The aphids were reared at 18 + 2°C, 65% r.h., 16L:8D on their host plants. A range of different instars, apterae and alatae were used in all experiments.

Females of *A. rosae* were four days old when used in experiments and they comprised (a) naive females which were kept for four days with males but without access to aphids, and (b) experienced females which were kept for two days with males and then transferred for two days to a glass/gauze cages (35 cm x 35 cm x 35 cm) containing colonies of *M. rosae*. Experienced females used for the wind tunnel experiments were removed from aphids 12 hours before the beginning of a test. Parasitoids were given access to honey and water at all times.

Assays in a wind tunnel. The test chamber of the wind tunnel was 160 cm long, 65 cm wide and 65 cm high (for details see Keller, 1990). Laminar air flow was maintained at 18 cm/sec and 23-26°C. All plants were of the same height (30 cm) and as similar as possible in shape. Shoots of *Rosa sp.,* var. Tea hybrid 'McGredy's sunset', 4th stage (Maelzer, 1977) were cut two days before they were used, and if required infested with 50 large nymphs or adults of aphids. Since the adults kept reproducing during these days the colonies comprised a natural mix of all stages at time of experiments. Other plant species were cut just before tested. All shoots were kept fresh by immersion in water

supplemented with fertiliser for cut flowers (Flower Fresh ™, Flower Fresh Products, Glengowrie, South Australia) at 18°C. In between flights, the positions of plants were swapped.

The parasitoids were released 110 cm downwind of the plant odour sources from glass tubes (23 mm in diameter, 70 mm long) with cotton stoppers on both open ends and the opening was directed into the air flow. Before the cotton was removed the wasps were allowed to acclimatise for two minutes. Wasps which did not fly after 4 minutes were excluded from further tests. Every wasp was used four times in most experiments but this was not always possible since some were injured or irritated by handling; these were not included in the analysis. The number of wasps used in experiments was eight, 21 or 24. For each individual the preference for one of the two choices was determined and a two-tailed sign test was used to analyse the data.

To demonstrate the distribution of odour plumes in the flight chamber smoke was produced by dripping Ethylene-diamine on to Acetic acid soaked cotton wool.

No-choice and choice tests in petri dishes. Aphids of each species were established on young leaves of their host plant and exposed to *A. rosae* in 8.5 cm diameter glass petri dishes for two hours. Each petri dish was considered as a replicate and comprised 40-50 aphids and 3 parasitoids which were released into the petri dish one hour after the aphids. Six replicates were used per experiment. Within a dish, each female parasitoid was observed for six periods of 10 s in which the occurence of attacking behaviour was recorded. Only obvious attacking behaviour against aphids were recorded, i.e. when the parasitoid started to bend its abdomen forward in preparation for oviposition in an aphid. After three minutes, the observations were carried out on the next petri dish. Every petri dish was observed 4 times and consequently 72 observations per petri dish were obtained. In the first series, naive parasitoids were tested. In a second series, experienced females were tested only against aphid species which were attacked by naive females in the first series.

Variances were unequal among treatments, so data were ranked and the ranks were analysed by ANOVA and the Student-Newmann-Keuls test.

A choice test was conducted using the same techniques as in the no-choice tests. Only the three aphid species which occur naturally on rose plants, *M. rosae*, *M. euphorbiae* and *R. porosum* were used. 30 aphids of each of these species were placed together on rose leaves and exposed to three females of *A. rosae* in a petri dish. In six replicates, the attacking behaviour of female wasps on the different aphid species were recorded using the same method as above.

Extended choice test. To reduce the unnatural conditions of the petri dishes, experienced parasitoids were tested in a glass/gauze-cage (35 cm x 35 cm x 35 cm) in which they were able to choose among (a) a rose shoot infested with *M. rosae*, (b) a rose shoot infested with *M. euphorbiae* and *M. rosae*, (c) a rose shoot infested with *M. euphorbiae* and (d) a potato shoot infested with *M. euphorbiae*. Each shoot was the same length (25 cm), was kept fresh by immersion in water and was infested with 50 aphids.

10 parasitoids were released in the middle of the cage and every five minutes observations were made to determine their locations and behaviour. Every exposure lasted 30 minutes and was repeated four times over a period of two hours with the same wasps. The positions of the shoots were chosen at random and changed with every exposure. This experiment was replicated three times.

Variances were unequal among treatments, so data were ranked and the ranks were analysed by ANOVA and the Student-Newmann-Keuls test.

Host-suitability of M. euphorbiae. 40 third instars each of *M. rosae* and *M. euphorbiae* were exposed one by one to females of *A. rosae* in 1.0 ml gelatine capsules, and were observed under a dissecting microscope. Only after an obvious insertion of the ovipositor were the aphids removed and reared on roses at 18°C. After 6 days, the aphids were dissected to determine the frequency of parasitoid development.

6.3. Results

Given a choice between roses and different plants in the wind tunnel, *A. rosae* was strongly attracted only to roses (Table 6.1). The parasitoid was not able to distinguish between infested and uninfested roses when the shoots were 30 cm apart. But females were able to find and land on the infested shoot when the shoots were placed only 5 cm apart, regardless of whether *M. rosae* or *M. euphorbiae* were used (Table 6.2). There was no difference in the frequency of landing when wasps were presented with a choice between shoots infested with *M. rosae* or *M. euphorbiae.*

It may be argued that learning in successive flights could have influenced the outcome of the wind tunnel experiments (e.g. Grasswitz & Paine, 1993). However, under the assumption that foraging parasitoids make the best possible choice between two alternative landing sites, the clear preference in some experiments and the lack of preference in other tests showed that the set up of choices presented to wasps were the most important determining factor for the outcome.

In the no-choice test with naive parasitoids, only the two *Macrosiphum* species were frequently attacked (Table 6.3). Nearly seven times more attacks were counted on *M. rosae* than on *M. euphorbiae.*

When experienced females of *A. rosae* were presented with the same range of hosts, the rate of attack on *M. rosae* did not change, but no attacks were observed on the other species. In contrast, experienced parasitoids attacked *M. euphorbiae* when exposed together with *M. rosae* in a choice test but not *R. porosum.* In the extended choice test in a cage, *A. rosae* spent more time on shoots infested with *M. rosae* or a mix of *M. rosae* and *M. euphorbiae* than on plants infested with only *M. euphorbiae* (Table 6.4). The great majority of attacks was counted on *M. rosae.*

Table 6.1 Two-choice test of *Aphidius rosae* in a flight tunnel between shoots of *Rosa sp.* var. Tea hybrid 'McGredy's sunset' and various plant species. Eight female wasps were used four times in each test. Plants were swapped between flights.

Test plant	Sign of preference for option *Rosa*, no. of wasps for each choice-ratio (Rosa vs test plant)					
	+		0	-		
	4 : 0	3 : 1	2 : 2	1 : 3	0 : 4	
Pyrus malus (Rosaceae)	6	2	0	0	0	*
Pelargonium sp. (Geraniaceae)	8	0	0	0	0	*
Solanum tuberosum (Solaniaceae)	7	1	0	0	0	*
Callistemon sp. (Myrtaceae)	6	2	0	0	0	*
Medicago sativa (Leguminosae)	6	1	1	0	0	*

* indicate significant difference in choice of individual wasps for *Rosa* (two-tailed sign test, $p < 0.01$).

Table 6.2 Two-choice test of *Aphidius rosae* in a flight tunnel with infested and uninfested rose shoots, *Rosa sp.* var. Tea hybrid 'McGredy's sunset', using the aphid species *Macrosiphum rosae* and *Macrosiphum euphorbiae*. Female wasps were used four times in each test. Plants were swapped between flights.

Infestations (A vs B)	Distance between shoots	n †	Sign of preference for option A, no. of wasps for each choice-ratio (A vs B)					
			+		0	-		
			4 : 0	3 : 1	2 : 2	1 : 3	0 : 4	
M. rosae vs uninfested	25 -30 cm	21	1	3	9	8	0	ns
M. rosae vs uninfested	5 cm	21	8	11	2	0	0	***
M. euphorbiae vs uninfested	5 cm	8	5	1	2	0	0	*
M. rosae vs *M. euphorbiae*	5 cm	24	1	7	12	4	0	ns

† n refers to the number of wasps used in each test.
-statistical differences in the choice of individual wasps within each pair of shoots (two-tailed sign test,
ns = not significant, * = $p < 0.05$, *** = $p < 0.001$)

Table 6.3 Attacking behaviour by *Aphidius rosae* on different aphid species established on young leaves in choice and no-choice conditions in 8.5 cm diameter glass petri dishes.

Aphid species	Plant	Number of periods in which attacking behaviour occurred per petri dish. Mean ‡ (SD)
A. No-choice test with naive† parasitoids		
Macrosiphum rosae	rose	31.50 (9.73)[a*]
Macrosiphum euphorbiae	rose	4.83 (4.58)[b]
M. euphorbiae	potato	4.50 (4.37)[b]
Rhodobium porosum	rose	0.17 (0.41)[c]
Acyrthosiphon kondoi	lucerne	0.67 (1.03)[c]
Myzus persicae	lettuce	0
Aphis gossypii	hibiscus	0
Hyperomyzus lactucae	sow-thistle	0
B. No-choice test with experienced† parasitoids		
M. rosae	rose	28.5 (5.96)
M. euphorbiae	rose	0
M. euphorbiae	potato	0
R. porosum	rose	0
A. kondoi	lucerne	0
C. Choice test among three hosts with experienced† parasitoids		
M. rosae	rose	17.67 (6.15)[a]
M. euphorbiae	rose	4.00 (0.41)[b]
R. porosum	rose	0.16 (0.41)[c]

Three wasps were tested per petri dish. Observations lasting 10 s in which the occurence of attacking behaviour was recorded were taken on 24 occasions on each of these wasps in two hours. Six replicates with a total of 432 observations were taken per test. No. of aphids/petri dish = 40-50 in tests A and B or 90 (30/species) in test C.
‡ Mean number of periods in which attacking behaviour occurred by three wasps per petri dish. Only obvious attacking behaviour against aphids was recorded, i.e. when the parasitoid started to bend its abdomen forward in preparation for oviposition in an aphid.
† Naive wasps had no contact to any aphids beforehand whereas experienced wasps were kept for two days with the host *M. rosae*.
*ANOVA on ranks with Student-Newmann-Keuls test. Means followed by the same letter are not significantly different ($p < 0.05$).

Table 6.4 Choice of *Aphidius rosae* between four combinations of different plant shoots infested with *Macrosiphum rosae* and *M. euphorbiae*, and observed attacking behaviour against these aphids in a cage test.

Plant shoot	Aphid species 50 aphids/shoot.	No. of observations of wasps presence on shoot Mean † (SD)	No. of observations of attacking behaviour Mean † (SD)
rose	*M. rosae*	34.00 (10.82)[a*]	13.33 (0.58)[a]
rose	*Mix of M. rosae / M. euphorbiae*	37.33 (13.87)[a]	12.00 (9.54)[a] 3.00 (3.00)[b]
rose	*M. euphorbiae*	9.67 (4.51)[b]	0.67 (0.58)[b]
potato	*M. euphorbiae*	7.33 (3.79)[b]	1.33 (2.31)[b]

† Ten wasps were released in a cage for two hours and 240 observations were taken of their momentary residence and behaviour in the cage. Three replicates were carried out. Only residence on shoots and attacking behaviour against aphids on these shoots are shown in the table. Only obvious attacking behaviour against aphids was recorded, i.e. when the parasitoid started to bend its abdomen forward in preparation for oviposition in an aphid. Mean refers to the total number of observations per cage.

*ANOVA on ranks with Student-Newmann-Keuls test. Means followed by the same letter are not significantly different ($p < 0.01$).

Six days after parasitoid attack 34 out of 40 *M. euphorbiae* aphids were alive and no larvae of *A. rosae* were found when these were dissected. In comparison, 34 parasitoid larvae were found in 36 aphids of *M. rosae*.

6.4. Discussion

Host selection. In the wind tunnel females of *A. rosae* were strongly attracted to roses if given the choice between a host plant and a non-host plant. They did not distinguish between uninfested shoots and shoots infested with the host-aphid *M. rosae* if shoots were 30 cm apart, but they distinguished clearly if shoots were only 5 cm apart. An attraction towards infested shoots also occurred when the non-host *M. euphorbiae* was used.

The attraction of Aphidiinae to plants or the odour of plants that normally harbour the host aphid is well documented (Powell & Zhi Li, 1983; Read *et al.*, 1970; Schuster & Starks, 1974; Sheehan & Shelton, 1989; Singh & Sinha, 1982; Wickremasinghe & van Emden, 1992). Kennedy (1977) divided the response to cues from a certain source habitat into long range and short range attraction (within a few centimetres of the source of the chemical cues).

The results suggest that *A. rosae* uses roses as a long range odour source but not aphid kairomones or host induced synomones. When infested and uninfested shoots were placed 30 cm apart, their odour clouds mixed 60 cm behind the source (demonstrated with smoke). This marks the point at which a wasp must make the decision which way to fly (Fig. 6.1). Individuals of *A. rosae* were obviously not able to detect their hosts from this distance. To enable them to distinguish between uninfested and infested buds, the shoots had to be placed closer together. Now, the wasps could delay making a choice until they were only 10 cm from the source of odour. Hågvar & Hofsvang (1989) observed *Ephedrus cerasicola* Stary examining plants by flying very close to them and suggested that volatiles involved in host location of this aphidiine wasp may work only over very small distances. In Y-olfactormeter tests it was shown that *Aphidius ervi* Haliday, *Aphidius rhophalosiphi* De Stefani-Perez, *Lysiphlebus fabarum* (Marsh.), *Trioxys sp.* and *Praon sp.* responded more strongly to a combination of aphid and plant than to host plant or host alone (Wickremasinghe & van Emden, 1992). However, there is much less dilution of odours in an olfactormeter than in a wind

tunnel and therefore a comparison between these results and the present investigations is inappropriate. Guerrieri *et al.* (1993) demonstrated with no-choice tests in a wind tunnel a greater response of *A. ervi* towards infested plants and host-damaged plants than to plants or aphids alone. This suggests that *A. ervi* is attracted towards host-induced synomones or host products. Since odour source and the release point of the wasps were only 20 cm apart in these experiments, they can not be directly compared to the present work either. Only in the present experiments was a method used that could show the distance over which wasps could distinguish between attractive cuues.

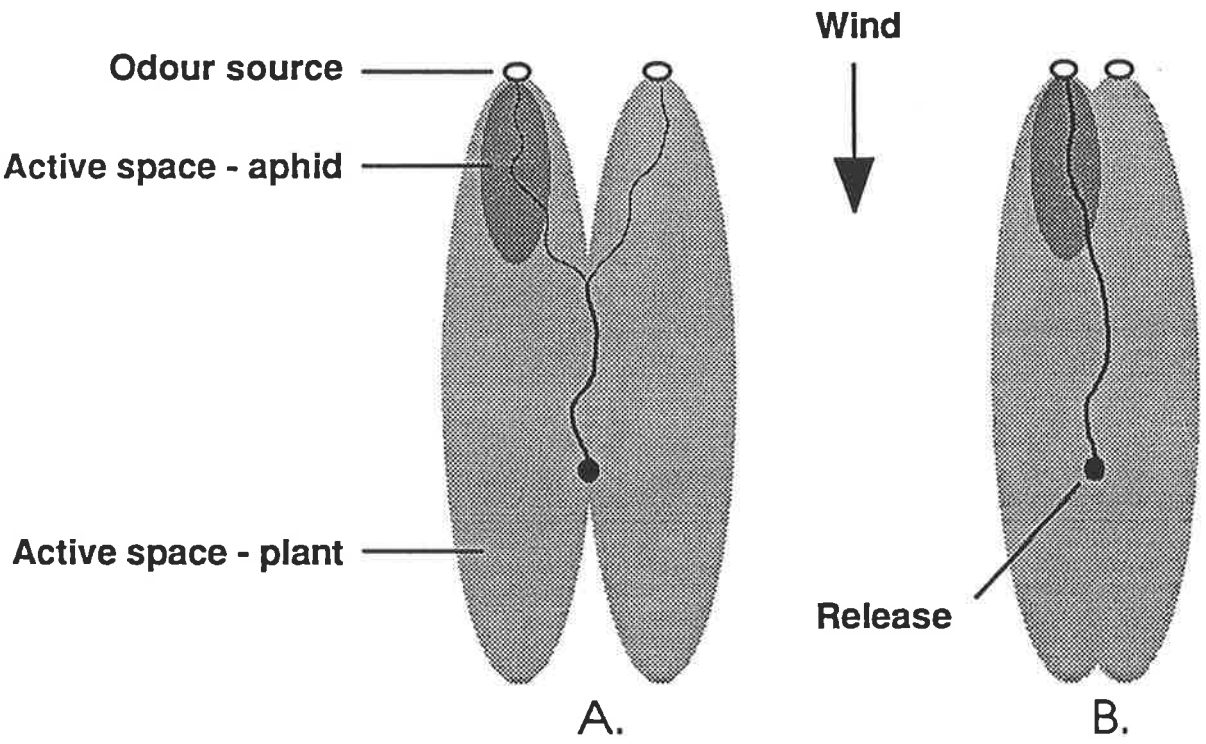


Fig. 6.1 The active space is the region within which an insect can detect an odour. In the model shown here, the plumes of odour are shown in the idealised Gaussian form. In both cases the wasp is in the active space of rose odour at the point of release but the point at which a wasp must make the decision which way to fly lies A) outside the active space of odour associated with aphids and B) inside.

A. rosae did not show significant differences in attraction to *M. rosae* or *M. euphorbiae* on roses during flight. But the wasp clearly distinguished between different aphid species when contact was made with the antennae. Hosts or host by-products give important chemical cues for Aphidiinae during antennation (Ayal, 1987; Bouchard & Cloutier, 1984; Budenberg, 1990; Cloutier & Baudin, 1990; Gardener & Dixon, 1985) and at this stage visual cues might be involved in host evaluation as well (Michaud & Mackauer, 1994). From all aphid species tested only *M. euphorbiae* was attacked in moderate numbers, but under all conditions the attack rate was much higher on *M. rosae*. *A. rosae* is obviously strongly attracted to roses but occasionally it will happen that some individuals land on different plants and come into contact with non-host species. But even under the most extreme laboratory conditions (inexperienced parasitoids with a heavy egg-load and a strong urge to oviposit, many aphids and close confinement) hardly any aphids other than *Macrosiphum* species were attacked. These few attacks on aphids in other genera, which do not necessarily indicate that oviposition has occurred, should be considered as an experimental artefact. It should be expected that parasitoids in the field would respond differently, perhaps by dispersing (Mackauer, 1983b). Host selection must be considered only in the context of habitat selection (van Alphen & Vet, 1986). Only on roses would host selection operate at the lowest level of foraging, when *A. rosae* is actually choosing between different aphid species. In choice tests, even experienced females of *A. rosae* attacked *M. euphorbiae* to a certain degree, but they did not do so in the no-choice tests. Vinson (1976) postulated that contamination of an otherwise unacceptable host by the odour of the preferred host may result in an attack of the unacceptable host. This occasional 'confusion' of *A. rosae* occurred only between the two closely related *Macrosiphum* species, but not with *R. porosum*.

Specificity assessment of A. rosae in Australia. The Aphidiinae are specific to aphids (Stary, 1970). In the past more than a dozen species of Aphidiinae were released into Australia (for a summary see Hughes, 1989), including polyphagous species such as *A. ervi*. There is no record of any of these introduced parasitoids parasitising species other

than non-native aphids. In Australia, of 156 known aphid species, only 20 are indigenous and the majority of the rest are exotic and mostly pestiferous (Carver, 1989). The few species of aphids which are native to Australia are found on plants quite different to roses and live mainly in habitats which are very unlikely to attract *A. rosae.* It is expected that *A. rosae* will rarely encounter most aphid species occurring in Australia. Only on roses would the wasp have to distinguish between different aphid species. *M. euphorbiae* is the only aphid species other than *M. rosae* expected to be occasionally parasitized by *A. rosae,* but in this case offspring would not complete development. The long sympatric existence of *M. euphorbiae, M. rosae* and *A. rosae* on roses in other parts of the world indicates that no sudden change of host suitability is likely. Because of the specificity of *A. rosae*, the special composition of the Australian aphid fauna and the history of introduced aphidiine wasps into Australia, environmental risks resulting from the release of the control agent are extremely low.

Assessment of host specificity in biological control. Most host specificity tests deal mainly with the final steps of host selection, i.e. host acceptance and suitability, in which an intensive list of hypothetical host species is tested (e.g. Wapshere, 1974). I believe that more attention should be paid towards the entire host selection process from location of host habitats to location of hosts, host acceptance and host suitability. This will not only help to reduce the number of species to be tested, but will also help to develop a more realistic assessment of host specificity, in which host selection will be better understood in its entirety. Recently, the assessment of behaviour in wind tunnels has become routine in behavioural research (e.g. Drost *et al.*, 1986; Keller, 1990). The results obtained can give better predictions about the aspected theoretical performance of an insect in the field. Thus the use of wind tunnels to assess host specificity and environmental risks should be promoted.

Intra-patch foraging of *Aphidius rosae*

7.1 Introduction

Foraging of parasitoids is characterised by a behavioural hierarchy adapted to spatial scales (see Chapter 6). Hassell & Southwood (1978) distinguished three levels within the foraging parasitoid's environment, the habitat, the host, and between these the basic unit of foraging activities, the patch. The border of a patch depends on the parasitoid's perception of its environment. In general it may be described as a portion of its environment, the border of which, when crossed by a parasitoid, triggers a change in behaviour (Waage, 1979). Such a patch is considered as the smallest unit in which a parasitoid is able to display a typical series of responses (Ayal, 1987). Because of the complexity of this topic and the vast amount of existing literature, even on Aphidiinae alone, the reader may be referred to comprehensive reviews, e.g. Godfray (1994).

Völkl (1994) considered a patch for *Aphidius rosae* Haliday as a single rose bud and compared it to Ayal's (1987) 'elementary unit of foraging', the smallest environmental scale in which the parasitoid can find food, hosts and shelter. *A. rosae* moves between shoots by flight, but landing on a shoot triggers a typical change in behaviour.

This chapter describes the foraging behaviour of *A. rosae* around single rose buds in the field. Possible consequences for the life history of the parasitoid and its effectiveness in biological control are discussed.

7.2 Methods

All observations were carried out on rose bushes *Rosa sp.*, var. Tea hybrid 'McGredy's sunset', that were located at the principle investigation site of this project in the orchard on the Waite Campus (Chapter 10). Observations were made only on 4th stage rose buds that were moderately to heavily infested with *Macrosiphum rosae* (L). In the morning (around 10 am) and late afternoon (around 5 pm) a flying female was arbitrarily chosen. After landing on a rose shoot her behaviour was observed until she left the shoot, or in case she did not leave for a maximum of 45 minutes. This procedure was repeated on 20 days in autumn 1994. Observations took place only on sunny days at temperatures between 20 and 23°C.

Six categories of behaviour were distinguished a) resting - no noticeable movements of the wasp, usually positioned on the lower side of a leaf, b) grooming - intensive cleaning of mostly antennae and legs, c) searching - behaviour assumed to be related with searching activities, indicated by either erected and moving antennae when standing, or predominantly walking and drumming the substrate with the antennae, d) flight - short distance flight, e.g. from bud to leaf, e) encounter with host - head directed towards nearby host f) attack - abdomen with ovipositor bent and directed towards host, thrust and sting. Only resting, grooming and searching were quantified by timing. Flights, encounters and attacks were counted but not timed and are included in the foraging time.

The procedure allowed observations of behaviour of *A. rosae* without disturbance or interference. On the other hand, these observations had the disadvantage that they took place under uncontrolled conditions. Among other factors, the ages of females, their experience with hosts and the numbers of parasitized aphids on the shoots at the start of observations were unknown. These factors can have profound effect on patch-residence times and the behaviour of the wasps (e.g. Godfray, 1994). Statistical comparison of results obtained in the morning

morning and results obtained in the afternoon was undertaken by SAS using the nonparametric NPAR1WAY procedure (Wilcoxon rank-sum test)(SAS, 1985)

7.3 Results

Females of *A. rosae* landed both on the tip of the bud (24 times) and on the first leaf following the bud (16 times). Upon landing the wasps immediately started drumming the plant with the tips of their antennae. An encounter with a host was often accompanied by wing fanning (Fig. 7.1). In a manner typical of Aphidiinae, a female bent its abdomen underneath the thorax with the ovipositor directed towards a potential host when attacking it (Fig 7.2). Wasps remained in this position until the oviposition attempt took place. This usually took a few seconds, but in some instances wasps were observed in this position for longer than a minute. Normally, the actual sting took less than a second.

A. rosae attacked aphids only at the edge of the colony or isolated individuals. Females were never observed to walk through a dense colony. Attacks on aphids in the middle of a colony were undertaken in cases where leaves or stalks of leaves were running parallel to aphid colonies on the shoot. In these instances wasps were able to sting aphids without entering the colony (Table 7.1).

In general, a wasp undertook a series of attacks on aphids in the colony and then walked away, usually to the lower side of a leaf where it rested or groomed (Fig. 7.3). This alternation between resting periods and active periods was the typical pattern of activity of *A. rosae* in host patches. Parasitoids were regularly observed to take short flights and then land on the same or a different plant structure on the same shoot, e.g. they undertook a flight from the tip of the bud to a leaf.

Females spent more time on a single rose shoot in the afternoon (1952 s) than they spent in the morning (1390 s)(Table 7.1). It has to be emphasised that these means do not represent the true patch-residential time of parasitoids since observations were stopped after a maximum of 45 minutes. Patch-times of six

females had to be censored in the morning compared to 12 females in the afternoon. However, the statistical analysis was undertaken on ranks and since equal data are transferred to the same rank value all observations lasting 45 minutes or longer were equalised in the analysis.

Females were less active in the afternoon than in the morning. They spent most of their observed patch-residential time resting (1353 s or 69 %) and grooming (433 s or 22 %) instead of searching which occupied only 176 s of their time (9 %). In contrast, females in the morning spent more time for searching (780 s or 56 %) and less for resting (429 s or 31 %) and grooming (181 s or 13 %). In the morning parasitoids stung hosts more frequently (16.1 attacks/hour) compared to 4.9 attacks/hour in the afternoon. The mean number of attacks per rose shoot was 6 in the morning and 3 in the afternoon. The percentage of aphids rejected for attack was 10 % in the morning and 12 % in the afternoon.

Fig. 7.1 Female of *Aphidius rosae* foraging on a bud of *Rosa sp.*, var. Tea Hybrid 'McGredy's sunset'. The parasitoid is drumming with its antennae on the bud. Aphids on top of the bud consist mainly of second instars. An apterous adult of *M. rosae* is located on the lower left side of the bud.

Fig. 7.2 Female of *Aphidius rosae* attacking a younger instar of *Macrosiphum rosae* on a bud of *Rosa sp.*, var. Tea Hybrid 'McGredy's sunset'.

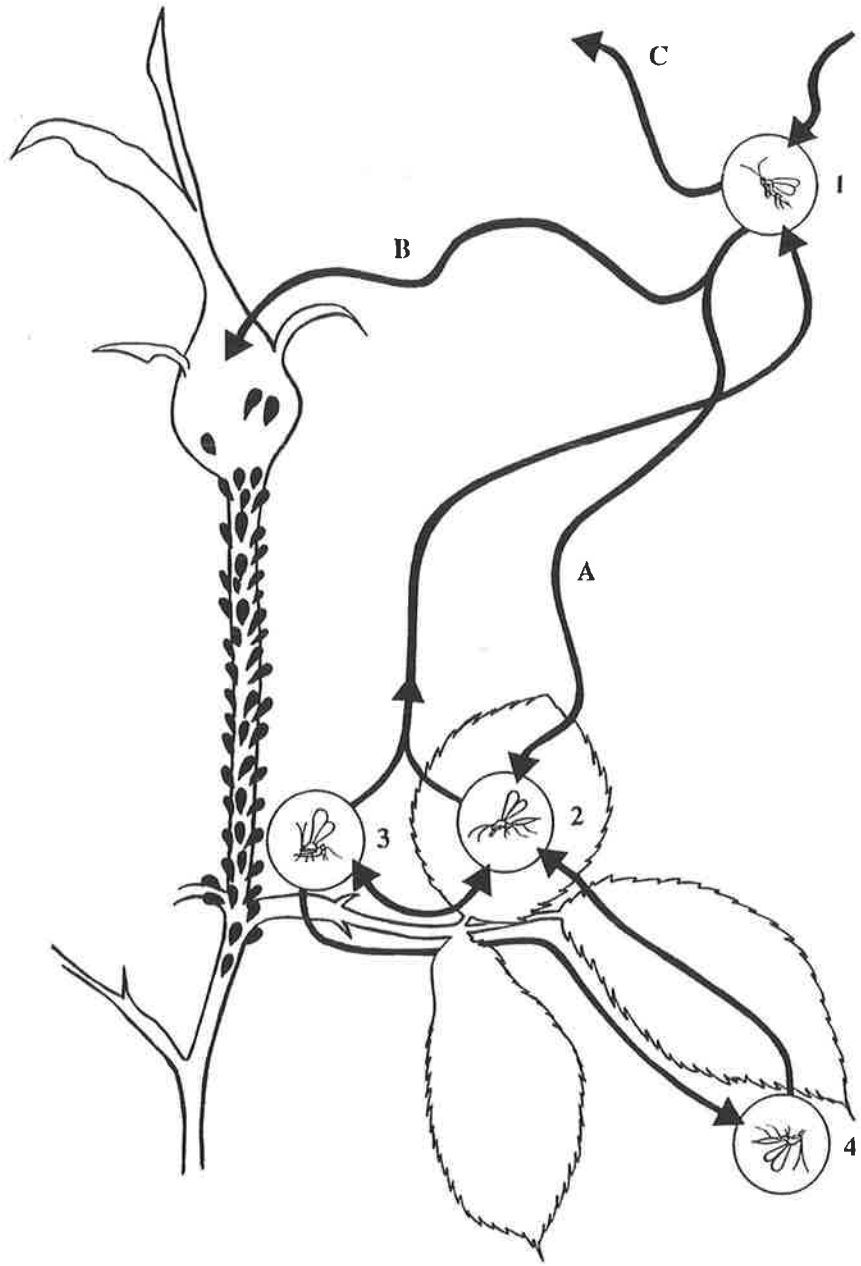


Fig. 7.3 The principle behavioural pattern of *Aphidius rosae* on a rose shoot infested with its host *Macrosiphum rosae* (stylised in drop shape). A wasp approaches a shoot exclusively by flight (1). It lands on the first leaf following the bud (A), the bud itself (B) or flies away (C). After landing on bud or leaf a female walks towards the colony of *M. rosae* (2→3). After encountering hosts, a series of attacks take place (3). The number of attacks is variable. After a while, the parasitoid usually walks away from the place of attack. *A. rosae* may take off (3→1) to leave the patch or to land on another plant structure (e.g. bud→leaf). Females walk regularly to a lower side of a leaf to rest or to groom (3→4). Resting times are variable. After a while females start to forage again (4→2) which may lead to further oviposition (2→3) or to a take off (2→1). This alternation between resting and foraging periods seems to be typical for the activity of *A. rosae* on a shoot. Parasitoids do not enter dense colonies and only parasitize aphids at the edges of a colony.

Table 7.1 Behaviour of *Aphidius rosae* on shoots of *Rosa sp.*, var. Tea hybrid 'McGredy's sunset' infested with *Macrosiphum rosae* in the morning (10 am) and afternoon (5 pm). Except for row one and two, results show means and SE of mean (n =20).

	Morning	Afternoon	
First landing bud : leaf	11 : 9	13 : 7	
Number of censored observations †	6	12	
patch time (s) †	1390 ± 241	1952 ± 224	p = 0.043
Activities in patch (s)			
(% of patch time)			
searching	780 ± 142 (56%)	176 ± 15 (9 %)	p = 0.0001
resting	429 ± 133 (31 %)	1353 ± 201 (69 %)	p = 0.0035
grooming	181 ± 46 (13 %)	433 ± 79 (22 %)	p = 0.0078
Number of attacks in patch	6.0 ± 1.0	3.0 ± 0.3	p = 0.147
Aphids encountered but rejected	0.7 ± 0.2 (10 %)	0.4 ± 0.1 (12 %)	
Attacks / hour	16.1 ± 1.4	5.0 ± 0.9	p = 0.0001
In-patch flights	1.0 ± 0.2	0.5 ± 0.2	
Attacks on aphids from a plant structure different to the structure attacked aphids were feeding on.	1.0 ± 0.4 (17 %)	0.4 ± 0.2 (13%)	

† Females were observed until they left the patch or observations were censored after a maximum of 45 minutes. Hence, means do not represent the true patch-residential time of parasitoids.

The study was carried out in the field and took place on sunny days at temperatures between 20 and 23°C. Analysis was undertaken with Wilcoxon rank-sum test.

7.4 Discussion

Females of *A. rosae* landed 24 times on the tip of the bud and 16 times on the first leaves following the bud. This result contrasts with results of Völkl (1994) who found that *A. rosae* first landed exclusively on the tip of the bud. This difference could have resulted from considerable differences in plant morphology in rose varieties used. Tea hybrid roses used in the present study have long exposed bud shoots compared to dog roses with shorter bud shoots used in Völkl's study.

Females of *A. rosae* were never found to walk through dense colonies of *M. rosae* and parasitized only aphids on the edges of colonies. This behaviour is likely to be related to aphid defence. Koume & Mackauer (1991) demonstrated that defensive behaviour of aphids, e.g. kicking, can help aphids to avoid attacks of parasitoids. Also, the secretion of aphid cornicle wax can be a serious threat to attacking *A. rosae* females (Chapter 5). Therefore, the careful attacking behaviour of parasitoids may be seen as adaptation to the potential danger aphid defence represents. The telescopic potential of the abdomen of Aphidiinae used during attack (Stary, 1970) may reflect the importance of keeping away from the immediate vicinity of aphids. As a result of this restricted access to a colony, aphids in the middle of dense colonies on roses with long bud shoots like Tea hybrid 'McGredy's sunset' are virtually out of reach of *A. rosae*. This may explain in part the inverse density-dependent response of the parasitoid (Chapter 10).

However, by using leaves, and stalks of leaves running adjacent to aphid colonies, the parasitoids were able to increase the numbers of accessible aphids. On younger bud stages and different rose varieties with only short bud shoots this may be an important strategy and may enable *A. rosae* to exploit aphid colonies more efficiently. The structure of a plant has been shown to be an important factor in the foraging success of aphidiine wasps. Völkl (1994) showed that *A. rosae* undertook 17.3 % of all attacks on *Sitobion fragariae* (Walker) from plant structures other than those where the attacked aphid was resident. Weisser (1994) demonstrated that

removal of leaves from brown knapweed *Centaurea jacea* L. decreased the oviposition rate of *Aphidius funebris* Mackauer on *Uroleucon jaceae* L. feeding on the stem of the plant.

The results suggest that *A. rosae* shows more oviposition activity in the morning than in the afternoon. The average rate of 16.1 attacks/hour for the morning would, if continued over the whole day, suggest average daily fecundity rates more than double the maximum daily oviposition rate displayed by individuals in the laboratory (Chapter 5, Fig. 5.8). Consistent with laboratory findings, a change of oviposition activities over the day was found, with a significantly lower afternoon attack rate of 4.9 attacks/hour. Collins & Dixon (1986) showed under laboratory conditions that the number of ovipositions of *Monoctonus pseudoplatini* (Marshall) decreased significantly over a time period of six hours. In contrast, Völkl (1994) found that the oviposition rate of *A. rosae* did not change significantly between earlier and later visits to colonies. However, in his experiment the time differences between first and last visit were on average 144 minutes compared to 420 minutes in the present study.

A significant change occurred also in the pattern of behaviour between afternoon and morning. In the morning *A. rosae* spent 56 % of its patch-residential time searching whereas females allocated only 9 % of their patch-time searching in the afternoon. This result corresponds to the work of Völkl (1994) who found that females of *A. rosae* spent 49 % of their time searching in earlier patch-visits compared to 25 % in later visits. Such a decrease in oviposition activity could be explained by e.g. decreasing egg-load of females (Rosenheim & Rosen, 1991) or females' experience (Visser et. al, 1992). Whatever caused these differences in behaviour, the results indicate that changes in the behaviour of *A. rosae* can occur over the course of the day. In future experiments time of the day will be an important factor in assessing quantitative aspects of behaviour of *A. rosae* in the field.

Number released and success in establishment of *Aphidius rosae* in Victoria.

8.1 Introduction

Control agents establish in just 34 to 39 % of biological control attempts with arthropod agents (Hall & Ehler, 1979; Waage, 1990) and only 60% of these provide some degree of control (Hall et al., 1980). These low rates of success, and especially the inability to explain failure in many cases, are one of the major criticisms against biological control and help to create substantial scepticism. Hypotheses for the failure of establishment range from poor selection of control agents and inadequate rearing programs to insufficient release techniques and adverse conditions at the time of release (e.g. DeBach, 1964; Van den Bosch & Messenger, 1973; Huffaker & Messenger, 1976; Mackauer et al., 1990).

Among these hypotheses, the inadequate numbers of introduced organisms has received considerable attention. Van den Bosch (1959) postulated that a minimum 'inoculation charge' is needed if a reasonable chance for parasitoid establishment is to be expected. Beirne (1975) analysed retrospectively Canadian introductions of parasitoids and predators for biological control and reported that nearly 80% of species established when more than 30 000 individuals were released per site but only 10% of species established when the totals were under 5 000. However, he pointed out that by world standards these lower figures contain atypically few data from control agents of homopteran species on which control agents colonise relatively easily (Beirne, 1985). Ehler & Hall (1982) also found that the success rate of establishment rises when higher numbers of control agents are released.

Hopper & Roush (1993) analysed the relationship between numbers released and successful establishment in introductions of parasitoids of lepidopteran pests. Additionally they developed a reaction-diffusion model to test the hypothesis that biological control introductions fail because an Allee effect drives small, introduced populations to extinction (Allee, 1931). At low densities parasitoids become so rare due to dispersal that females and males fail to encounter each other. The outcome of both approaches suggested a general threshold of about 1000 released insects at a single site and time to gain a high probability of establishment.

I tested if this hypothetical threshold would be valid for the establishment of *Aphidius rosae* Haliday. *A. rosae* was released in different numbers at eight cities throughout Victoria, Australia in September 1993.

8.2 Methods

Insects. The parasitoids used in this experiment were reared in six gauze cages (1.2 m x 1.2 m x 1.2 m) in the field. Rose bushes inside cages were sprayed with Pyrethrum (CRG LTD, 25 ml insecticide[4 g/L Pyrethrins & 16 g/L Piperonyl Butoxide] : 1 L water) to kill any predators. One week later the plants were reinfested with *M. rosae* and the aphids were allowed to increase in numbers for another week before release of the parasitoids took place in the cages. Two days after the first mummies were observed, the roses were completely pruned back to the stems and cuttings were taken to the laboratory. Mummies were collected and stored at 8°C for 2 days until start of releases. The remaining aphids were kept on rose shoots at 18°C and additional mummies that formed were used in the experiment as well. A mixture of mummies from each cage was used at each release site.

Release. Releases took place from the 9 to 11 Sept. 1993. Over the three days of transport to the release sites, the mummies were stored in cool boxes during the day (8 -12°C) and in refrigerators at night (4 - 7°C). Insects were released as mummies,

most of them still attached to leaves or stems. The material was simply placed underneath rose bushes at the sites of release.

All release sites were community gardens with at least 40 rose plants. Communities and gardeners co-operated by not spraying any insecticides in the first year following the release. The eight chosen cities (Fig. 8.1) have similar climates during the periods of main abundance of rose aphids (spring and autumn) and had populations of 6600-12200 citizens (Appendix.3). Cities were separated by rural countryside and at least 40 km apart. The number of mummies released varied from 16 to 1024 in a geometric series (Table 8.1).

Surveys. To detect the establishment of *A. rosae* a first survey was undertaken in autumn, six months after release during 21 - 24 March 1994. At this time, local populations of *A. rosae* had survived the summer, a period when aphids are either absent or present in very low numbers (Maelzer, 1977). A second survey followed in late spring, during 25 - 29 November 1994. By then, populations had also survived the winter and would have experienced the complete range of seasonally adverse conditions within a whole year.

Samples of the recovered mummies were reared to confirm the identity of emerging parasitoids. The survey was not restricted to the release sites but included rose gardens in the vicinity within each city as well. In most cases the number of mummies recovered was too low to undertake quantitative analysis. Additionally a high variation in aphid abundance among sites restricted this aim. However, all mummies encountered during one hour of intensive search were counted as an index of the abundance of *A. rosae*.

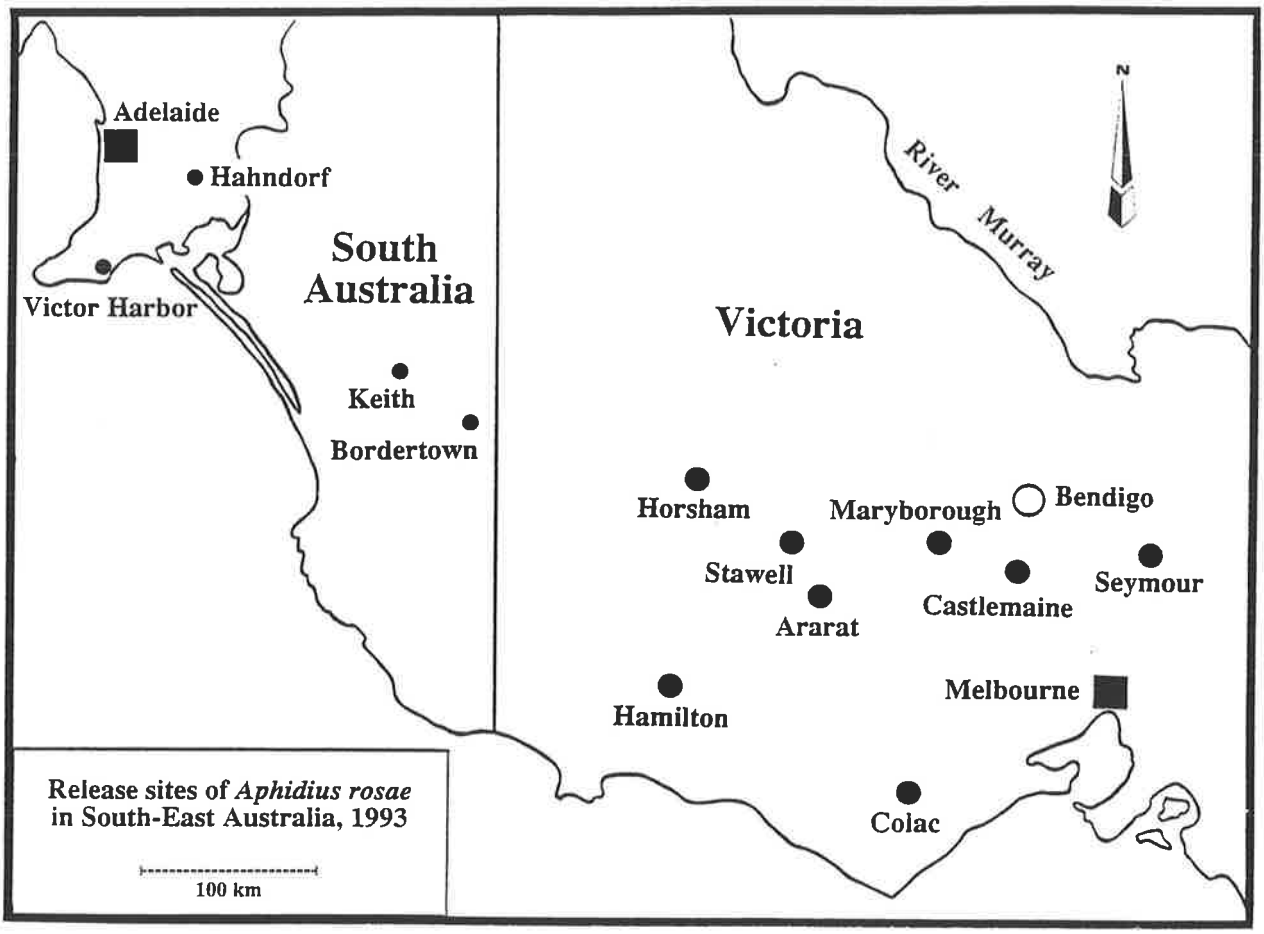


Fig. 8.1 Map of South-East Australia, displaying the release sites of *A. rosae* in 1993. ● = Cities which were used in the release experiment. ● = Cities with additional releases. ■ = Capital cities with release sites. ○ = No mummies were released in Bendigo but mummies of *A. rosae* were detected.

8.3 Results

There was no clear pattern in establishment versus numbers of mummies released (Table 8.1). 14 months after initial release, mummies were found in Hamilton (16 mummies released) but the parasitoid was not detected there eight months earlier. No recoveries of *A. rosae* were made in Colac (16 mummies released). In Seymour (64 mummies released) mummies were detected in both surveys, but in Stawell, where 64 mummies were also released, no recoveries of *A. rosae* were made. No mummies or rose aphids were found in Ararat in the second survey, even though *A. rosae* was detected in this city during the first survey. In Maryborough (1024 mummies released) no recoveries were made in autumn but a few mummies were found in spring. Castlemaine (1024 released mummies) revealed the highest abundance of *A. rosae* and *M. rosae* in the first survey as well as in the second. Mummies of *A. rosae* were also found in Bendigo (no release), 35 km away from the release site in Castlemaine during the second survey.

Table 8.1 Numbers of mummies of *Aphidius rosae* released in eight cities in Victoria, Australia, and recoveries of mummies after 6 and 14 months. Numbers of recoveries represent all mummies counted during 60 minutes.

Town	No. released in September 1993	Recoveries in March 1994		Recoveries in November 1994	
		A. rosae	Abundance of *M. rosae* ‡	***A. rosae***	Abundance of *M. rosae* ‡
Colac	16	**0**	medium	**0**	low
Hamilton	16	**0**	low	**3**	low
Stawell	64	**0**	high	**0**	low
Seymour	64	**105**	high	**165**	medium
Horsham	256	**0**	low	**0**	low
Ararat	256	**231**	medium	**0**	none
Maryborough	1024	**0**	medium	**34**	low
Castlemaine	1024	**1498**	high	**1171**	high

‡ The abundance of the host *Macrosiphum rosae* is estimated; *high* = aphids were present on most bushes, *medium* = aphids were present on the minority of bushes, *low* = aphids were found occasionally on bushes, none = no aphids found.

8.4 Discussion

Six months after release, mummies of *A. rosae* were recovered at three sites where 64, 256 and 1024 parasitoids were released, indicating that 1000 insects were not necessary for initial establishment. In the second survey, *A. rosae* was even recovered in a site with only 16 released mummies. However, results indicate also that even 1000 released insects/site may not reliably achieve establishment.

Rainy and stormy weather occurred throughout the release area for the first two weeks after release. It is possible that locations where low numbers were released suffered disproportionably more from these adverse conditions since their initial low density could easily have been lowered below a critical threshold. Better results in establishment might have been achieved, if the weather had been more favourable.

The chosen release technique was most practicable for the experiment but did not favour establishment. The actual number of parasitoids in the field was assumable to be even lower than the numbers released, due to natural variation in time to emergence and possible predation on mummies.

At Maryborough, Victoria (1024 released), no recoveries were made after six months but mummies were found after 14 months. Since Maryborough is only 40 km away from Castlemaine where the parasitoid established most successful, these recoveries could have been the offspring of spreading females, especially since *A. rosae* was also found at Bendigo (no release) (Fig. 8.1), 35 km away from Castlemaine.

At Ararat (256 released) a substantial number of mummies were found in autumn after six months but not in spring after 14. According to David Clark (Alexander Gardens, Ararat release site) no aphids or parasitoids were found during the whole of spring, normally the peak time for aphid abundance. This could be due to a cold wet winter with unusually long frost periods in this area. *M. rosae* probably does not produce sexual forms in Australia (Wöhrmann et al., 1991) and therefore does not produce eggs, the stage in aphid life cycles which is best adapted to frost.

Diapausing parasitoids in mummies are well adapted to low temperatures and offspring from the observed population in autumn probably survived the winter. But individuals emerging in spring might have dispersed in the absence of hosts.

At none of the sites where only 16 mummies were released were recoveries made after the first six months, but three mummies of *A. rosae* were found in Hamilton 14 months after release. It is hard to judge if these individuals were offspring of the released parasitoids. In the first survey parasitoids may not have been found because of low and patchy abundance, or they could have spread naturally or by human activities from another release site. Aphidiinae can display a great dispersive power (e.g. Stary, 1988). Distances between release sites clearly could have restricted movements but the possibility of few far spreading individuals can not be excluded.

However, the result of initial establishment with low numbers is supported by findings from additional releases in Bordertown and Keith (Fig. 8.1). These two country towns along the Dukes Highway have a warmer and drier climate than the chosen cities in Victoria and therefore were not included in the experiment. In both towns additional releases of 16 mummies were undertaken on 9 Sept. 1993. No mummies were recovered after six months but after one year six mummies were found in Keith and eight mummies in Bordertown. Again, it can not be excluded that these mummies result from spreading *A. rosae*.

Additionally support for the finding that a released number of 1000 insects per release was not necessary to achieve establishment of *A. rosae*, was obtained from two release sites in Adelaide, South Australia (Fig. 9.1) each in which over a period of four weeks 200 adults were released weekly into cages, resulting in establishment and wide spread (Chapter 9). Furthermore, a single release of 300 adults without cages at Hahndorf, South Australia (Fig. 8.1), resulted in establishment but three releases with a total of 1200 adults in cages at Victor Harbor, South Australia (Fig. 8.1), did not result in establishment during the unfavourable summer period.

Campbell (1976) released between 10 and 1000 individuals of *Aphytis melinus* De Bach, a parasitoid of *Aonidiella quarantii* (Mask.), per release site in South Australia. Under unfavourable conditions 1000 insects were necessary to achieve establishment, but released at the right time of the year 10 -100 adults were sufficient. In California, Olkowski et al. (1982) achieved establishment of *Trioxys complanatus* (L.), a parasitoid of the linden aphid *Eucallipterus tiliae* L., by releasing only 160 wasps. Data from Canada and the USA (Beirne, 1985) show that the number of accidentally introduced and established parasitoids about equals the number that have been established in classical biological control attempts. Since accidental introductions are not thought to involve high numbers of individuals, this might be taken as an indication for the relative ease with which small populations of some species are able to establish.

Aphidius sonchi Marshall was released in Australia as a control agent of the sow thistle aphid *Hyperomyzus lactucae* (L.) and established widely (Carver & Woolcock, 1986). In most releases thousands of insects were used, but in two cases only 500 and 800 parasitoids were released. Establishment took place in these instances as well.

Hopper & Roush (1993) conceded that low release numbers may only reflect either a problem of or commitment to handling or rearing the control agent. This point is also emphasised by Greathead (1986), and DeBach (1964) suggested that success in biological control is achieved in proportion to the effort invested.

The success rate of biological control agents is highest against Homoptera compared to pests from other taxonomic groups (Greathead, 1986; Kfir, 1993). Homoptera tend to occur in patchy, large concentrations and Beirne (1985) saw in this pattern the key for the relatively high colonisation rate of parasitoids of this group. As a result of host aggregation, the parasitoids also occur in clumps and do not have to disperse too widely to find their targets.

Rose aphids virtually disappear during certain times of the year from host plants and aphidiine wasps have mainly adapted to this situation by entering diapause

(Stary, 1970, 1988). Even if the parasitoid is well synchronised to its host, in the beginning of seasonal aphid outbreaks, parasitoids will have to face a thin distribution. The ability to disperse effectively and to aggregate at early, patchy aphid outbreaks may be one of the key factors to overcome this situation. Therefore, the ability to recolonise sites even in low numbers might be crucial for the survival and spread of *A. rosae* and Aphidiine populations in general.

In most biological control programs, as many insects as possible are released into the field, exceeding the hypothesised threshold of 1000 manyfold according to the rule "more is better". Nevertheless, the application of low release numbers is relevant for many programs that have limited labour and/or material capacity for mass rearing. The question then is, would it be better to release many insects at a few sites to increase chances for mate finding, or should limited numbers of control agents be released in a variety of places to cover potential host and environmental variation ? Campbell (1976) showed with the release of *A. melinus* that a tenfold increase in number of released parasitoids only doubled the likelihood of successful establishment. Hence, releasing more than the minimum of 100 parasitoids required, had little effect on the outcome and was judged by him as a waste of parasitoids.

The release of *A. rosae,* as well as examples with other aphidiine wasps, shows that this group of parasitoids is able to establish in numbers far below the hypothesised threshold of 1000 insects per single release site if the quality of released insects and release techniques favour establishment. It appears that *A. rosae* can easily be distributed to Institutions and commercial rose growers because this species can establish when relatively small numbers are released.

The findings from the releases of *A. rosae,* as well as examples from the literature indicate the limitations of broad retrospective analysis of biological control attempts, which in this case was not sufficient to predict failure or success in establishment under specific circumstances. Rather than using data from hundreds of control attempts in which the outcomes are influenced by a wide range of factors, predictions may be more precise if only few closely related case studies are analysed.

Establishment and spread of *Aphidius rosae* in Adelaide.

9.1 Introduction

The ability of parasitoids to disperse is important in biological control since control agents can not be released everywhere. Insect populations are usually clumped in time and/or space, and the distribution of these clumps can change from season to season. The patchy distribution of pest outbreaks may disrupt synchrony of parasitoids with their host population in space, even if they may be in synchrony with the host in time (Vinson, 1981). These uncertainties, combined with other decisions of the parasitoid about host patch use (reviewed e.g. by Van Alphen & Vet, 1986; Godfray, 1994), make dispersal a critical issue for the success of parasitic wasps in biological control.

Most studies of insect dispersal have been undertaken on pest insects rather than their parasitoids (for summaries see Johnson, 1969; Pedgley, 1982). Marked insects are often used to investigate short range/duration dispersal of insects (e.g. Bishopp & Laake, 1921; Stern et al., 1965; Messing et al., 1994). To investigate long range/duration dispersal this method is only useful if the insects show a pronounced migratory behaviour and/or are relative long lived, e.g. the monarch butterfly, *Danaus plexippus* (L.) (Urquhardt, 1941). Therefore most data for non-migratory long range/duration dispersal have been obtained when biological control agents have invaded new environments. In these instances, dispersive movements might be more correctly called spread, since they result in a major modification of a species geographic distribution (Smith, 1959).

Data on long range/duration dispersal of control agents, including Aphidiinae, usually indicate only the maximum extend of spread. E.g. one year after

release *Aphidius ervi* Haliday was found as far as 300 km away from the nearest release site (Milne, 1986), and *Praon palitans* Muesebeck spread from a single release site over an area of 2000 km^2 (Van den Bosch et al., 1959). *Lysiphlebus testaceipes* (Cresson) was released in Antibes in 1973 and 1974. In the following 14 years the parasitoid became widespread over southern France, Spain, Portugal and Italy (Stary, 1988). These data are useful in demonstrating the dispersive power of a population, but they do not contain much information for economic entomologists since they do not reveal local movements of a population in a specific target area (Johnson, 1969). The dispersive movements of an established population of control agents in a patchy environment is of special interest at the beginning of a new season, especially in classical biological control attempts.

More detailed data on the spread of Aphidiinae are available from the release of *Aphidius eadyi* Stary, Gonzales & Hall in New Zealand (Cameron et al., 1981) and *Trioxys complanatus* Quilis (= *utilis* Muesebeck) in South Australia (Wilson, 1982), but in both releases, field samples were not designed to investigate underlying mechanisms of spread and therefore interpretation is difficult.

However, by analysing dispersive movements of insects after their release it should be considered that insect behaviour may not be independent of release techniques. For example, adult insects kept in captivity show a strong tendency to fly off and away after release (Hughes, 1989). The enormous number of control agents used in most releases of parasitoids maximises the chance of establishment and economic effectiveness but in like manner creates overpopulated patches with strong migratory tendencies. Ruth et al. (1975) and Sinha & Singh (1980) found in laboratory experiments using overpopulated patches that repeated interactions between aphidiine wasps resulted in an increased tendency to disperse. It was assumed that wasps under field conditions would have left the patch. Therefore, data collected following mass releases can give important information for biological control, but such information does not necessarily indicate patterns of natural dispersal.

Parasitoids of aphids spread mainly by 1) active flight, 2) passive flight as larvae inside alatae, 3) as mummies attached to litter displaced by wind and by 4) human activities (Stary, 1970). Low densities of the aphid host and/or mutual interference between individuals of the same parasitoid species are assumed to cause aphidiine wasps to disperse (Van den Bosch et al., 1957; Stary, 1970, 1988). Additionally, Höller et al. (1994) showed that abundant hyperparasitoids could cause dispersal of aphidiine wasps.

The release of *Aphidius rosae* Haliday in Adelaide, South Australia, presented an opportunity to monitor the spread of a small population of aphidiine wasps in a new environment. Since there was no urgent need to control this pest, I had the rare opportunity to release only small numbers of wasps and therefore to minimise the unnatural conditions associated with mass releases. The release was intended to simulate a small clumped population of aphid parasitoids after an initial build up early in the season and to investigate their spread over the following year.

9.2 Methods

Release. 800 individuals of *A. rosae* were released in each of two rose gardens in the metropolitan area of Adelaide (Fig. 9.1). One rose plot was located at the Waite Campus and consisted of 128 rose bushes. The second rose garden (Veale Gardens) was situated near the City centre and comprised approximately 1000 rose bushes at the time of release. Insects were released weekly, in groups of 200 per site (27 Aug. to 17 Sept. 1993). At each site 100 males and 100 females were released into 2 screened cages measuring 1.2 m x 1.2 m x 1.2 m in size. The cages were placed around rose plants two weeks before release at the release site on the Waite Campus . The plants were sprayed with Pyrethrum (CRG LTD, 25 ml insecticide [4 g/L Pyrethrins & 16 g/L Piperonyl Butoxide] : 1 l water) to kill predators. One week after this treatment the plants were reinfested with *M. rosae* and the aphids were allowed to breed for another week before a release of parasitoids took place. The

wasps were released in the early morning and the cages were not removed before sunset on the second day. Parasitoids were then free to disperse.

Plants were not treated with insecticide at the public Veale Gardens. Cages were simply placed over roses with heavy aphid infestations on the day of release. The parasitoids were released in the morning and cages were removed after sunset of the same day when most of the parasitoids were resting beneath leaves.

Monitoring. The monthly spread of *A. rosae* from the release site at the Waite Campus was assessed by searching for mummies on rose buds. Only very sporadically does *A. ervi* form comparable mummies on roses in the Adelaide region. In the year preceding the release of *A. rosae* only 3 mummies were found on roses. Therefore, mummies were only collected and reared for identification when found in low numbers in questionable locations. Counting the mummy stage does not interfere with the spread. Since the mummies are relatively persistent on the plant and are easy to find, it is possible to detect the previous presence of aphidiine wasps even at very low densities. At each site the maximum time spent searching was 10 minutes. Sampling sites had at least 20 rose plants and consisted of community gardens, school gardens and home gardens. They varied from month to month, depending upon aphid infestations and the actual spread of the parasitoid. Special attention was given to sites along three axes from the release point : towards North-East (NE) (23°-68°), South-West (SW) (203°-248°) and towards North-West (NW) (293°-338°). The NE and SW axes run along the foothill suburbs of the Adelaide Hills. The whole metropolitan area of Adelaide over which this survey was undertaken can be considered to have a regularly distributed high abundance of roses. In contrast, there are only few and patchy rose gardens to the South-East (SE)(135°) of the release site, in the Adelaide Hills. The spread of *A. rosae* towards SE is not documented in this paper because of this fundamental difference in the environment.

In April 1994, eight months after the initial release, a more quantitative assessment was carried out. As an index of the density of reproducing wasps, mummies on 25 randomly-chosen, heavily-infested 5th stage rose buds (Maelzer, 1977) were counted at each of 104 sites. If no mummies could be found, searching was continued for the remainder of 10 minutes. If during this time mummies could be found, the site was still included as a site of establishment but it had no influence on the assessment of density. From this survey, density curves of *A. rosae* mummies dependent upon distance from the release point on the Waite Campus were fitted along three axes. Smooth curves that best fit the data were fitted using Cricket Graph.

This chapter deals only with research on local spread of *A. rosae* over a range up to 23 km. It was not attempted to detect wasps that might fly up and away from the release point and beyond the limits of the sampling area.

Winds. The direction of prevailing winds was analysed by using data from the Bureau of Meteorology, Kent Town, South Australia. Wind direction is given in 16 compass points. The direction of winds were measured every hour in the periods from 28 Aug. 1993 to 15 Dec. 1993 and 15 Feb. 1994 to 20 Apr. 1994 between 6 am and 9 pm. The winds during these periods should have been the most influential on the spread of *A. rosae* since hosts and adult parasitoids were not abundant during the middle of summer and aphidiine wasps generally do not fly short/medium distances at night (Stary, 1970). For each hour only the predominant wind direction was given. Hours for each direction were summed and prevailing winds were determined by comparison of the sums.

9.3 Results

By 8 October, three weeks after the last release of *A. rosae,* the parasitoid was scarce around the release point at the Waite Campus. Mummies were found occasionally at only four sites not further than 500 m away from the release site. In November, mummies were abundant near the release point and were found in a 3 km long belt running from NE to SW (Fig. 9.1). Three mummies were found at a large rose garden in the Pasadena Cemetery, 6 km away from the release point (Fig. 9.1). In December aphid abundance dropped to nearly zero and it subsequently became very difficult to find mummies. This situation lasted the entire summer. In March 1994 the over-summer survival of the parasitoid population was apparent, and also substantial spread was observed. The occupied area was a belt spreading in the same direction as November, up to 5 km NE of the release site and up to 3 km towards the SW, parallel to the foothills of the Adelaide Hills (Fig. 9.2).

In April, over a period of 5 days, the survey was expanded over wide parts of the metropolitan area, including the release-point at Veale Gardens. Although the areas of spread of *A. rosae* from both sites were merging, the distribution of patches with higher densities indicated in both cases that the main spread was in the same direction (Fig. 9.3). From the release point at Veale Gardens no substantial spread towards S and SW was observed. At this time, *A. rosae* covered an area of approximately 200 km^2 and was found as far as 18 km away from the nearest release site.

When the declining density of mummies along three axes from the release point at the Waite Campus in April was examined (Fig. 9.4), the dispersal gradients towards NE and NW showed typical shapes for the relationship between density and distance of dispersing insects (Wolfenbarger, 1975). The curve towards SW displayed a fundamental difference with the build up of high densities of mummies around the Pasadena Cemetery.

After the final survey in April it was impossible to conduct further investigations without excluding considerable spread caused by human activity. At two sites, mummies were found on roses in commercial nurseries. Furthermore, rose cuttings bearing mummies were transported to dumping places around the city of Adelaide at the end of autumn. Also, coverage of the release by the media led enthusiastic home gardeners to distribute mummies to friends and family members.

By October 1994, 13 months after the first release, *A. rosae* was found all over the metropolitan area of Adelaide.

The analysis of data from the Bureau of Meteorology showed that prevailing winds were from the WSW to S (Fig. 9.3).

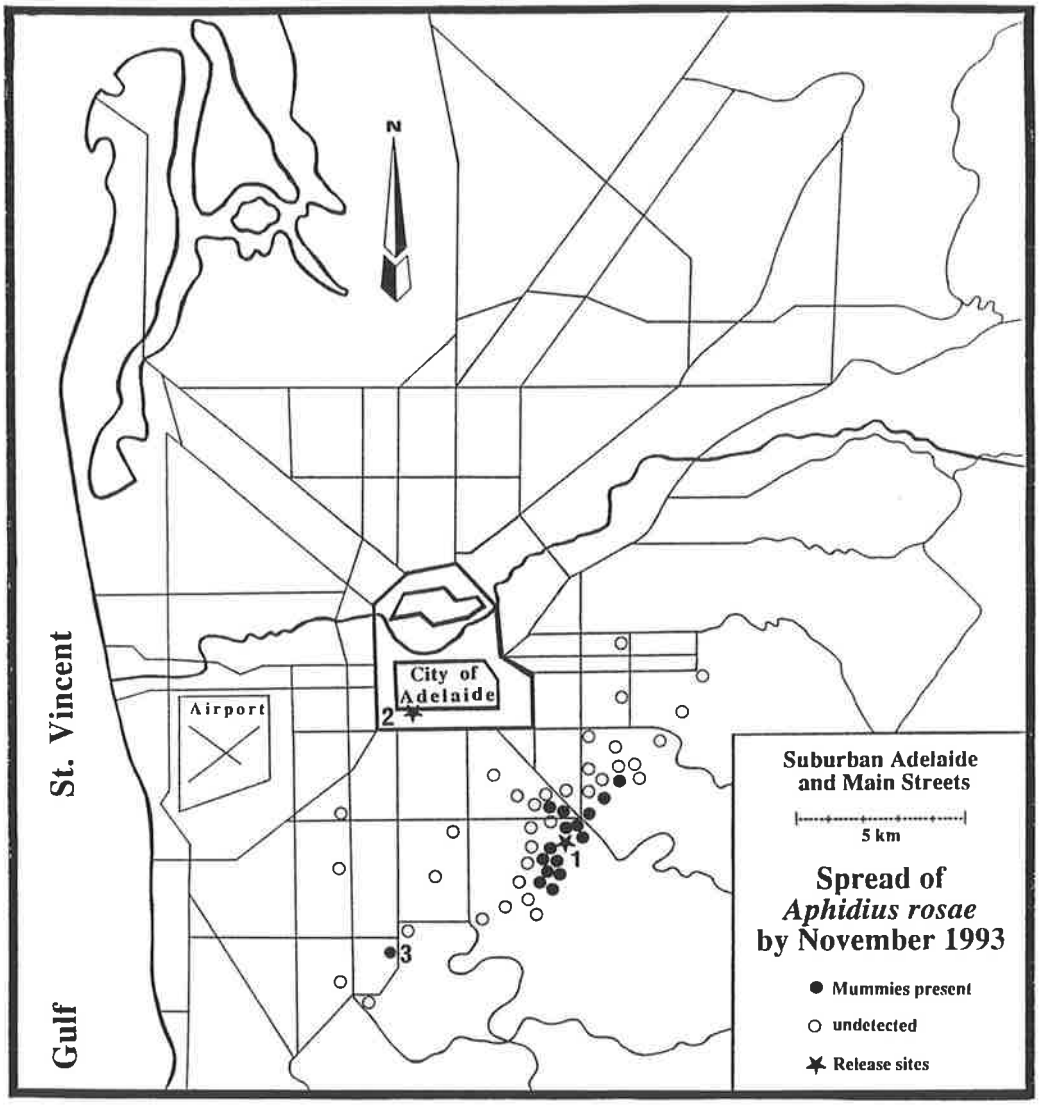


Fig. 9.1 Spread of *Aphidius rosae* from the Waite Campus by 16 Nov., 12 weeks after first release. Release sites : (1)Waite Campus and (2) Veale Gardens; (3) Pasadena Cemetery (no release site).

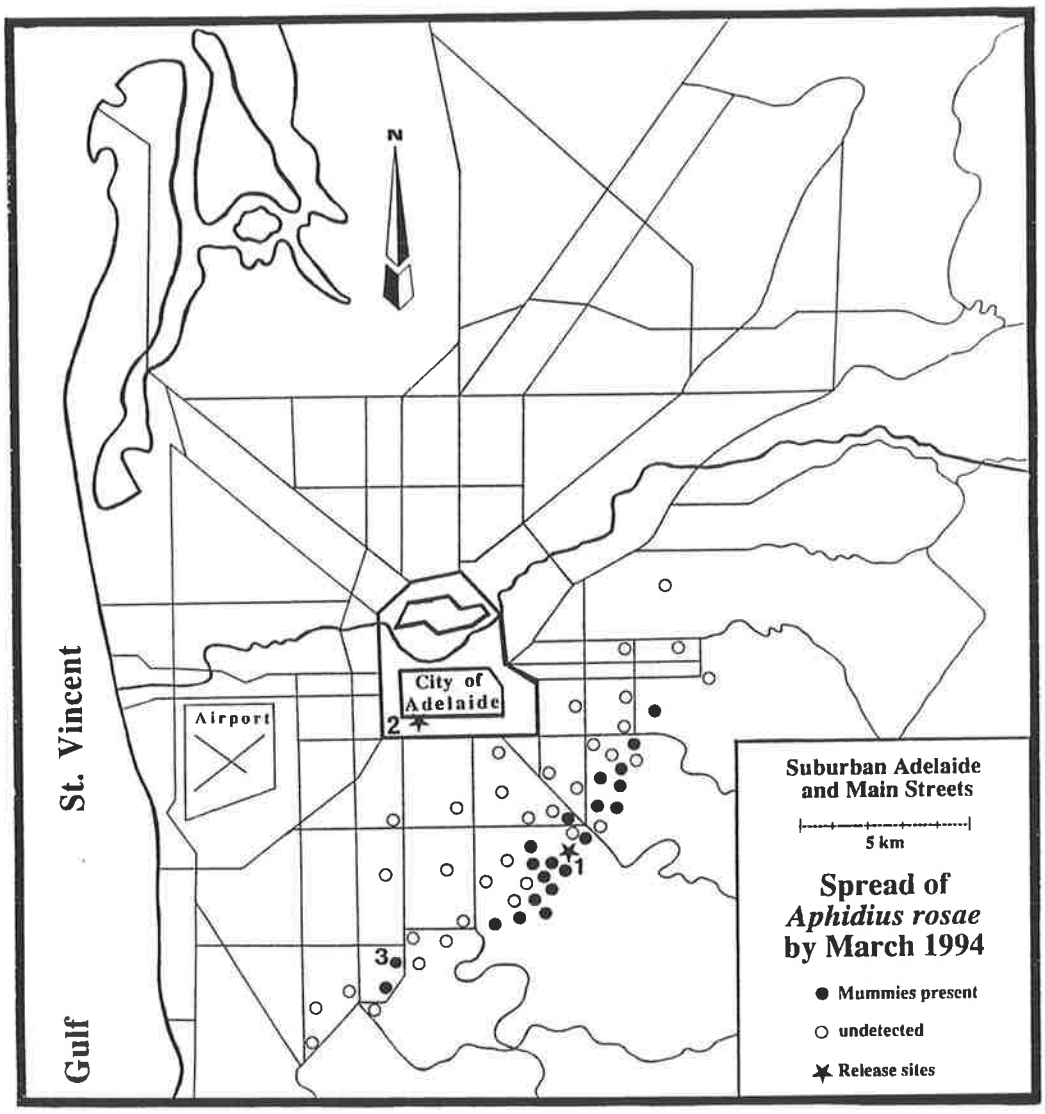


Fig. 9.2 Spread of *Aphidius rosae* from the Waite Campus (1) by 19/20 March 1994, seven months after first release.

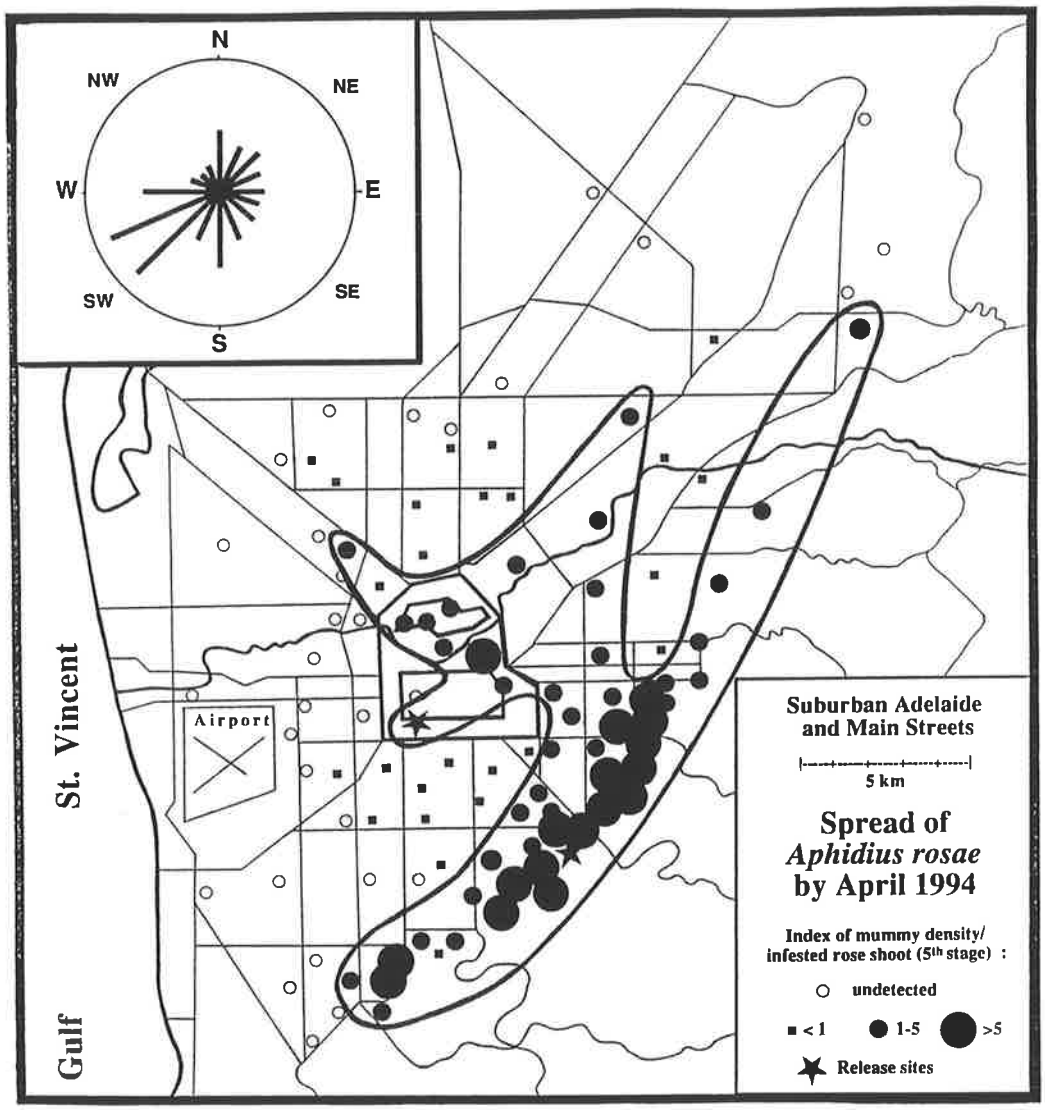


Fig. 9.3 Spread of *Aphidius rosae* in Adelaide with bordered area of higher densities, 18-22 April 1994, eight months after first release.

Inset : wind directions from the Bureau of Meteorology, Kent Town, South Australia, from the 10 Sept. 1993 to 15 Dec. 1993 and 15 Feb. 1994 to 20 Apr. 1994 between 6 am and 9 pm. The radius of the polar-graph represents 400 hours. Length of bars represent the sum of hours in which winds were prevailing from a particular direction.

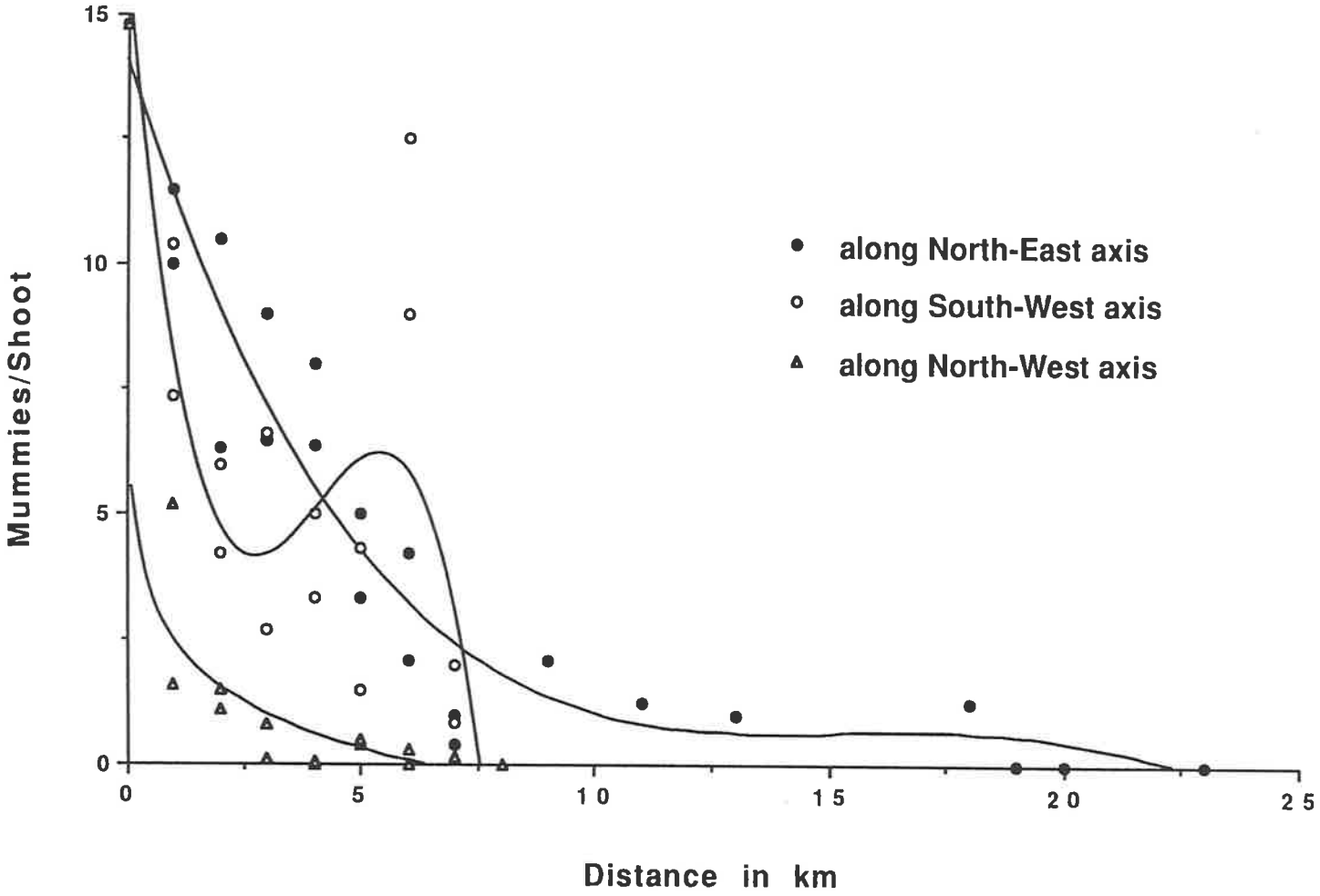


Fig. 9.4 Density of *Aphidius rosae* mummies dependent upon distance and direction from the release point on the Waite Campus in April 1994, eight months after initial release. Smooth curves that best fit the data were fitted using Cricket Graph.
North-East axis, $y = 14.12 - 2.927x + 0.22513x^2 - 0.007065x^3 + 0.00007087x^4$, $r^2 = 0.915$;
South-West axis, $y = 15.874 - 10.24x + 2.79x^2 - 0.22661x^3$, $r^2 = 0.565$;
North-West axis, $y = 2.4842 + (-3.111 \log [x])$, $r^2 = 0.951$.

9.4 Discussion

The concentration of mummies decreased as the distance from the point of release increased but the shape of the gradient in each of the three directions was different (Fig. 9.4). The shape of curves imply that dispersal was not random (Taylor, 1978). By analysing the density-distance data of various dispersing insect species, Taylor (1978) showed that random dispersal is generally unrealistic for insects. Random dispersal would occur only if the insects move independently without any repulsion, attraction or other influences from their environment.

For several species of parasitoids, it has been shown that wind plays a major part in determining the direction of dispersal (e.g. Anderson & Paschke, 1970; Keller et al., 1985). These findings are supported by the spread of *A. rosae* as determined in the April survey. From both release sites, parasitoids spread mainly in the direction of prevailing winds, i.e. towards the NE (Fig 9.3.)

On the other hand, winds are the carrier of odour cues for searching parasitoids. Since *A. rosae* is attracted to roses (Chapter 6), the high abundance of roses to the SW of the release site at the Waite-Campus might explain why lesser but still substantial spread occurred in a direction against the prevailing winds. On the other hand, individuals from the population which had established at Pasadena Cemetery soon after release at the Waite Campus (Fig. 9.1), may have spread back to the Waite Campus. Consequently, they may have contributed to the observed pattern of spread against prevailing wind from the initial release site. However, at the release site in Veale Gardens no roses are grown for about one kilometre towards SW, presumably resulting in prevailing winds carrying few if any attracting odours. No substantial spread of parasitoids was observed in this direction, suggesting that the absence of roses restricted major spread against the prevailing wind direction at this release site.

The slope of the density gradients suggest that the majority of reproducing females did not spread far. Densities of *A. rosae* declined steadily from the release point at the Waite Campus towards NE and NW whereas the slope of the density gradient towards SW was irregular (Fig. 9.4). The finding of a well established population at Pasadena was the consequence of the arrival and multiplication of *A. rosae* soon after release (Fig. 9.1). This pattern of establishment might be expected if individuals dispersed over distances up to several kilometres. However, this pattern of spread was isolated to this one site and this suggests that this mode of spread was exceptional.

Overall, the findings suggest that most individuals must have moved in a quite controlled manner in or near the insect boundary layer of their garden habitats, exploiting abundant hosts. Taylor (1974) defined the insect boundary layer as the space near the ground where wind flow is retarded and within which the insect's airspeed exceeds the wind speed. Above this the insect can no longer prevent displacement by the wind and is consequently carried with it.

Evidence from other species indicates that parasitic wasps fly predominantly within the boundary layer and avoid flying in high winds. Decker et al. (1993) caught more aphidiine wasps when traps were placed in between wheat tillers, rather than above the plants, outside the insect boundary layer of the wheat plants. Keller (1990) showed that flight by the parasitoid *Cotesia rubecula* (Marshall) was inhibited by increasing wind speed. It is known that aphidiine wasps prefer to run rather than fly under unfavourable whether conditions (Stary, 1970). This range of behaviour effectively enables parasitoids to avoid unwanted displacement. Even small insects are not completely transported passively by wind. The direction of travel might depend upon the wind, but to travel in this manner the insect must launch itself into the air and then keep itself airborne by persistent wing flapping, the duration of which partly determines the distance travelled (Johnson, 1969; Kennedy, 1975).

In spite of the low release numbers, high densities of *A. rosae* were recorded in a localised area around the release site (Fig. 9.3), suggesting that most parasitoids did not spread far and tended to exploit abundant aphids in their immediate vicinity. This behaviour would also have assisted in mate finding, a crucial aspect for establishment in the early phases of release. Newly introduced insect species dispersing into a new environment may become so thinly distributed that males and females often fail to encounter each other, especially when released in low numbers (Allee, 1931; Hopper & Roush, 1993).

The population of *A. rosae* not only survived the most crucial hot summer period when hosts were scarce, but also increased rapidly in numbers and spread over a vast area one year after release.

The spread of *A. rosae* in Adelaide has given important information for further releases of this control agent. The chosen release technique proved to be sufficient and mass rearing was not necessary. Like many other species, *A. rosae* covered wider areas in the direction of the prevailing winds but most individuals did not spread far. Therefore, optimum sites for first releases might be those that are adjacent to other host infested sites which would attract dispersing individuals. Dispersal can be costly and providing searching parasitoids the opportunity to find new habitats near by would allow more individuals to exploit new resources, to optimise their reproductive success and therefore to maximise the chance to establish the species in large numbers around the release site. Perimetrical dispersion is desirable but not likely to occur if prevailing winds are present (Hendricks, 1967). Therefore, good knowledge of wind conditions are essential for choosing optimum sites for release.

The changes of numbers of *Macrosiphum rosae* and *Aphidius rosae* in the field

10.1 Introduction

The size of aphid populations can change dramatically from year to year. Therefore, the change of their numbers should be surveyed for several years before and after the introduction of a biological control agent to be sure that the introduction results in a real change (Dixon, 1985; Hughes, 1989). Such periodical censes of pest populations were judged by Legner (1969) as the only reliable method to assess the impact of a biological control agent. Alternatively, experimental methods can be used to evaluate the impact of natural enemies; these are discussed in Chapter 11.

Only few cases of biological control attempts showed dramatic decreases of pest populations easy to interpret (DeBach & Rosen, 1991). In the majority of biological control attempts, the task is difficult. Especially aphid/parasitoid systems, with overlapping, fast reproducing generations create substantial problems in assessing the rate of parasitism in the field and even more problems in the assessment of the impact this parasitism is causing on the pest population. Impacts may be masked by the influence of other factors, such as the host-plant, hyperparasitoids and weather.

Research on aphid and parasitoid population dynamics in the field ranges from purely descriptive studies, through the application of life-tables and key-factor analysis, to the development of advanced computer-models. However, it is not always possible to apply the most powerful analytical method available, since the more advanced methods involve intensive study of the target species.

Phenology of aphids and their natural enemies. Many investigators of aphid populations stress the independence of aphid population dynamics from the controlling effects of predation. Wellings & Dixon (1987) emphasised the importance of favourable weather for optimal build up of aphid populations and cited many examples in which heavy rain greatly reduced aphid numbers. They judged weather conditions as the major variables governing aphid populations, either through direct effects on the aphids themselves or through effects mediated by the host plants. For example, Maelzer (1977) recorded more than 80 % mortality for adults of *M. rosae* after heavy rainfall. Likewise, wind was shown to a have tremendous effect on sycamore aphid (Dixon, 1985). The role of abiotic components was also stressed by Gilbert (1980a,b). Key-factors for population growth were mainly based on host plant - aphid interaction or weather. Losses to natural enemies were judged as having no substantial influence.

One of the main reasons for the relative ineffectiveness of natural enemies observed in many systems lies in their requirement for a generally higher temperature threshold for development than that of the pest (e.g. Campbell et al., 1974; Maelzer, 1981; Lajeunesse & Johnson, 1992). As a result, low temperatures at the beginning of a season reduce the effectiveness of natural enemies and can create a transient enemy free space lying within a temperature band in which aphid populations are able to increase (Wellings & Dixon, 1987).

However, natural enemies control aphids in other systems. For example, some populations of *Aphis fabae* Scopoli are regulated by coccinellid beetles (Dixon, 1985). The number of successful classical biological control programs against aphids gives further evidence of the regulating influence natural enemies can have on aphid population (e.g. Hughes, 1989; DeBach & Rosen, 1991).

In general, aphids reproduce as quickly and efficiently as possible on time limited favourable plant resources. They produce high numbers of dispersing alatae, to ensure high rates of colonisation, before declining plant quality and high numbers of voracious predators reduce the reproductive success in established colonies (Gilbert et al., 1976; Dixon, 1985).

Phenology of Macrosiphum rosae. In South Australia, *M. rosae* breeds anholocyclicly on roses with peaks in spring and summer. Maelzer (1977) concluded that the fate of a colony of rose aphids is a function of temperature, rainfall, predation, the time during which the bud remains favourable for the aphids, the density-dependent production of alatiforms and dispersal of apterae by walking or dropping off. The population dynamics of *M. rosae* was also investigated by Eggers-Schuhmacher et al. (1979) and Tomiuk & Wöhrmann (1980, 1982) in Germany. They concluded that perhaps plant quality was the most important factor for realising high reproductive rates. They also suggested that natural enemies were mainly responsible for the decline of a population towards the end of the season. This regulating effect of predators towards the end of autumn was also stressed for populations of *M. rosae* in Adelaide (Maelzer, 1977).

Assessment of parasitism. It is difficult to analyse the phenology of a single species, but a series of complications arise in coupled two-population aphid/parasitoid systems. With aphidiine wasps, the effect of parasitism is delayed so parasitized aphids remain available for census on the plant. This allows comparison between numbers of parasitized and unparasitized aphids, but gives rise to the problem of assessing the fate and mortality of aphids during the larval developmental time of the parasitoid. Counts of mummies still containing parasitoids and adult aphids present on a plant provides a quick estimation of parasitism but is not precise (Van Emden, 1963). E.g. the period of presence of a mummy on a bud is variable when hyperparasitism and diapausing individuals are taken into account (Rabasse, 1986). Furthermore, large numbers of parasitized aphids are often observed to leave the plant to form mummies in litter or soil (Behrendt, 1968) and thereby may not be included in the census. Parasitoid attacks may disturb aphid colonies, resulting in dislodgment of large numbers of aphids which are actually not parasitized but do suffer mortality (Tamaki et al., 1970). Sampling adults by traps or with sweep nets gives an estimation of relative changes of parasitoid populations over time (e.g. Boyd & Lentz, 1994; Brodeur & McNeil, 1994), but

reveals only very little of the true population size and nothing about the impact of natural enemies on the host.

These difficulties have let most workers to base estimations of rate of parasitism on dissection of alive aphids and counts of immature parasitoid stages in them. Tomiuk & Wöhrmann (1980) used starch gel electrophoresis to detect parasitoids in rose aphids. This procedure allowed them to distinguish between two parasitoid species, namely *A. rosae* and *Ephedrus sp.*. The method was not able to detect eggs or first larval instars of the parasitoids (Wöhrmann, pers. communication). The eggs of parasitoids are difficult to find and therefore most methods include a rearing period to allow the first instar larvae to hatch before the dissection of sampled aphids takes place. Day (1994) stressed his concerns about disproportionate mortality of parasitized hosts by diseases, oviposition trauma and stresses that occur during the rearing process. However, this sensitivity of parasitized hosts occurs more in some species (e.g. lepidopteran hosts) but rarely in aphids when rearing conditions are favourable (e.g. Cloutier & Mackauer, 1979).

There are several methods for estimating the rate of parasitism from the number of parasitized and unparasitized aphids. For example, Hughes et al. (1981) measured the rate of mummy formation in fourth instar nymphs sampled, whereas Rabasse (1986) suggested to concentrate on second and third instars. In both methods the theoretical reproduction rate of the sample, if no parasitism would have occurred, was compared with the actual observed reproduction rate to assess the impact.

To assess accurately the rate of parasitism and construct field life-tables, Van Driesche (1983), Bellows et al. (1988a) and Van Driesche et al. (1991) suggested an unbroken series of back to back sample intervals for a particular host stage which is suitable for parasitism. In this 'recruitment' methods all host which enter this particular stage are counted and compared to the losses of this stage to parasitism. Lopez & Van Driesche (1988) used this method successfully in an aphid parasitoid system.

Analysis of stage-frequencies data, i.e. Southwood and Jepson's graphical method, was shown to be subject to large and complex biases and therefore seemed to

be only appropriate in cases were assumptions and any mortality factors other than parasitism are small (Bellows et al., 1989b).

In this chapter, the intensive and extensive sampling methods that were employed for monitoring the phenology of roses, *M. rosae*, *A. rosae* and associated species is described and the results are discussed with respect to the capacity of *A. rosae* to control rose aphids. Data collected before the release (Maelzer, 1977) are compared to data collected subsequently.

10.2 Methods

Over a period of two years after initial release of *A. rosae* the changes of numbers of the parasitoid and its host were investigated in the field. Intensive monitoring was performed at the rose plot of initial release at the Waite Campus. In the second year, a more extensive survey was carried out after *A. rosae* had spread. During summer time, potentially summer hosts of *M. rosae* were examined.

Sampling methods for rose aphids and their parasitoids are labour intensive. E.g. the effort invested in a single sample day, including counting and collection of shoots, identification and separation of different aphid species and instars, rearing and dissection of aphids and preparation and cleaning of equipment was approximately 20 hours, an average of one hour per shoot. The structure of rose aphid and parasitoid populations with high reproductive rates and overlapping generations would have required sample intervals of four to six days during the time of aphid abundance (e.g. Lopez & Van Driesche, 1988), which was impossible. Therefore, the aim of this study was restricted to a descriptive survey to monitor the initial performance of *A. rosae* in the field in Adelaide.

10.2.1 Intensive monitoring

The rose plot and its maintenance. The rose plot at the Waite Campus consisted of 128 rose bushes planted in seven rows covering 35 m x 15 m on a southern slope. Only the five easterly rows with a total of 86 rose bushes, *Rosa sp.*, var. Tea hybrid 'McGredy's sunset', were used for sampling. Two additional rows consisted of 15 bushes of the former variety and 27 bushes of *Rosa sp.*, var. 'Indigo', which is normally used as rootstock. These additional roses were only used for shoot supply in rearing and experiments.

Due to major infrastructual changes on the Waite Campus, the whole rose plot had to be relocated during winter 1993, three months before the initial release of *A. rosae*. Bushes used in the sample program were 25 years old when their roots were moved to their new location.

All roses used in the sampling program were pruned back hard to about one third of original length during their winter dormancy in the beginning of each July. Weak shoots were removed. Otherwise, roses were only pruned occasionally if a bush was going to grow out of shape. Before hips formed, faded blossoms were removed to promote an optimum of new growth. Shoots produced by the rootstock were regularly cut back.

Irrigation was provided by a dripping system and was adjusted according to temperature and rainfall. Weeds between bushes were cut back four times per year. Osmocote® Controlled Release Fertiliser 'Outdoors, trees and shrubs' (Grace Sierra Australia Pty Ltd, 89 Cevil Avenue, Castle Hill, Nsw 2154) was applied early each spring. Additionally, a circle of mulch, approximately 50 cm in diameter, was placed around the bases of rose bushes to inhibit weed growth in early spring and autumn. A copper based fungicide (Copper Oxy 500, active constituent 500 g/kg Copper oxychloride; Farmoz Chemicals PTY LTD, 1/116 Cabramatta Rd, Cremorne, NSW 2090) was applied only in late winter.

Number of favourable shoots. The number of favourable shoots was counted on 20 randomly chosen rose bushes. Plants were selected using a table of random numbers (Cavalli-Sforza, 1981) as follows. The field was divided in rows and columns. The starting point in the field and in the table were chosen arbitrary. To select the plants to count, a continuous sequence of numbers was used. Two numbers characterised the next plant to sample. The first number represented the number of bushes to move along in the row, and the second number represented the number of bushes to move along in a column. If the end of a column or row was reached, counting continued in the next column or row in opposite direction.

Using Maelzer's (1977) classification of rose shoots, the number of first/second, third/fourth, fifth stage rose shoots and flowers were counted (Fig. 10.1). Shoots were counted as infested or uninfested.

Sampling of shoots. Even a large rose garden has only a limited number of aphid infested shoots to collect. This is a fundamental difference to most agricultural or horticultural situations were sample units can be considered as unlimited. In situations with unlimited resources, sampling does not interfere with the phenology of species in the field but as soon as sample units become limited, care has to be taken not to remove to large proportions for the census. It was decided to limit the sample size to a maximum of 10 % of total rose aphid colonies in the plot at any time. This was considered to have no substantial effect on the phenology of species in the field. The amount was roughly estimated by sampling not more than 10 % of the estimated number of infested buds in the field which were calculated by multiplying the average number of infested buds per plant times 86, the total number of plants in the plot.

Rose shoots were sampled randomly in proportional strata. Only infested buds were sampled. This was justifiable because it minimised the variance and because the proportion of infested and uninfested shoots was known. The ratio of different shoot stages sampled was proportional to the number of infested shoot stages counted. E.g. if

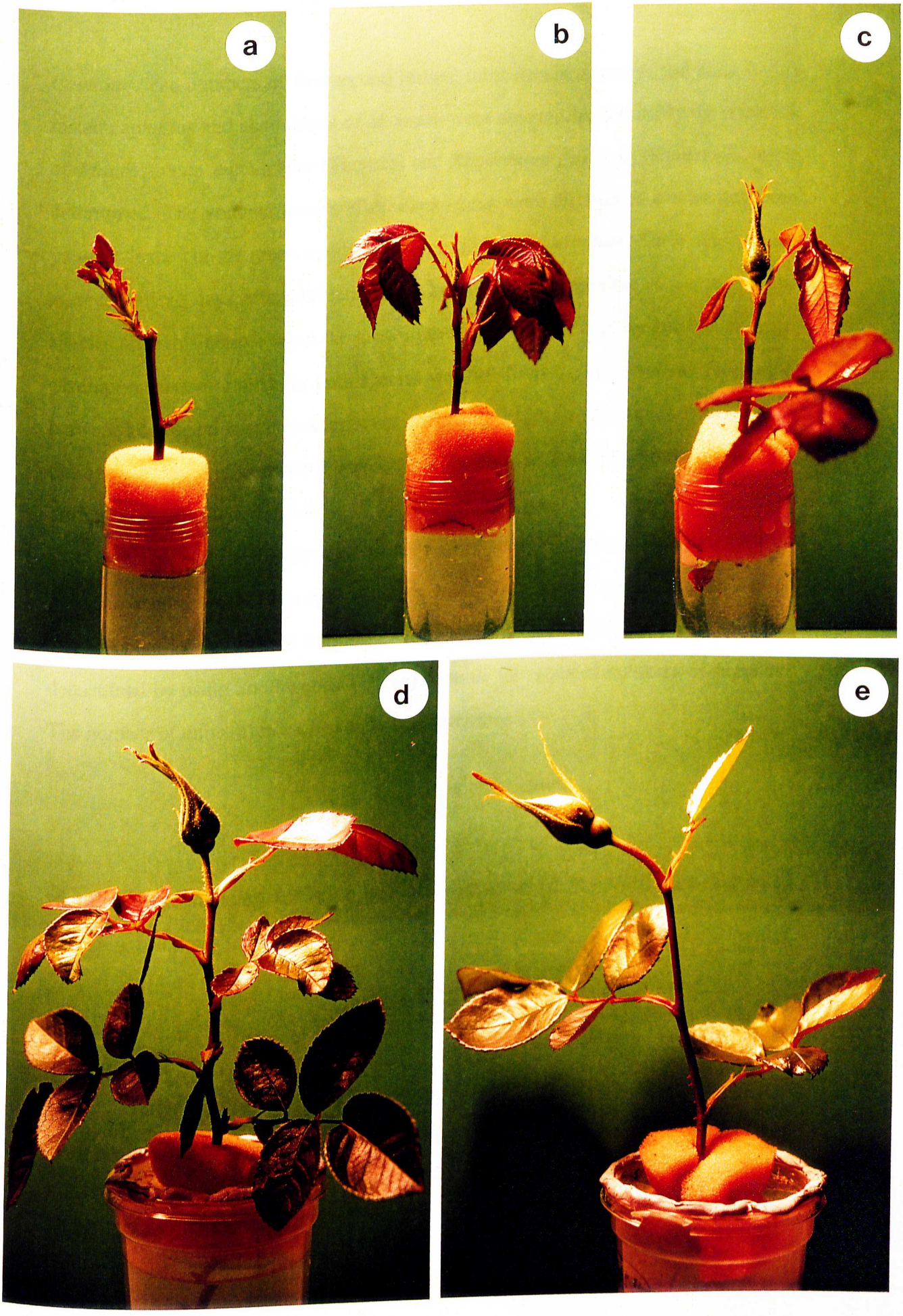
the number of infested rose shoots consisted of more fifth stages, then samples were collected from more fifth stages.

A maximum of 20 shoots was collected per sample. Shoots were taken from the same plants that were used for counting.

A maximum of one randomly chosen shoot was cut per plant. For this purpose, a frame was constructed, consisting of two wood panels, each 1.35 m, joined together at a right angle. Each panel had ten holes all of them 13 cm apart and numbered from one to ten. This frame was placed around a chosen bush. With the help of the randomised number table two holes were chosen. A stick (1.30 m) was placed in each selected hole, fitted with a right angle pointer at the end. The pointers of both sticks crossed at a right angle over the plant and consequently represented coordinates. Their meeting point marked the spot where to look for a shoot. This simple construction was easy to shift from bush to bush. Often larger numbers of coordinates did not match the smaller size of the bush and coordinates met outside the covering area of the plant. In this cases the next number in the table was chosen. Shoots were not always found next to the selected position. Under these circumstances the position was thought of as lying on a radius starting from the centre of the bush. This radius was rotated until the first infested shoot of the desired stage was found. It was rotated clockwise if both coordinates summed up to an even number and anticlockwise if both numbers summed up to an odd number.

Shoots were collected in plastic bags, which were held underneath the shoot before cutting since aphids were easily disturbed and especially larger instars and adults tended to drop off. Shoots were cut just beneath the base of the first leave following the bud, or, in cases where several buds were produced by the same terminal shoot, just behind the branch. After cutting took place plastic bags were temporarily stored in a cool box with ice until they were placed at 4°C.

Fig. 10.1 Different growth stages of rose buds *Rosa sp.*, variety Tea hybrid 'McGredy's sunset', favourable for development of *Macrosiphum rosae* (after Maelzer, 1977). **a)** stage 1, only leaves evident; **b)** stage 2, flower bud first evident; **c)** stage 3, petiole of flower bud obvious; **d)** stage 4, flower bud well lifted away from leaves; **e)** stage 5, the sepals start to separate, last favourable growth stage.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d
e

Counting. The numbers of first/second instars, third instars, apterous and alate fourth instars, apterous and alate adults of *M. rosae* were counted. Additionally the numbers of *Macrosiphum euphorbiae* (Thomas) and *Rhodobium porosum* (Sanderson) were determined. The younger instars of *M. euphorbiae* were difficult to distinguish from younger instars of the green morph of *M. rosae* in mixed colonies. Since aphids were reared for five days after collection (see below), it was possible to identify these species after this period, when the grown instars were easier to distinguish. Under the assumption that mortality for both species was not different, the resulting proportion between both species after rearing were applied to the originally counted numbers on the sample day and data were corrected. However, under most circumstances the original numbers were accurate.

Because of the generally high variances, data were log transformed (Zar, 1984).

An accuracy of 0.15 in total numbers of *M. rosae* per infested shoot in a sample was selected as the goal of sampling. The degree of precision in each sample was determined by using Southwood's (1978a) formula for required numbers of samples . The number of units (n) in each sample was determined by

$$n = \left(\frac{s}{xE} \right)^2$$

where s is the standard deviation and x is the mean. E represents the degree of precision. It follows from that

$$E = \frac{s}{x\sqrt{n}}$$

A sample size of 20 shoots turned out to be sufficient to achieve the desired accuracy in mean numbers of aphids/shoot. In cases were the preset 10 % of maximum collected population size interfered with the sample size, lower accuracy was accepted, rather than an increase of n.

All egg, larval and adult stages of associated predators were counted. Mummies were collected from the sampled shoots and reared separately to determine sex-ratio and hyperparasitism. Empty shells from which emergence had already occurred still revealed valuable data about the inhabitants (Kitt & Schmidt, 1993). The edge of the emergence hole represented a more or less clean cut if *A. rosae* had emerged, but appeared much more jagged when hyperparasitoids had emerged (Appendix 4). The aphid instar in which mummification took place was noted.

Some mummies appeared to be victims of predation but this appearance could also have been due to mechanical destruction. It was not possible to determine if it had occurred before or after emergence of the parasitoid.

Rate of parasitism. To determine the rate of parasitism, aphids of each shoot were reared in separate instar classes (L1/L2, L3/L4, adults) for five days on rose shoots in gauze cages (Fig. 3.1c).

After five days the number of newly formed mummies was counted and aphids killed in the freezer. A maximum of 40 aphids per class and shoot was dissected. If the number of aphids per shoot exceeded this threshold, a sub sample was taken. Aphids were emptied in a petri dish with a pie-type counting grid and selected with the help of the random number table. In general L3/L4 instars contained the most wasp larvae (Appendix 5) and represented an indicator of the rate of parasitism (see below). The rate of parasitized third/fourth instar aphids per shoot (P) was then calculated as

$$P = \left(1 - \frac{A_{mum}}{A_{col}}\right)\frac{A_{par}}{A_{sur}} + \frac{A_{mum}}{A_{col}}$$

where A_{col} represents the number of third/fourth aphid instars collected before rearing, A_{mum} the number of mummies formed during rearing, A_{sur} the number of aphids alive after rearing and A_{par} the number of aphids containing parasitoids (data already transformed for cases in which sub samples were taken). This assumed that mortality between parasitized and unparasitized aphids was the same.

Every shoot sampled was infested with *M. rosae*, but in some cases no third/fourth instars were found. For the calculation of the rate of parasitized third/fourth instar aphids per shoot, only shoots on which those aphid stages were found were used. As a result n for sampled infested shoots was different to n used for calculation of parasitism in some cases (Appendix 6).

It was desirable to achieve an accuracy of 0.25 in total numbers of all larval stages of *A. rosae* per infested shoot. This allows detection of at least a doubling of the population in the field (Southwood, 1978a). Pilot samples indicated that 20 shoots per sample were sufficient to achieve this goal.

The density-dependent numerical response of *A. rosae* was analysed with SAS CORR procedure (SAS, 1985). Only sample dates from autumn were used since colonies were large during this time. Data for %-parasitism per shoot were transformed to their arcsine (Zar, 1984).

10.2.2 Samples before release of *A. rosae*

In the year before the release of *A. rosae*, various sampling methods were tested for their practicality. One method was very similar to the one used following the release and results gave virtually the same data on the phenology of aphids. The major difference was that samples of different shoot stages were not taken proportionally to their occurrence in the field but taken in fixed numbers per class. Ten shoots each of 1st/2nd bud stage, 3rd/4th bud stage and 5th bud stage were sampled. However, by multiplying the means of each shoot class with the proportion of their occurrence, a crude mean infestation on a nominal average shoot was estimated, which is comparable to data obtained from 1993 to 1995.

The sampling method of Maelzer (data 1969) was described in the literature (Maelzer, 1977). An important difference is that these data were log transformed by myself from the given mean, whereas data from the census 1992-95 represent the mean

of already log transformed data. As a consequence, data from 1969 are likely to be slightly exaggerated, compared to data from pervious years.

10.2.3 Extensive monitoring

At four additional rose gardens an extensive survey was undertaken to estimate the numbers of rose aphids in spring 1994 and autumn 1995. The Urrbrae rose garden is located at the Waite Campus and is distinguished by its selection of older heritage rose varieties. This garden (500 m distance from main survey plot) together with the Mercedes College Rose garden (1.5 km distance) and the Urrbrae High School Rose garden (1 km distance) represent the closest large rose gardens in the neighbourhood of the release plot. The fourth garden was Veale Gardens in which the second initial release of *A. rosae* took place.

In each garden, eight rose beds were randomly chosen and in each bed ten plants selected. On each plant the number of aphid colonies on 5 shoots were counted. Therefore shoots were selected randomly. Like a clock, plants were divided in 12 sectors. Continuously following single numbers from the randomised number table were compared with the hours on the clock and consequently represented a pointer. The shoot closest to this pointer was selected for counting. Since single numbers of the table only allowed selection among 10 pointers, for each new shoot the 12 o'clock radius was shifted to the former 10 o'clock radius to avoid biases. If a shoot was infested by two or more species, colonies were counted for each species.

In the summer of 1994/95 two surveys were undertaken to search for rose aphids on potential summer hosts of *M. rosae* in the Adelaide region.

10.3 Results

Phenology of rose plants. In the experimental rose plot in the Waite orchard, the growth of Tea hybrid roses in 1993/94 and 1994/95 was characterised by peaks of flowerbuds in spring, early summer and autumn (Fig. 10.2). In this rose garden, favourable bud stages for *M. rosae* were available all year round except for a few weeks in February when all plants were in summer dormancy. At this time, temperatures were highest with mean maximum temperatures of nearly 30°C.

The ratio of different bud stages changed over time. Typically, a peak of favourable bud stages was preceded by strong shooting of young shoots which grew into older stages over time. Consequently, the initiation of bud flushes was characterised by a high percentage of first/second bud stages whereas the end of peaks were characterised by a majority of fifth stage buds and a following flush of flowers. This pattern was especially clear in spring.

The spring and early summer peaks of favourable buds in 1993/94 were higher than in the following year. Both spring peaks in 1993/94 appeared around one month later than their counterparts in the following year.

Sample accuracy. The restriction of sampling only a maximum of 10% of all colonies in the plot at any given time sometimes interfered with the desired degree of sample accuracy. Especially in the initial phase of aphid increases only a few colonies were in the field and the number of sampled shoots was low. E.g. on the 8 August 1994 eight shoots were collected, representing 10 % of all existing colonies in the plot at this time. The degree of sample accuracy for aphids turned out to be 0.32. To achieve the desired accuracy of 0.15, 35 shoots would have been needed, representing 40 % of all colonies. Such interference of the census with insect numbers in the plot was judged as more harmful than good. However, this was only important at times of low aphid abundance. At the majority of sample dates the 10 % threshold did not limit the sample size severely (Appendix 6). The same principle applied for the sample accuracy of *A.*

rosae which was in general lower than that of the host. By sampling only aphid infested shoots a preselection occurred which reduced the variance in aphid numbers. Since larvae of *A. rosae* were not instantly detectable in the field this procedure did not preselect for parasitoid numbers. However, only on three days was the sample accuracy beyond the desired 0.25 threshold.

Phenology of aphid species in the plot. Four species of aphids were found on roses during the survey. *M. rosae* was generally the predominant aphid on roses in this plot (Fig. 10.3). However, this was not the case during August and mid September 1994 when *M. euphorbiae* outnumbered the rose aphid. In this year, the potato aphid was abundant on roses throughout the spring. R. porosum was frequently found on roses during autumn and in lower numbers during spring. Occasionally a few individuals of *Aphis gossypii* Glover were collected, but did not form larger colonies as observed sporadically on other roses.

Changes of numbers of M. rosae. The phenology of *M. rosae* was characterised by peaks in spring and autumn in both years. The highest densities were observed during autumn. In summer, from December to March, the aphid virtually disappeared from the plot but maintained small numbers during winter (not quantified here). In both years the aphid peak in spring coincided with the first strong development of new shoots around September. Aphids were abundant over the whole of spring but numbers did not reach damaging levels in both years. By the time the second seasonal peak of rose buds took place in early summer, aphid abundance had already dropped to zero. In 1993/94 this event was late in the season and temperatures were already outside the favourable zone for aphid growth in Adelaide (15-17°C, Maelzer, 1981)(Fig. 10.4), but in 1994/95, the second peak of buds occurred while temperatures were still favourable (Fig. 10.5).

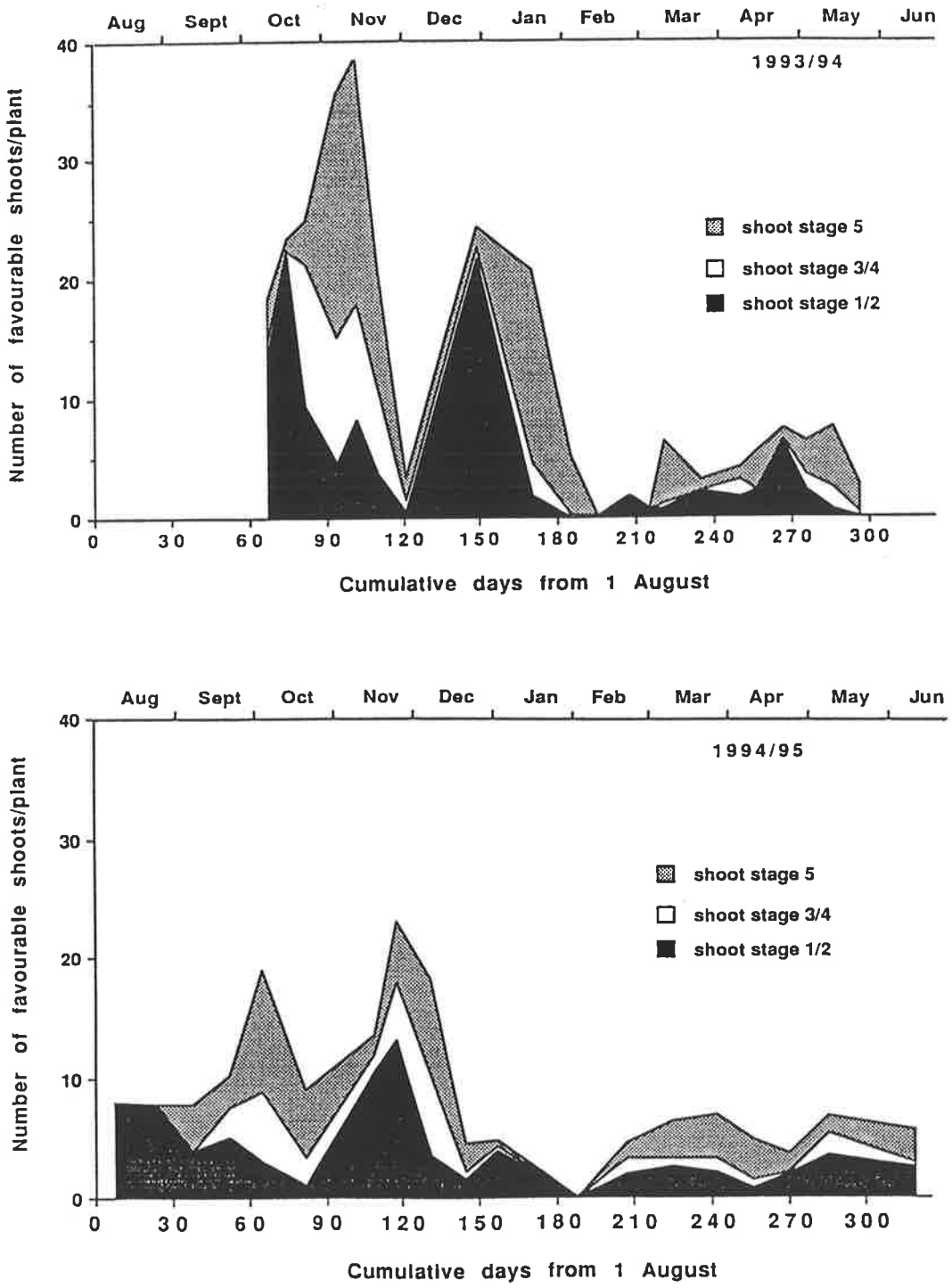


Fig. 10.2 The proportions of different rose bud stages on *Rosa sp.*, variety Tea hybrid 'McGredy's sunset', at the Waite Campus, Adelaide, South Australia, during the year. Shown are only bud stages favourable for the growth of the rose aphid *Macrosiphum rosae* (after Maelzer, 1977). Data were obtained by counting shoots on 20 plants. **a)** 1993/94, **b)** 1994/95.

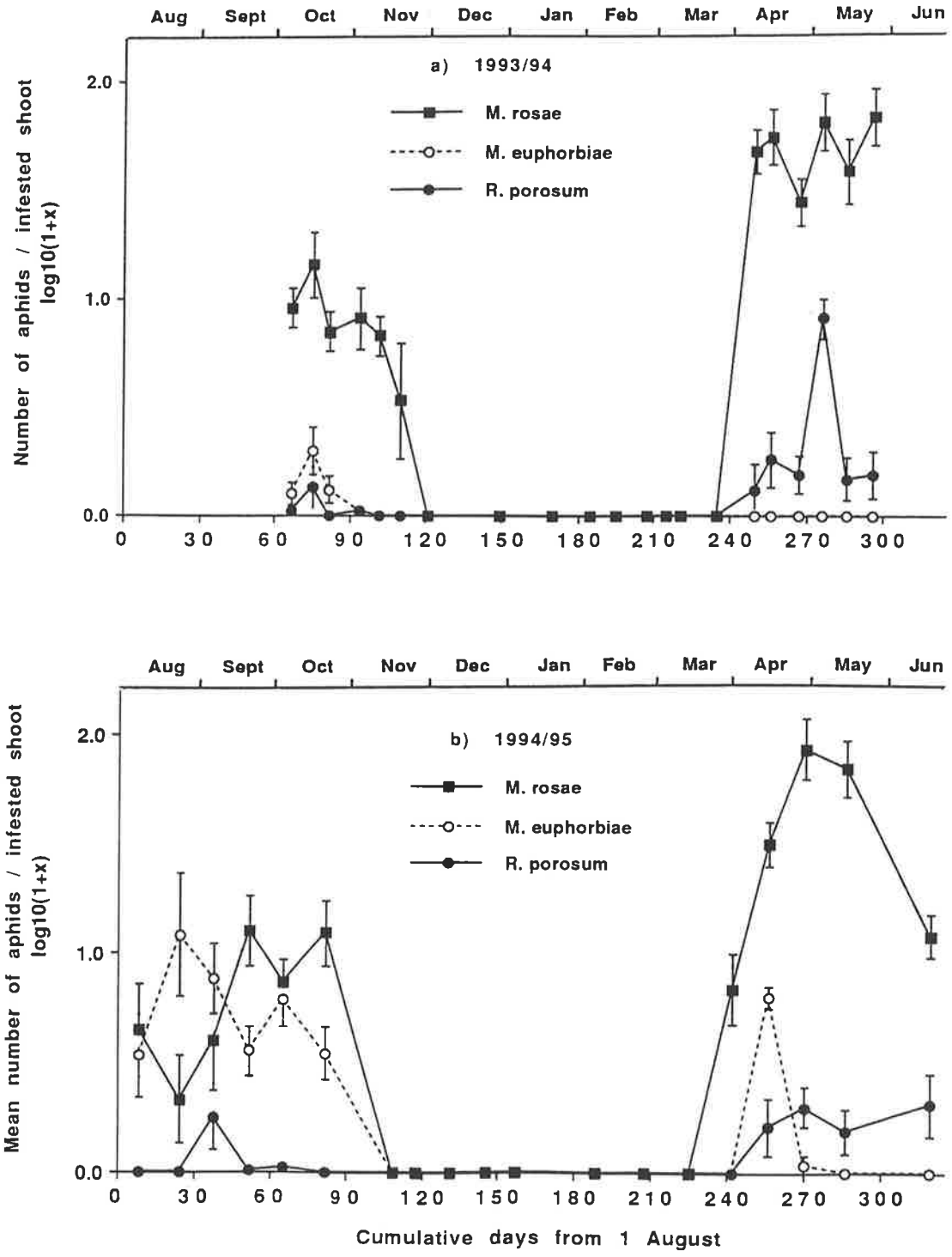


Fig. 10.3 Changes of numbers of three species of aphids, *Macrosiphum rosae*, *M. euphorbiae* and *Rhodobium porosum* on *Rosa sp.*, variety Tea hybrid 'McGredy's sunset', at the Waite Campus, Adelaide, South Australia, during the year. Error bars show SE of mean. N can be taken from Appendix 6. **a)** 1993/94, **b)** 1994/95.

Fig. 10.4 Seasonal weather conditions, the phenology of *Rosa sp.*, variety Tea hybrid 'McGredy's sunset' and the phenology of the rose aphid *Macrosiphum rosae* at the Waite Campus, Adelaide, South Australia, in 1993/94. Only heavy rainfall of more than 5ml/day is shown. The optimum temperature band of 15 to 17 °C for aphid growth in the Adelaide region is indicated by dotted lines. Temperatures are shown as average mean max. and mean min. temperatures between sample dates. Phenology of favourable shoots (stage 1-5)(Maelzer, 1977) was determined by counting all shoots on 20 plants. The number of shoots collected to determine the number of aphids/infested shoot was 20 except day 110, n=14; 250, n=9; 256, n=17; 267, n =18. Error bars show SE of mean. From 27 August to 17 September the initial release of 800 individuals of *Aphidius rosae* took place.

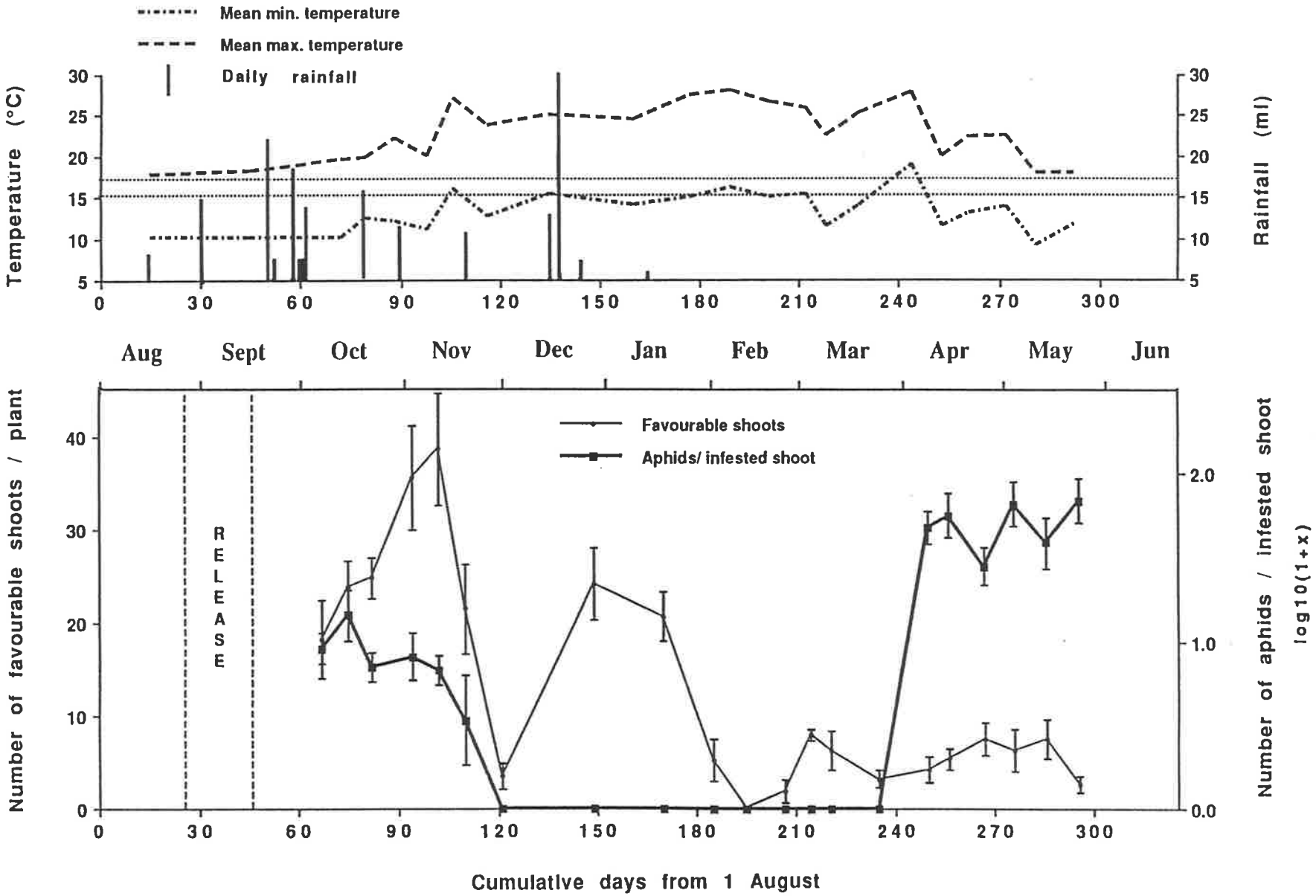
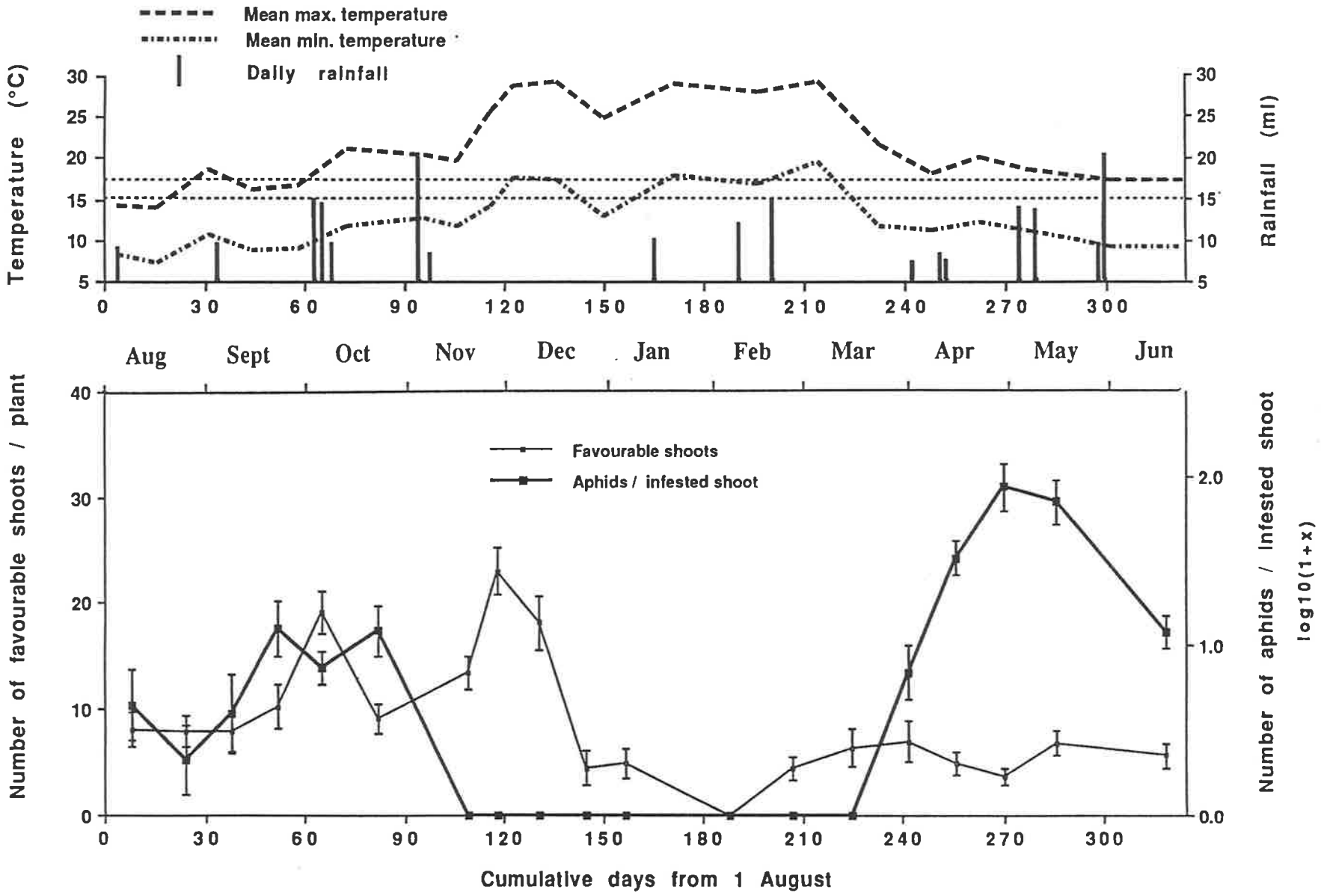

Mean min. temperature
Mean max. temperature
Daily rainfall
Temperature (°C)
Rainfall (ml)
Aug
Sept
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Favourable shoots
Aphids/ infested shoot
RELEASE
Number of favourable shoots / plant
Number of aphids / infested shoot
log10(1+x)
Cumulative days from 1 August

Fig. 10.5 Seasonal weather conditions, the phenology of *Rosa sp.*, variety Tea hybrid 'McGredy's sunset' and the phenology of the rose aphid *Macrosiphum rosae* at the Waite Campus, Adelaide, South Australia, in 1994/95. Only heavy rainfall of more than 5ml/day is shown. The optimum temperature band of 15 to 17 °C for aphid growth in the Adelaide region is indicated by dotted lines. Temperatures are shown as average mean max. and min. temperatures between sample dates. Phenology of favourable shoots (stage 1-5)(Maelzer, 1977) was determined by counting all shoots on 20 plants. The number of shoots collected to determine the number of aphids/infested shoot was 20 except day 8 n=8; 24, n=10; 52, n=5; 242, n =3; 256, n=19. Error bars show SE of mean.


Mean max. temperature
Mean min. temperature
Daily rainfall
Temperature (°C)
Rainfall (ml)
30
25
20
15
10
5
0
30
60
90
120
150
180
210
240
270
300
Aug
Sept
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Favourable shoots
Aphids / infested shoot
Number of favourable shoots / plant
Number of aphids / infested shoot
log10(1+x)
40
2.0
1.0
0.0
Cumulative days from 1 August

Heavy rainfalls took place in both springs. A period of six rainy days during which between eight and 22 ml daily rainfall occurred took place just before the first sample date (Fig. 10.4) in September/October 1993. The autumn of 1994 was free from heavy rainfalls but aphids in the following autumn were exposed to seven days of heavy rainfall.

In both years the rise of aphid numbers in autumn (March) occurred when mean temperatures fell back into the favourable range of 15-17°C.

The relatively low number of favourable shoots in autumn, together with high numbers of aphids led to an infestation of nearly all buds towards the end of this period (Fig. 10.6).

The proportion of apterous adults was high at most times (Fig. 10.7). This was not reflected in the proportion of apterous and alate third/fourth instars. A typical phenology for density-dependent dispersal was observed in both surveyed autumns. At the beginning of those seasonal outbreaks, nearly all offspring were apterous and represented the major source for rapid increase in aphid numbers. With increasing density, this ratio shifted strongly towards alatiforms. Such a clear pattern did not appear in spring when aphid numbers were relatively small.

Alternative hosts for Macrosiphum rosae. During summertime two surveys were carried out to investigate if *M. rosae* utilised other host plants. The species was not found on any of the surveyed plants (Table 10.1). *M. rosae* virtually disappeared from roses throughout Adelaide, but was still found in low numbers in the cooler Adelaide Hills (e.g. at Hahndorf).

Table 10.1 Survey of the abundance of *Macrosiphum rosae* on known potential summer host plants in Adelaide and surroundings, South Australia, in summer 1994. *M. rosae* was not present on any listed plants.

Scabiosa crinita Kotschy & Boiss.	Dipsacaceae	15.12.94, Royal Botanical Garden, Adelaide
Scabiosa gramuntia Brot..	Dipsacaceae	"
Knautia macedonia Griseb.	Dipsacaceae	"
Patrinia scabra Bunge	Valerianacae	"
Valeriana offcinalis L.	Valerianacae	"
Valeriana sambucifolia Eichner *var. fauriei*	Valerianacae	"
Centhranthus ruber (L.)	Valerianacae	"
Scabiosa atropurpurea L.	Dipsacaceae	22.12.94, road sides in Adelaide, Willunga, McLaren Vale, Victor Harbor.

However, the world first record of *M. rosae* feeding and reproducing on river red gum *Eucalyptus camaldulensis* Dehn. was made at two locations in autumn 1995 (Appendix 7)(Mary Carver, CSIRO Canberra, kindly confirmed the identity of *M. rosae*). Even though colonies were able to survive for several generations in a glasshouse on river red gum, they soon vanished in the field. This record was probably insignificant for the field ecology of *M. rosae* but demonstrated a potential flexibility in host relationships.

Rate of parasitism. The highest rate of parasitized aphids was found in third/fourth instars on 17 out of 20 sample dates. In general the lowest rate of parasitism was found in first/second instars (Appendix 5).

3rd/4rth instars represented the stages which accumulated parasitoid larvae of different ages. Most aphids parasitized as first and second instars, will continue to develop and die as third or fourth instars (Fig. 4.9). Additionally, third/fourth instars

can be parasitized as well and therefore can also contain young parasitoid larvae. Therefore, third/fourth instar aphids were exposed to parasitism for much longer than first/second instars and simply had a greater chance of being parasitized at time of collection. Even though younger instars were obviously preferred for oviposition (see below), many of those collected may not have been encountered by parasitoids because of their short time in the field.

Because mainly younger instars were parasitized, many parasitized aphids died before they reached adulthood. Consequently the rate of parasitism in adults was generally lower than in third/fourth instars because relatively more unparasitized aphids than parasitized aphids reached adulthood.

In summary, the number of parasitised third and fourth instars gave the best indication of parasitism and was therefore taken as a representative figure for parasitoid activity. Because of the wide age structure of parasitoid larvae in third/fourth instar, the sample method did not reveal the true rate of parasitism (Van Driesche et al., 1991) but represented an index of the accumulative pressure of parasitoids on aphids during their life-time from birth to sample day.

Phenology of Aphidius rosae. The changes of numbers of larvae of *A. rosae* were strictly related with the changes of numbers of hosts (10.8). Peaks of parasitized aphids occurred together with peaks of aphid abundance.

During both surveyed springs, the majority of third/fourth instar aphids was parasitized, whereas the rate of parasitism was lower in autumn (Fig. 10.8, Appendix 5). However, in absolute numbers, more *A. rosae* larvae were present in the plot in autumn because of higher aphid numbers (Fig. 10.9).

At times of heavy aphid infestations in autumn the percentage of parasitism in third/fourth instars per shoot was inversely density-dependent with the number of aphids per shoot (Fig. 10.10).

The sex-ratio of *A. rosae* emerging from field collected mummies was 0.36 in the 1993/1994 season and 0.42 in 1994/95 (Table 10.2).

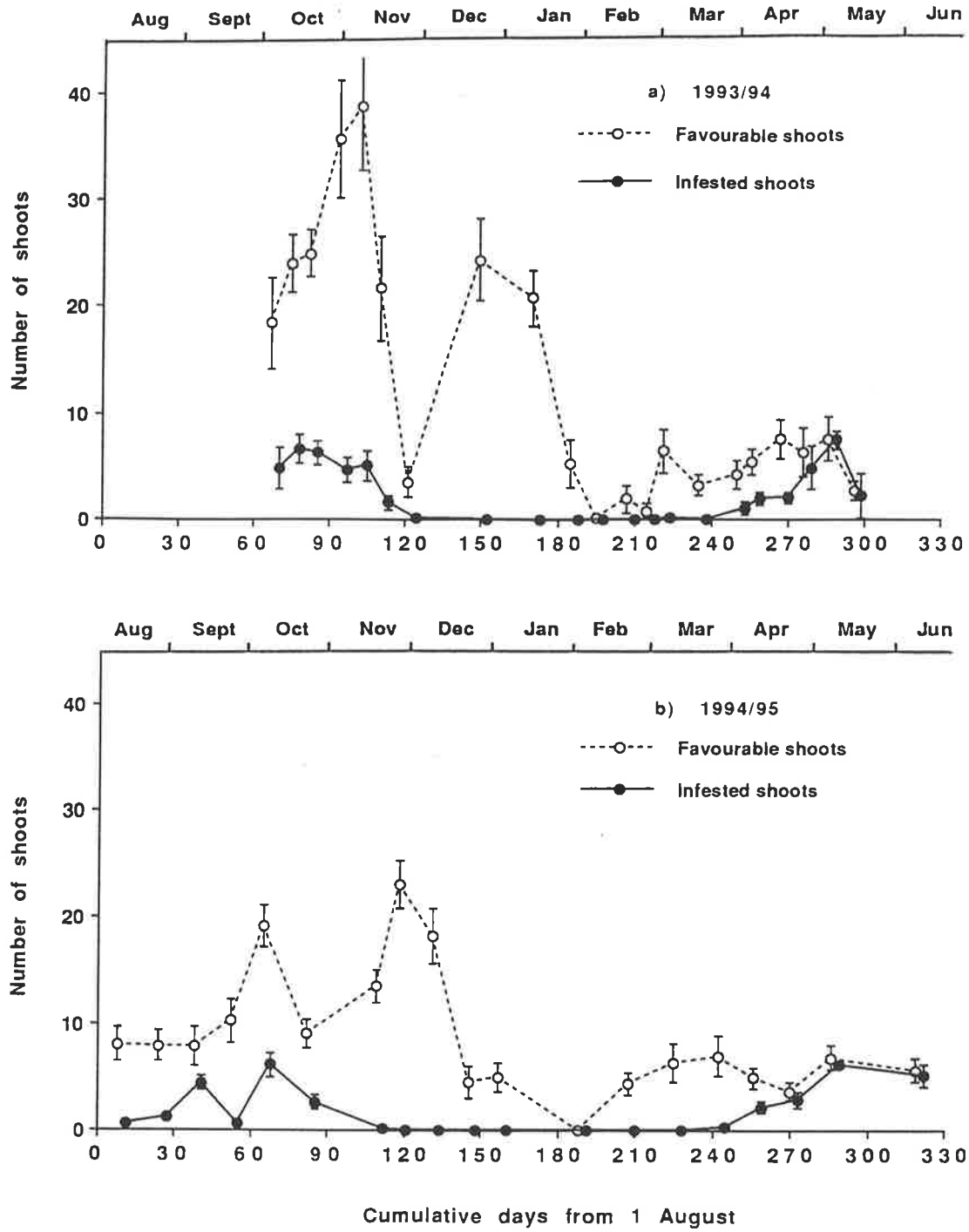


Fig. 10.6 Comparison between aphid infested and uninfested shoots of *Rosa sp.*, variety Tea hybrid 'McGredy's sunset', at the Waite Campus, Adelaide, South Australia, during the year. Only shoot stages 1 to 5, favourable for the growth of the rose aphid *Macrosiphum rosae*, are shown (after Maelzer, 1977), n = 20 plants. Error bars show SE of the mean. Data for infested shoots are shifted to the right by three days. **a)** 1993/94, **b)** 1994/95.

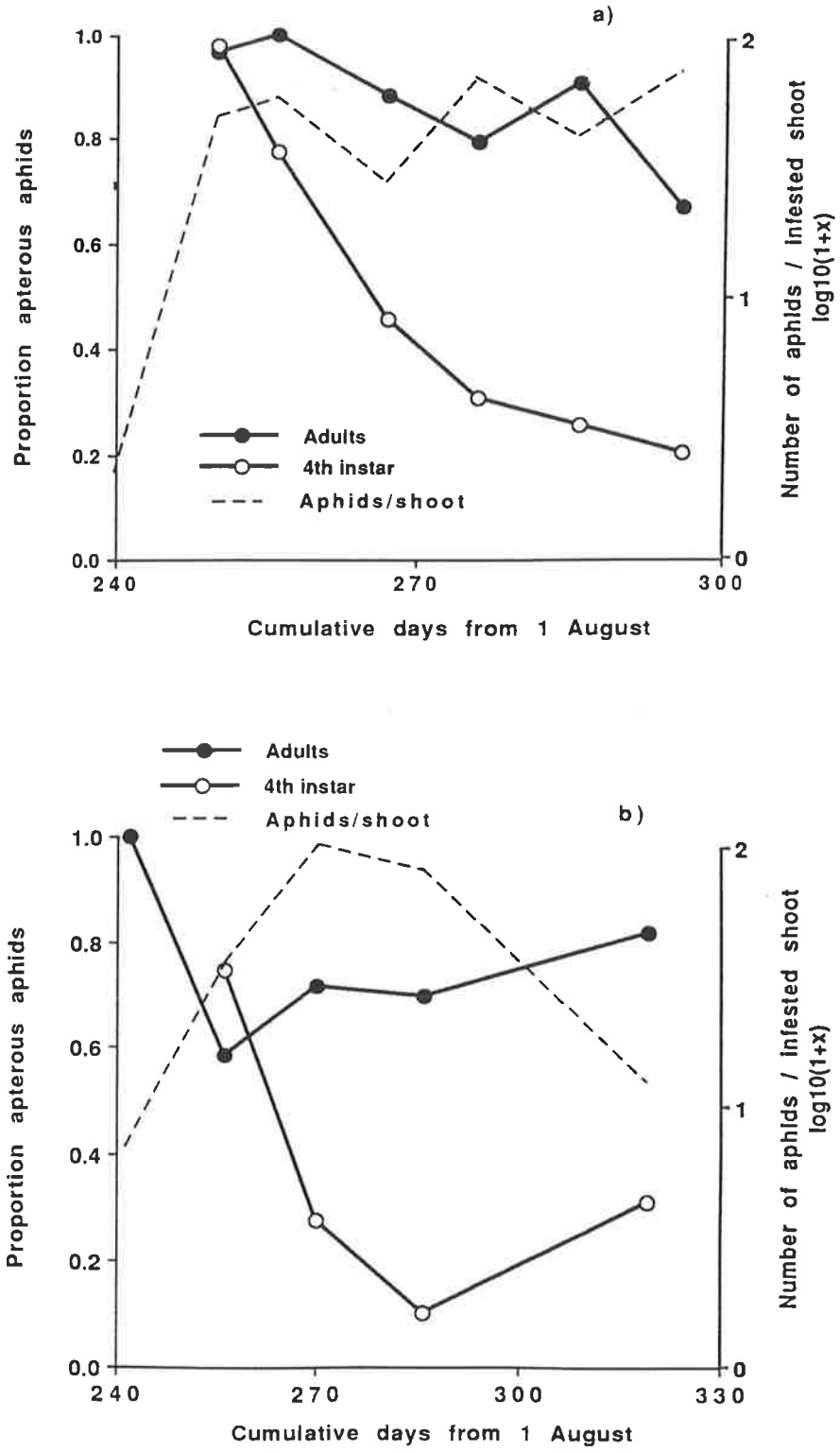


Fig. 10.7 The proportion of apterous and alate adults vs. apterous and alate fourth instars of *Macrosiphum rosae* on *Rosa sp.*, variety Tea hybrid 'McGredy's sunset', at the Waite Campus, Adelaide, South Australia. Dotted lines show the mean number of aphids/shoot in the plot at the same time. **a)** Autumn 1993/94, day 256, n shoots =17; 267, n=18; 276, n=20; 286, n=20; 296, n=20; **b)** Autumn 1994/95, day 242, n=3; 256, n=19; 270, n=20; 286, n=20; 319, n=20.

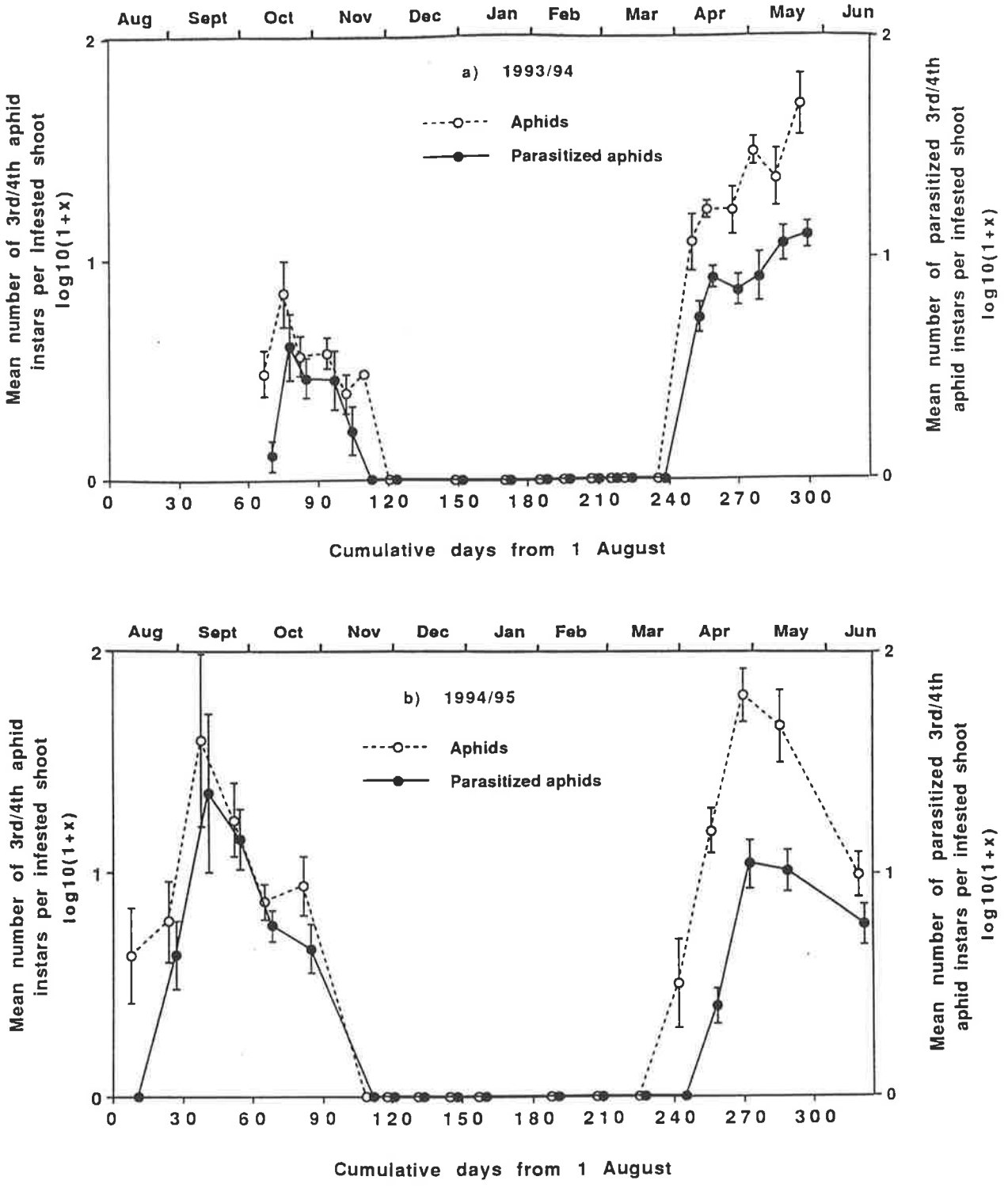


Fig. 10.8 The mean numbers of third/fourth instars of *Macrosiphum rosae* per rose shoot infested with these stages, compared to the mean number of parasitized third/fourth instars by *Aphidius rosae*. Investigations were undertaken on *Rosa sp.*, var. Tea hybrid 'McGredy's sunset', at the Waite Campus, Adelaide, South Australia. Data for parasitized aphids are shifted by three days to the right. Error bars show SE of mean. N can be taken from Appendix 6. **a)** 1993/94, **b)** 1994/95.

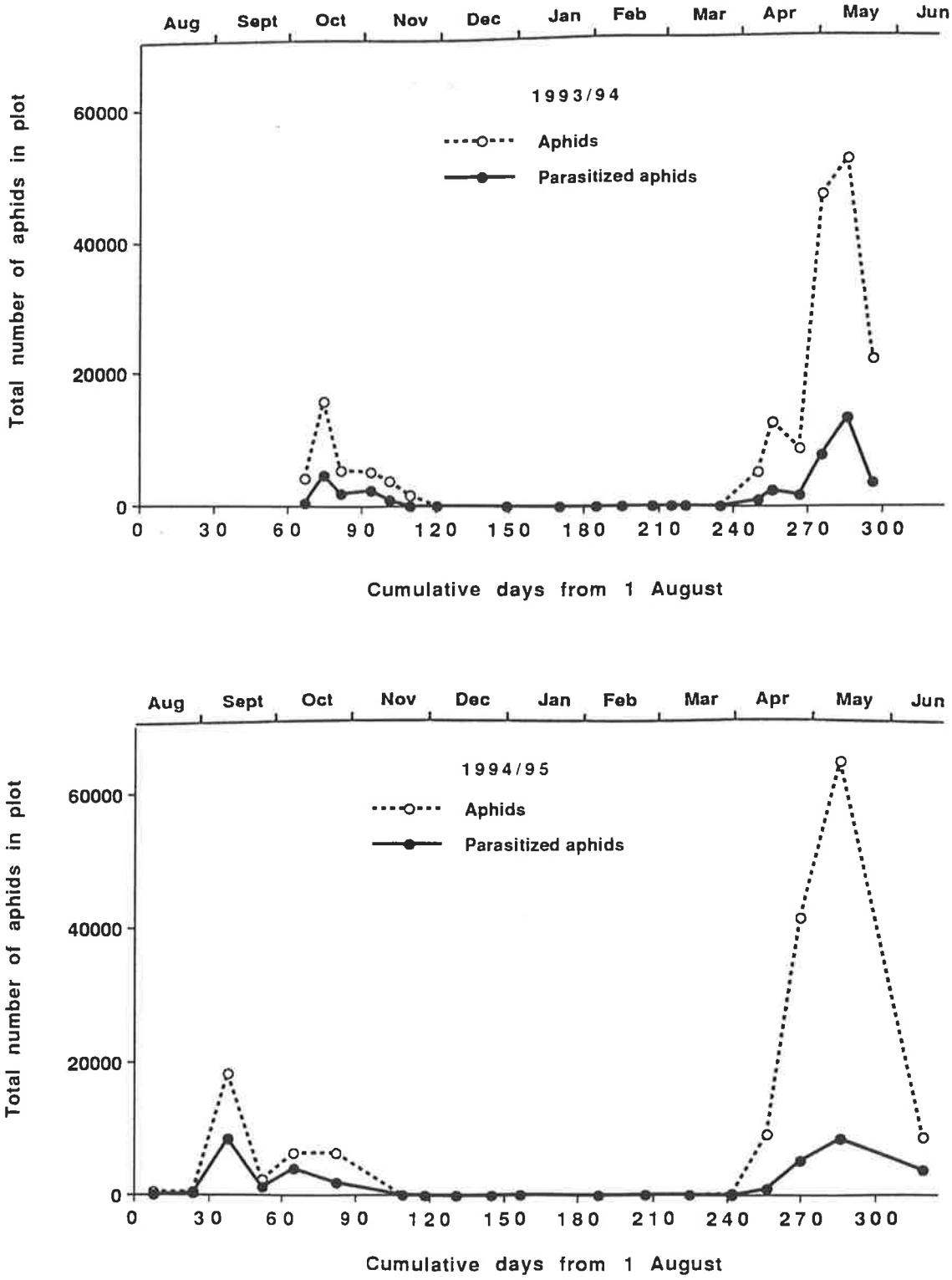
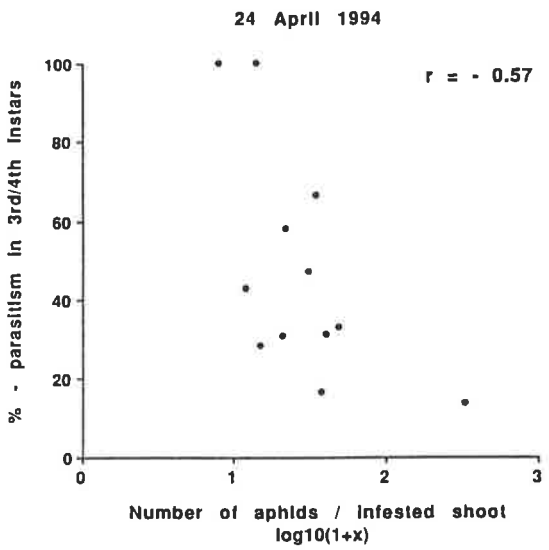
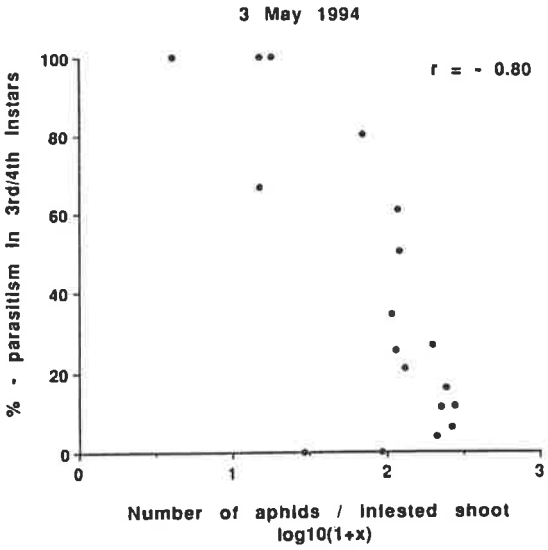


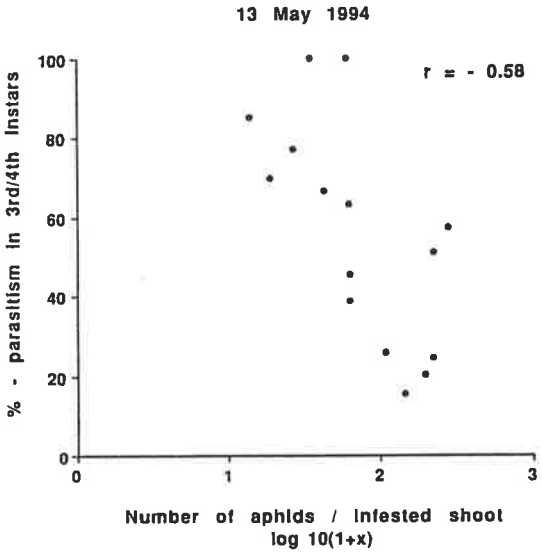
Fig. 10.9 Phenology of *Macrosiphum rosae* and its parasitoid *Aphidius rosae* in a rose plot consisting of 86 bushes *Rosa sp.*, variety Tea hybrid 'McGredy's sunset', at the Waite Campus, Adelaide, South Australia. Data were calculated by multiplying the mean number of aphids/shoot, the mean number of infested shoots/plant and number of plants in the plot.



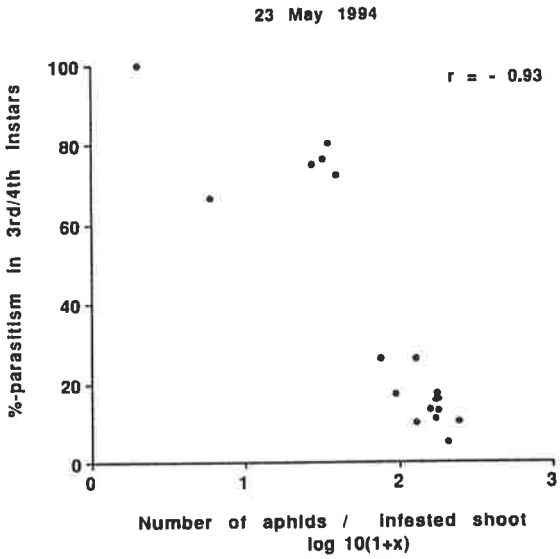
a) n = 12.



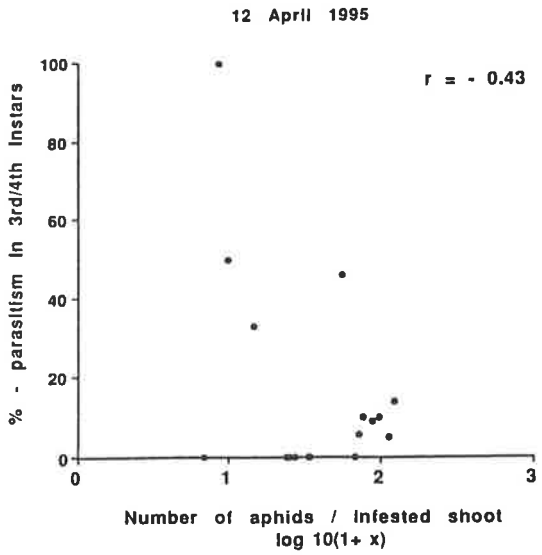
b) n = 18.



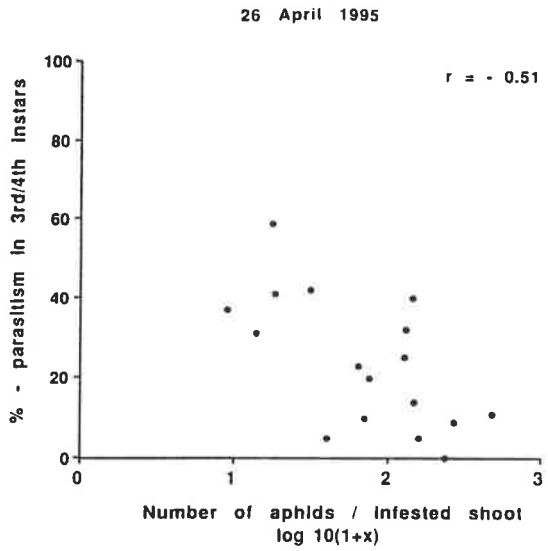
c) n = 15.



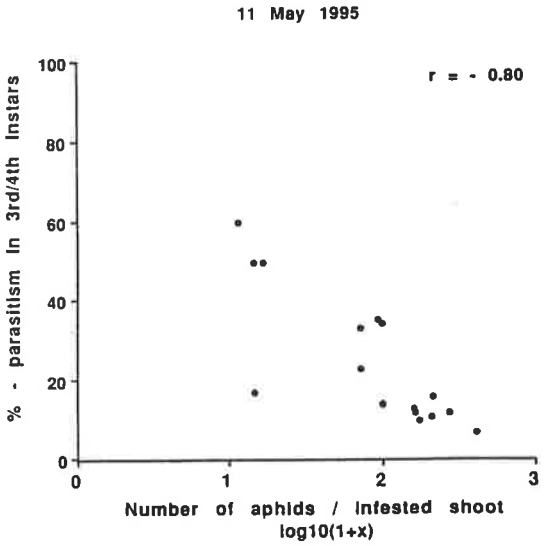
d) n = 18.



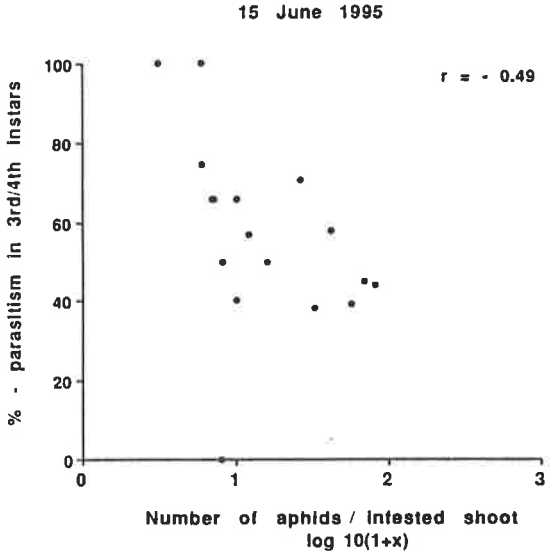
e) n = 17.



f) n = 17.



g) n =16.



h) n =18, data points
x/y = 0.9 / 50 and
x/y = 0.84 / 67
occur twice.

Fig 10.10 Correlation between density of *Macrosiphum rosae* per infested shoot and % - parasitism in third/fourth instars by *Aphidius rosae* on separate sample days. Investigations were carried out on *Rosa sp.*, var. Tea Hybrid 'McGredy's sunset' at the Waite Campus, Adelaide, South Australia. Only data from autumn are shown when numbers of aphids and parasitoids were high. Each data point represents one shoot. Data of % - parasitism were transformed to their arcsine for the correlation analysis.

The majority of mummies detected on the shoots consisted of aphids that had died as third and fourth instars (Table 10.3), indicating that parasitism must have taken place when hosts were first or second instars (Chapter 4). First and second instars were not the most abundant stages of *M. rosae* in the field (Appendix 5), so these must have been parasitized more frequently than older aphids. In both years only 26 percent of mummies consisted of adult forms which indicate parasitism of older instars.

The typically dark, thick diapause form of mummies was found over most of the time of parasitoid abundance (Table 10.4). The number of diapausing mummies were low at the beginning of a season in spring and autumn and increased steadily towards the end.

Phenology of hyperparasitoids. The first hyperparasitoid of rose aphids was discovered on the 21 October 1993, two months after the initial release (Table 10.5.). This species, *Phaenoglyphis villosa* (Hartig)(Charipidae : Cynipoidea) with a sample total of 68 individuals was the most abundant hyperparasitoid over the period of survey. 34 individuals of the second most common species, *Pachyneuron aphidis* (Bouché)(Pteromalidae: Chalcidoidea) were found. Only five *Dendrocerus aphidum* (Rondani)(Megaspilidae: Proctotrupoidea) emerged out of mummies from 24 April 1994. The shape of the emergence holes from mummies where wasps had already emerged revealed a further 75 cases of hyperparasitism. Overall, hyperparasitoids or traces of hyperparasitism were recorded from 15.4 % of mummies. Hyperparasitoids increased in a delayed density-dependent manner to *M. rosae* and *A. rosae*. During spring the percentage of hyperparasitoids that emerged from mummies never exceeded more than 10 %. In contrast, hyperparasitism reached peaks of 36 % in the autumn of 1994 and 44 % in the autumn of 1995.

Predators were rarely found (Table 10.6). Syrphids were the most commonly discovered predators on buds. More individuals were found in spring than in autumn.

Table 10.2 Sex-ratio (proportion males) of *Aphidius rosae*, emerged from field-collected mummies of *Macrosiphum rosae* on *Rosa sp.*, var. 'McGredy's sunset' in Adelaide, South Australia.

a) October 1993 to May 1994.

	Unbiased sample					Total of biased and unbiased sample		
Sample date	Mummies per sample day (n shoots)	Males emerged	Females emerged	**Sex-ratio**	Additional mummies collected (biased) (†)	Males emerged	Females emerged	**Sex-ratio**
7 October 1993	5 (20)	2	1	**0.67**	10	5	6	**0.45**
14 October	0 (20)	-	-	**-**	30	16	7	**0.70**
21 October	2 (20)	0	2	**0**	24	10	10	**0.50**
2 November	0 (20)	-	-	**-**	35	8	18	**0.33**
10 November	17 (20)	4	3	**0.57**	15	10	11	**0.48**
18 November	0 (14)	-	-	**-**	27	7	4	**0.64**
7 April 1994	3 (9)	1	1	**0.50**	13	4	7	**0.36**
13 April	48 (17)	16	19	**0.46**	-	16	19	**0.46**
24 April	78 (18)	23	31	**0.43**	-	23	31	**0.43**
3 May	97 (20)	14	42	**0.25**	-	14	42	**0.25**
13 May	107 (20)	14	32	**0.30**	-	14	32	**0.30**
23 May	234 (20)	17	31	**0.35**	-	17	31	**0.35**
Total	591	91	162	**0.36**	591 +154	144	218	**0.40**

b) August 1994 to June 1995.

	Unbiased sample					Total of unbiased and biased sample		
Sample date	Mummies per sample day (n shoots)	Males emerged	Females emerged	**Sex-ratio**	Additional mummies collected (biased) (†)	Males emerged	Females emerged	**Sex-ratio**
8 August 1994	0 (8)	-	-	**-**	-	-	-	**-**
24 August	0 (10)	-	-	**-**	-	-	-	**-**
6 September	60 (20)	24	29	**0.45**	-	24	29	**0.45**
20 September	5 (5)	4	1	**0.8**	37	15	23	**0.39**
4 October	24 (20)	5	14	**0.26**	26	22	22	**0.5**
20 October	29 (20)	8	11	**0.42**	34	14	25	**0.36**
12 April 1995	6 (19)	3	3	**0.5**	48	24	29	**0.45**
26 April	60 (20)	23	31	**0.43**	-	23	31	**0.43**
11 May	43 (20)	8	14	**0.36**	-	8	14	**0.36**
15 June	65 (20)	3	4	**0.43**	-	3	4	**0.32**
Total	292	77	107	**0.42**	292 + 145	133	177	**0.43**

(†) On sample dates where the sample procedure allowed encounter of only a few mummies, an additional sample of mummies was used, collected from the same plot. Collection of these mummies was not strictly random and therefore should be considered as biased. Contents of mummies without emergence of *A. rosae* can be taken from Table 10.5.

Table 10.3 Aphid instar form of mummies of *Aphidius rosae* parasitizing the rose aphid *Macrosiphum rosae* on *Rosa sp.*, var. 'McGredy's sunset' in the field, in Adelaide, South Australia.

a) October 1993 to May 1994.

	Unbiased sample					Total of unbiased and biased sample		
Sample date	Mummies per sample day (n shoots)	3rd instar form	4th instar form	Adult form	Additional mummies collected (biased) (†)	3rd instar form	4th instar form	Adult form
7 October	5 (20)	1	3	1	10	4	8	3
14 October	0 (20)	-	-	-	30	5	18	7
21 October	2 (20)	0	2	0	24	9	10	7
2 November	0 (20)	-	-	-	35	11	9	15
10 November	17 (20)	6	5	5	15	4	22	5
18 November	0 (14)	-	-	-	27	7	16	2
7 April 1994	3 (9)	1	1	0	13	5	8	2
13 April	48 (17)	17	21	10	-	17	21	10
24 April	78 (18)	21	38	19	-	21	38	19
3 May	97 (20)	43	31	22	-	43	31	22
13 May	107 (20)	30	57	18	-	30	57	18
23 May	234 (20)	51	95	82	-	51	95	82
Total	591	170	253	157	591 + 154	207	333	192
Proportion of instars		0.29	0.44	0.27		0.28	0.45	026

b) August 1994 to June 1995.

	Unbiased sample					Total of unbiased and biased sample		
Sample date	Mummies per sample day (n shoots)	3rd instar form	4th instar form	Adult form	Additional mummies collected (biased) (†)	3rd instar form	4th instar form	Adult form
8 August 1994	0 (8)	-	-	-	-	-	-	-
24 August	0 (10)	-	-	-	-	-	-	-
6 September	60 (20)	21	32	7	-	21	32	7
20 September	5 (5)	3	1	1	37	18	15	9
4 October	24 (20)	9	9	6	26	9	25	16
20 October	29 (20)	7	8	11	34	10	26	22
12 April 1995	6 (19)	0	6	0	48	12	33	9
26 April	60 (20)	13	26	21	-	13	26	21
11 May	43 (20)	12	19	10	-	12	19	10
15 June	65 (20)	19	27	17	-	19	27	17
Total	292	84	128	73	292 + 145	114	203	111
Proportion of instars		0.29	0.45	0.26		0.27	0.47	0.26

(†) On sample dates where the sample procedure allowed encounter of only a few mummies, an additional sample of mummies was used, collected from the same plot. Collection of these mummies was not strictly random and therefore should be considered as biased. Some collected mummies were destroyed and did not reveal the aphid stage. Numbers can be taken from Table 10.5.

Table 10.4 Proportion of diapause form mummies of *Aphidius rosae* parasitizing the rose aphid *Macrosiphum rosae* in the field. Mummies were collected on *Rosa sp.*, var. 'McGredy's sunset' at the Waite Campus, Adelaide, South Australia.

a) October 1993 to May 1994.

Sample date	Unbiased sample				Total of unbiased and biased sample			
	Mummies per sample day (n shoots)	Live mummies	Mummies in diapause form	Proportion diapause form	Additional mummies collected (biased) (†)	Live mummies	Mummies in diapause form	Proportion diapause form
7 October	5 (20)	3	0	0	10	11	0	0
14 October	0 (20)	-	-	-	30	23	2	0.09
21 October	2 (20)	2	0	0	24	21	1	0.05
2 November	0 (20)	-	-	-	35	28	3	0.11
10 November	17 (20)	10	6	0.60	15	24	7	0.30
18 November	0 (14)	-	-	-	27	11	5	0.45
7 April 1994	3 (9)	2	0	0	13	13	0	0
13 April	48 (17)	45	0	0	-	45	0	0
24 April	78 (18)	67	3	0.04	-	67	3	0.04
3 May	97 (20)	74	7	0.09	-	74	7	0.09
13 May	107 (20)	54	20	0.37	-	54	20	0.37
23 May	234 (20)	72	49	0.68	-	72	49	0.68

b) August 1994 to June 1995.

Sample date	Unbiased sample				Total of unbiased and biased sample			
	Mummies per sample day (n shoots)	Live mummies	Mummies in diapause form	Proportion diapause form	Additional mummies collected (biased) (†)	Live mummies	Mummies in diapause form	Proportion diapause form
8 August 1994	0 (8)	-	-	-	-	-	-	-
24 August	0 (10)	-	-	-	-	-	-	-
6 September	60 (20)	53	1	0.02	-	53	1	0.02
20 September	5 (5)	5	0	0	37	38	2	0.05
4 October	24 (20)	22	4	0.18	26	47	6	0.13
20 October	29 (20)	19	8	0.42	34	42	19	0.45
12 April 1995	6 (19)	6	0	0	48	54	0	0
26 April	60 (20)	58	2	0.03	-	58	2	0.03
11 May	43 (20)	24	8	0.33	-	24	8	0.33
15 June	65 (20)	39	28	0.72	-	39	28	0.72

(†) On sample dates where the sample procedure allowed encounter of only a few mummies, an additional sample of mummies was used, collected from the same plot. Collection of these mummies was not strictly random and therefore should be considered as biased. Contents of mummies without emergence of *A. rosae* can be taken from Table 10.5.

Table 10.5 Numbers of *Aphidius rosae* and associated hyperparasitoids from field collected mummies of the rose aphid *Macrosiphum rosae* at the Waite Campus, Adelaide, South Australia. Mummies were collected on *Rosa sp.*, var. 'McGredy's sunset'.

a) October 1993 to May 1994.

	Unbiased sample						Total of unbiased and biased sample						
Sample date	Mummies per sample day (n shoots)	*A. rosae*	Hyper-para-sitoids	Re-mains left only, *A. rosae* (#)	Re-mains left only, hyper-para-sitoids (#)	Mum-mies de-stroyed	Additional mummies collected (biased) (†)	*A. rosae*	Hyper-para-sitoids	Re-mains left only, *A. rosae* (#)	Re-mains left only, hyper-para-sitoids (#)	Mum-mies de-stroyed	Propor-tion hyper-para-sitoids
7 October	5 (20)	3	0	2	0	0	10	11	0	4	0	0	0
14 October	0 (20)	-	-	-	-	-	30	23	0	7	0	0	0
21 October	2 (20)	2	0	-	-	-	24	20	1	5	0	0	0.04
2 November	0 (20)	-	-	-	-	-	35	26	2	6	1	0	0.09
10 November	17 (20)	7	3	6	0	1	15	21	3	7	0	1	0.10
18 November	0 (14)	-	-	-	-	-	27	11	0	12	2	2	0.08
7 April 1994	3 (9)	2	0	0	0	1	13	11	2	1	1	1	0.20
13 April	48 (17)	35	10	3	0	0	-	35	10	3	0	0	0.21
24 April	78 (18)	54	13	5	6	0	-	54	13	5	6	0	0.24
3 May	97 (20)	56	28	5	7	1	-	56	28	5	7	1	0.36
13 May	107 (20)	46	8	45	6	2	-	46	8	45	6	2	0.13
23 May	234 (20)	48	24	129	27	6	-	48	24	129	27	6	0.22
Total	591	253	86	195	46	11	591 +154	362	91	229	50	13	

b) October 1994 to June 1995.

Sample date	Unbiased sample						Total of biased and unbiased sample						
	Mummies per sample day (n shoots)	*A. rosae*	Hyper-para-sitoids	Re-mains left only, *A. rosae* (#)	Re-mains left only, hyper-para-sitoids (#)	Mum-mies de-stroyed	Additional mummies collected (biased) (†)	*A. rosae*	Hyper-para-sitoids	Re-mains left only, *A. rosae* (#)	Re-mains left only, hyper-para-sitoids (#)	Mum-mies de-stroyed	Propor-tion hyper-para-sitoids
8 August 1994	0 (8)	-	-	-	-	-	-	-	-	-	-	-	-
24 August	0 (10)	-	-	-	-	-	-	-	-	-	-	-	-
6 September	60 (20)	53	0	7	0	0	-	53	0	7	0	0	0
20 September	5 (5)	5	0	0	0	0	37	38	0	4	0	0	0
4 October	24 (20)	19	3	1	1	0	26	44	3	2	1	0	0.08
20 October	29 (20)	19	0	5	2	3	34	39	3	13	3	5	0.10
12 April 1995	6 (19)	6	0	0	0	0	48	53	1	0	0	0	0.02
26 April	60 (20)	54	4	0	2	0	-	54	4	0	2	0	0.10
11 May	43 (20)	22	2	13	4	2	-	22	2	13	4	2	0.15
15 June	65 (20) (‡)	7	8	14	10	2	-	7	8	14	10	2	0.44
Total	292	185	17	40	19	7	292 + 145	310	21	53	20	9	

Numbers of wasps were obtained by counting emerging individuals, or in cases were wasps had already emerged, by the inspection of the typical shape of emergence holes (#).

(†) On sample dates where the sample procedure allowed encounter of only a few mummies, an additional sample of mummies was used, collected from the same plot. Collection of these mummies was not strictly random and therefore should be considered as biased.

(‡) 24 mummies from the 15 June were still in diapause when the project stopped.

Table 10.6 Aphidophagous predators of *Macrosiphum rosae*, collected on *Rosa sp.*, var. Tea hybrid 'McGredy's sunset' in the field, Adelaide, South Australia. Only aphid infested shoots were sampled.

a) October 1993 to May 1994.

Sample date	Sample size (shoots)	*Harmonia conformis*	*Micromus tasmaniae*	Syrphidae
7 October 1993	20	-	-	2
14 October	20	1	1 (2)	-
21 October	20	-	-	4 (7)
2 November	20	-	-	1 (1)
10 November	20	-	(1)	(1)
18 November	14	-	-	(4)
7 April 1994	9	-	-	-
13 April	17	-	-	2
24 April	18	-	1	-
3 May	20	1	-	-
13 May	20	-	1	-
23 May	20	-	-	-
Total	218	2	3 (3)	9 (13)

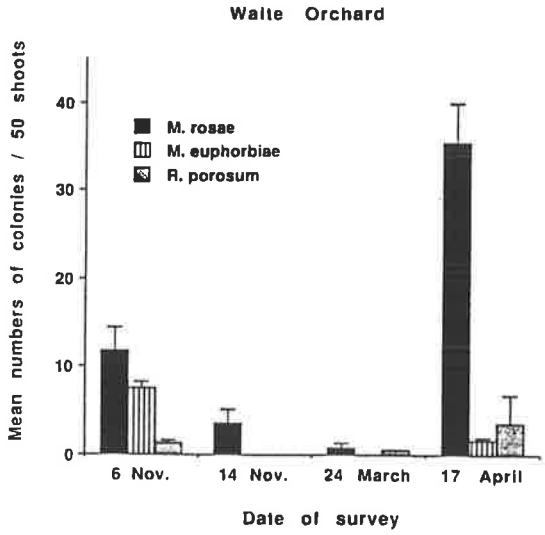
b) August 1994 to June 1995

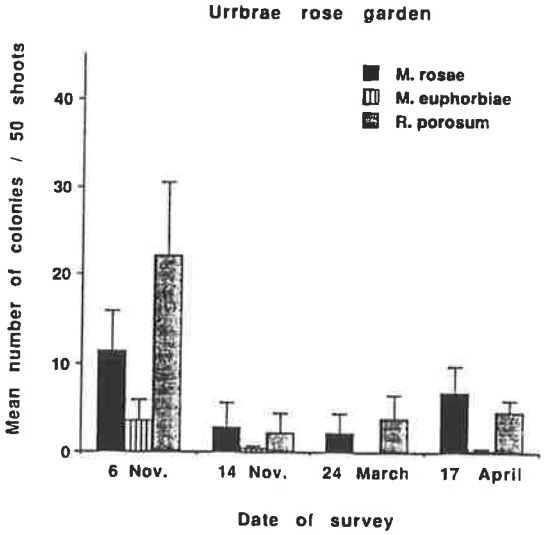
Sample date	Sample size (shoots)	*Harmonia conformis*	*Micromus tasmaniae*	Syrphidae
8 August 1994	8	-	-	-
24 August	10	-	-	-
6 September	20	2	1	(2)
20 September	5	-	1	1(2)
4 October	20	-	-	-
20 October	20	-	-	-
12 April 1995	19	-	-	-
26 April	20	-	1	-
11 May	20	-	-	(4)
15 June	20	-	-	-
Total	162	2	3	1(8)

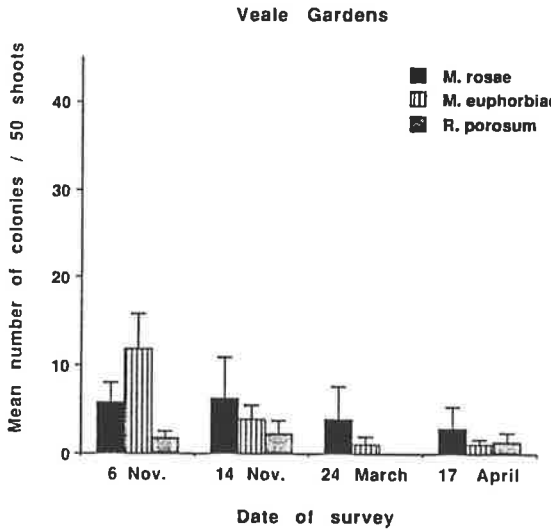
Numbers without brackets represent larvae stages, number in brackets show the number of eggs. Adults were not encountered on shoots.

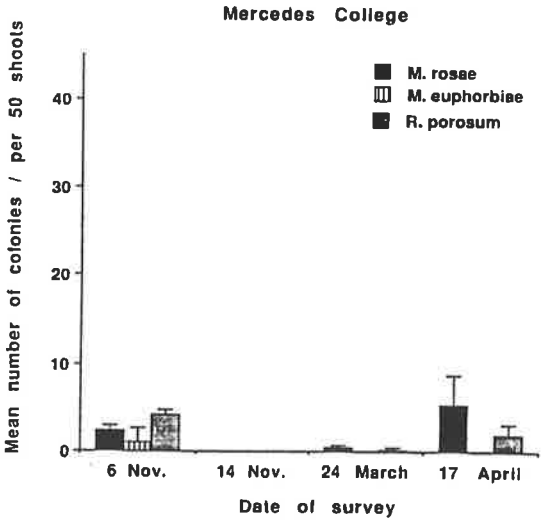
Extensive survey. A comparison of five rose gardens showed that aphid infestations displayed no uniform pattern (Fig. 10.11). At the end of spring 1994 (Nov.), *M. rosae* was not the predominant species on roses. In the Urrbrae House rose garden, *R. porosum* was the most abundant species, whereas at Mercedes College and Veale Gardens *M. euphorbiae* was most abundant. The only rose garden where *M. rosae* was the dominant species turned out to be the rose plot in the Waite orchard. In autumn (April), *M. rosae* was the predominant species in all gardens.

The rose garden in the Waite Orchard, the Urrbrae High School rose garden and the garden at Mercedes College had only very few or no aphids toward the end of spring and in the beginning of autumn. In comparison, aphid colonies in Veale Gardens and the Urrbrae House rose garden were abundant for a longer time at the end of spring and were present earlier in autumn.









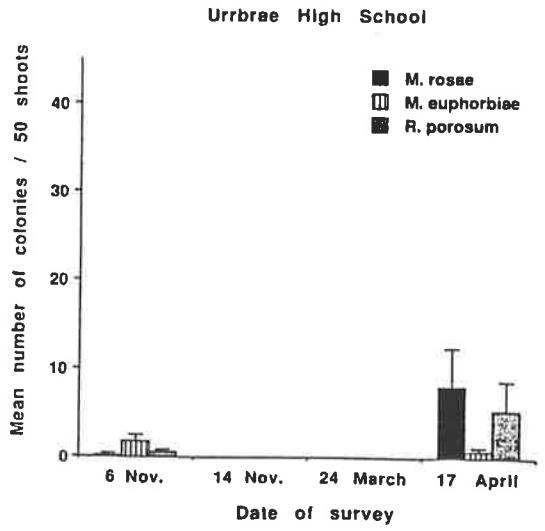


Fig. 10.11 Comparison of aphid colonies of *Macrosiphum rosae*, *Macrosiphum euphorbiae* and *Rhodobium porosum* in five rose gardens in Adelaide, South Australia, during four surveys in 1994/95. In each garden eight rose beds were randomly chosen and in each bed ten plants selected. On each plant the number of aphid colonies on 5 shoots were counted. In cases of mixed colonies each involved species was counted. Error bars show SE of mean.

Compared to data from years prior to the release of *A. rosae*, rose aphid numbers were lower in spring and started to decrease earlier towards summer (Fig. 10.12). In contrast, no reduction of aphids was apparent in autumn when aphids reached high densities.

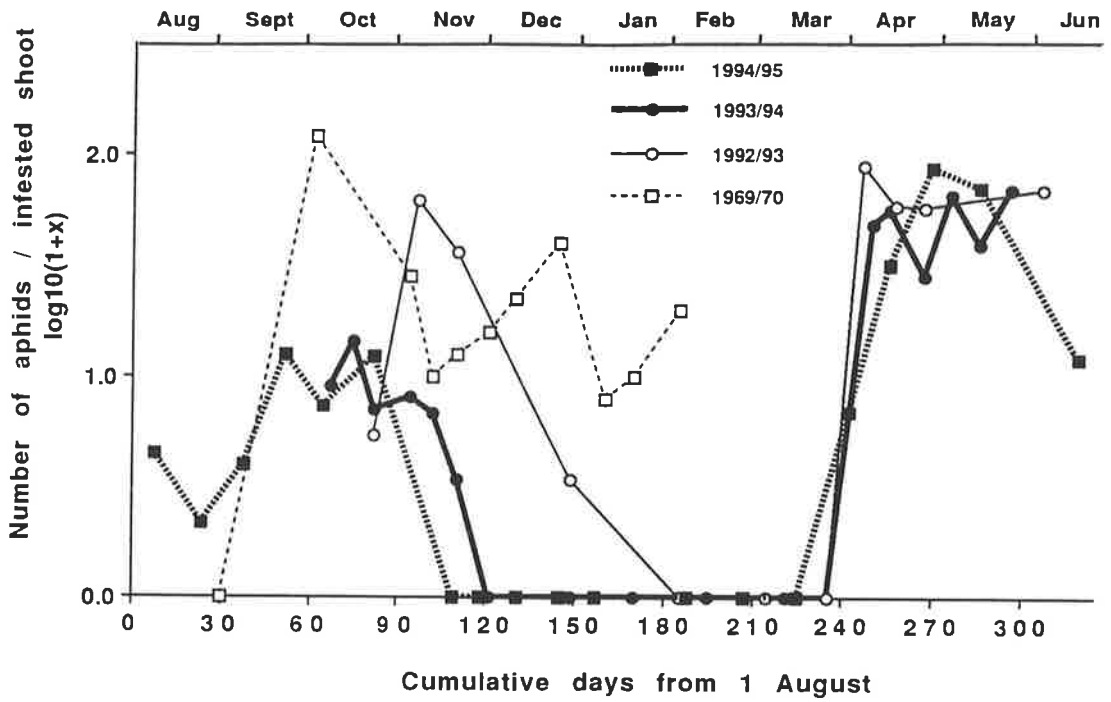


Fig. 10.12 The abundance of *Macrosiphum rosae* on *Rosa sp.*, variety Tea hybrid 'McGredy's sunset', at the Waite Campus, Adelaide, South Australia, before and after the release of the control agent *Aphidius rosae* in August 1993. In the survey in 1969/70 a ranking procedure was used (Maelzer, 1977)(expected error less than 10 %)(Maelzer, 1976). N ranged between 180 and 1080 shoots in 1969/70. During the surveys in 1993/94 and 1994/95 the sample sizes consisted mainly of 20 infested shoots (Appendix 6) whereas sample size of the survey in 1992/93 was 30 infested shoots. Data from 1969/70 were log transformed from the given means in the literature, whereas data from the census 1992-95 represent the mean of already log transformed data.

10.4 Discussion

The discussion examines the role of the control agent in the seasonal dynamics of the rose aphid. Several factors are examined, followed by a summary which combines the interactions among them.

Phenology of roses. Maelzer (1977, 1981) described the flushes of favourable rose buds under irrigated garden conditions in South Australia. For Tea hybrid roses he recorded peaks in early spring around September, in late spring around November and in mid summer, in January. Peaks were slightly different during the present survey but also concentrated in spring and early summer (Fig. 10.2). Mean numbers of favourable shoots reached a maximum of 39 per plant in 1993 and 23 in 1994, compared to 125 in 1969. These surveys were carried out on the same individual plants but the roots of rose plants were transferred to a new location in the winter preceding the survey in 1993. It is likely that stress of the old rootstock inhibited better shooting. However, shoot buds appeared healthy and showed no sign of stress.

Rose plants carried sufficient numbers of favourable buds to support aphid increases under most circumstances. This was not the case at the end of both autumns. At these times nearly all buds were infested, and represented a resource limitation for aphid population growth. E.g. in autumn 1994, a decrease of bud numbers between the last two sample days (Fig. 10.4) followed a rapid decrease of total aphid numbers in the plot (Fig. 10.9), even though this was not obvious from the mean numbers of aphids per shoot (Fig. 10.4) because aphids aggregated on the last remaining plant resources.

The role of temperature and rainfall. Maelzer (1981) stated that peaks of aphid species in the Adelaide region occurred when temperatures in the field were around a favourable range of 15 to 17 °C, even though maximum rates of increase were observed between 22 and 28°C in the laboratory. The phenology of *M. rosae*, observed

in the present study, supported those conclusions. Temperature itself may not be the primary factor that drives the population dynamics of *M. rosae*, but it may be seen as environmental indicator for complex phenological interactions in the field. In spring, the first peaks of aphids and parasitoids were associated with flushes of favourable growth of rose plants when mean temperatures reached around 15 to 17°C (Fig. 10.4 and 10.5). In autumn, aphid numbers increased when temperatures dropped back to the favourable range and roses overcame their summer dormancy. Tomiuk & Wöhrmann (1981) speculated that the most important factor for the potential increase in aphid numbers is the physiological stage of the rose plant, which is greatly influenced by seasonal conditions.

The time a rose shoot remains favourable for an aphid colony decreases disproportionately faster than the generation time of *M. rosae* with increasing temperature (Maelzer, 1977). Instead of supporting up to three generations per shoot in early spring, only two may be supported towards summer. Accordingly, Maelzer (1977) recorded smaller colonies of *M. rosae* with progressing season. However, this phenomenon did not play a significant role in the late springtime of 1993/94 and 1994/95 since infestations of rose buds were absent already in November, despite the presence of many favourable buds and, in 1994 continuing favourable temperatures. Therefore, it can not explain the observed decrease in aphid numbers.

Differences between the temperature developmental threshold of aphids and their natural enemies may effect their population dynamics. According to Maelzer (1977) the developmental threshold for *M. rosae* is around 10°C whereas the threshold for eggs and larvae of its coccinelid predator *Harmonia conformis* (Boisd.) is approximately 15°C (Maelzer, 1978, 1981). Differences occur between predator species, e.g. the lacewing *Micromus tasmaniae* (Walker) can be an effective predator when temperatures are still unfavourable for *H. conformis* (Maelzer, 1986). The lower developmental threshold for aphids compared to their predators or parasitoids is commonly seen as one of the major reasons for rapid aphid increase early in the season. However, during both survey years, the numbers of predators were low

regardless of temperatures (Table 10.6). Additionally, adults of *A. rosae* were observed next to *M. rosae* during the whole of winter in 1994 and 1995. Therefore, the role of temperature as a developmental barrier for natural enemies may be relatively unimportant in the interpretation of results. However, temperature may have had different physiological effects on the reproductive rate of aphid and parasitoid outside the favourable range, but this was not investigated.

During summer high temperatures caused rose plants gradually to enter dormancy. Maximum temperatures of more than 40°C restricted the survival of any aphids (Gilbert et al., 1976). The main problem facing aphid populations in South Australia is survival over summer when moisture deficits and especially extreme maximum temperatures threaten species with local extinction (Maelzer, 1981). Accordingly, *M. rosae* virtually disappeared from roses in Adelaide and so did *A. rosae* during summertime.

In both years aphid colonies were exposed to heavy rainfall (Fig. 10.4 and 10.5). Maelzer (1977) showed that aphid colonies can suffer up to 80 % losses of older instars and adults due to heavy rainfall. This may occur especially in older colonies on long exposed shoots (e.g. Appendix 1), but probably to a lesser degree on smaller buds or on rose varieties with short bud shoots.

Differences between seasonal rainfall were pronounced between the two autumn periods of study. In autumn 1994, no heavy rainfall occurred during the whole period of aphid infestation (Fig. 10.4), whereas seven days with heavy rainfall were recorded in autumn 1995 (Fig. 10.5). In both seasons aphid populations thrived, regardless of rain.

A field experiment (Chapter 11) showed that aphid numbers in autumn 1995 increased rapidly on rain sheltered plants but also on unsheltered roses, despite occasional heavy rainfalls. 1992 was the wettest year in 100 years in Adelaide (data not shown), but aphid colonies were able to build up high numbers (Fig. 10.12). Data suggest that at least in autumn 1993/94 and 1994/95, rainfall alone was not able to suppress an increase in aphid numbers over longer periods, even though an impact

over short periods can occur (Maelzer, 1977). It could be that maximum rates of increase achieved during dry periods are quickly counterbalanced by a sensitive, density-dependent mechanism (see below). Consequently rain may have great impact on the expansion and dispersal of aphid outbreaks, but may influence to a lesser degree population growth in a colonised rose garden. Tomiuk & Wöhrmann (1982) found that weather conditions were not the main factors that influence the population growth of *M. rosae*. Except under extreme climatic conditions, weather only modifies the increase or decrease, but does not cause the typical pattern of development of the population.

Density-dependent dispersal. During autumn, when the aphid density per shoot was high, the aphid population reacted with density-dependent dispersal. Maelzer (1977) showed that aphid numbers of more than 50 per shoot triggered an enhanced production of alatiforms. In autumn, these numbers were reached soon after the first appearance of *M. rosae*.

Tomiuk & Wöhrmann (1981) found constant ratios around five to 10 % of winged morphs in Germany throughout the seasons in three successive years and did not consider the production of alatae as the most significant factor for regulating population size. Low numbers of alatae were also observed in the present study, but in fact, they gave strong evidence for the regulation of population size by the production of alatae. Throughout autumn most adult aphids were apterae, but at the same time nymphs in the third and fourth instar were mostly winged morphs (Fig. 10.7). Differential mortality between apterae and alatae on the plant was unlikely. Therefore, the tremendous differences in ratios of alatiforms and apterous forms must be due to the greater dispersal of alatae. Soon after fourth instars moulted to alatae, they must have left the plot disproportionately more rapidly compared to their apterous counterparts which had only restricted means of dispersal. These figures demonstrated that the decline of numbers of aphids in autumn was probably due to a self-regulatory density-dependent mechanism of *M. rosae*.

The role of hyperparasitoids. It was not surprising to find hyperparasitoids in mummies of *A. rosae* soon after establishment of the primary parasitoid. Hyperparasitoids generally have a wide host range (Sullivan, 1988). When *Trioxys complanatus* Quilis was introduced into South Australia in 1977 (Wilson & Swincer, 1984), it was attacked by virtually the same guild of hyperparasitoids as found in mummies of rose aphids. Hyperparasitoids of *T. complanatus* emerged from a total of 8.7 % of all mummies collected during the first two years after initial release.

It is rather difficult to determine the impact of hyperparasitoids on the effectiveness of *A. rosae*. Many parasitized aphids leave the plant to mummify in concealed sites (Behrendt, 1968), thereby decreasing the likelihood of being encountered by hyperparasitoids. This alteration of aphid behaviour is especially common in aphids which contain parasitoids entering diapause (Brodeur & McNeil, 1989). For this reason, the figures obtained from collections of mummies from rosebuds may overestimate hyperparasitism, particular towards the end of a season. On the other hand, abundant hyperparasitoids may cause primary parasitoids to leave the patch and contribute to declining parasitism without causing direct measurable mortality (Höller et al., 1994).

Over a period of four years, Van den Bosch et al. (1979) noted a total of 51 % hyperparasitoids reared from mummies of the primary parasitoid *Trioxys pallidus* (Haliday) in California. Despite their abundance, the hyperparasitoids did not appear to impair the effectiveness of the control agent on its host, the walnut aphid, *Chromaphis juglandicola* (Kalt.). Schlinger (1960) reported up to 70 % hyperparasitism on mummies of primary parasitoids (*Aphidius nigripes* Ashmed; *Aphidius confusus* Ashmed; *Aphidius alius* Muesebeck; *Praon occidentalis* Baker; *Praon unicus* Smith; all non specific to *M. rosae*) of rose aphids in California in autumn. He concluded the impact of hyperparasitoids was one of the key factors for the relative ineffectiveness of the primary parasitoids. Similar conclusions were made by Borgemeister & Poehling (1990) who reported nearly 100 % hyperparasitism in cereal aphids towards the end of the season.

High percentages of hyperparasitism were not observed in the rose plot in the present survey. In spring, the peak of hyperparasitoid activity was delayed compared to *M. rosae* and *A. rosae*, whereas hyperparasitoids had more or less the same period of activity as primary parasitoids in autumn, a phenology commonly observed (Höller et al., 1991). The explanation for these findings may be the high temperature requirements of hyperparasitoids, which give them advantages during the seasonal change from hot summer to cooler autumn, but are a disadvantage to them during the seasonal change from cold winter to warmer spring. Even in autumn, the percentage of hyperparasitism was initially low in the present study and never increased dramatically. The observed numbers probably had only a minor impact on the initial increase of parasitoid numbers. However, towards the end of autumn the increase of hyperparasitism occurred at the same time with a decline of aphid numbers through self-regulatory density-dependent mechanism. Additionally the number of parasitoids entering diapause increased greatly close to the end of the season. At this time increasing hyperparasitism may have contributed to the ineffectiveness of *A. rosae* in the control of rose aphids.

The role of the Sex-ratio of A. rosae. The observed sex-ratios on different sample days were nearly always female-biased with a mean of 0.36 in 1993/94 and 0.42 in 1994/95. Similar ratios were found in laboratory cultures (Chapter 3). Other aphidiine species also have female biased sex-ratios (Mackauer, 1976b). Sex-ratios of 0.4 in *Aphidius ervi pulcher* Baker, 0.4 in *Aphidius smithi* Sharma & Subba Rao and 0.37 in *Praon pequodorum* Viereck were found in a sample of nearly 9000 mummified aphids from the field. In Aphidiinae, the sex-ratio favours the females, but it is influenced by variable environmental factors (Stary, 1988).

The observed ratio does not fit into Fisher's (1930) predicted principle of equal numbers of sexes. His principle implies that mothers gain the same fitness return for both sons and daughters resulting in an evolutionarily stable strategy of a sex-ratio of 0.5. But in cases were males compete intensively for mates, investment into sons will

not bring equal fitness returns. Under such circumstances, females will almost always be mated whereas males suffer severe competition and may not pass on their genes to the next generation. Such a situation may apply for Aphidiinae, and derivates of Hamilton's (1967) local mate competition model may be appropriate to explain female biased sex-ratios (Godfray, 1994). Fewer sons imply less competition among males and more daughters represent more mates for males.

Sex allocation in *A. rosae* is dependent on host quality, with more males emerging from small hosts than from large ones (Chapter 4). How far this adaptive pattern of sex allocation interacts with an evolutionarily stable strategy of female biased progeny allocation remains unclear.

Host instar preference. Most mummies found in the field revealed that oviposition of *A. rosae* must have taken place in younger instars of the host. Even though Aphidiinae are able to lay eggs in all stages of their hosts (Stary, 1988)(Chapter 4), various authors have shown in laboratory experiments that host preference in aphidiine wasps is shifted towards younger instars, mainly with second and third instars as most preferred host (Liu et al., 1984; Kouame & Mackauer 1991; Weisser, 1994; Kirsten & Kfir, 1991; Singh & Sinha, 1982). This pattern is partly explained by instar-specific aphid defence, with larger instars being more capable of avoiding parasitism (Liu et al., 1984; Koume & Mackauer, 1991)(note size differences between adult *M. rosae* and first/second instars compared to the parasitoid, Fig. 7.2). Even though offspring of *A. rosae* gain larger size and higher fecundity when eggs are oviposited into larger instars (Chapter 4), oviposition in smaller hosts may have advantages which are related to minimising the risk of larval mortality. Firstly, the older larvae normally survive intraspecific competition in cases of hyperparasitism. Since the detection of parasitized hosts is imperfect in Aphidiinae (e.g. Micha et al., 1992) an oviposition into a younger host increases the chances of being first. Secondly, by ovipositing into younger instars, the parasitoid decreases the larval developmental time of its offspring spent in larger instars. Lager instars and adults can suffer up to 80 % mortality rates in rain (Maelzer,

1977). E.g. an offspring allocated into a first instar host spent at least half of its larval developmental time in a host which is relatively less affected by rain, whereas a larva allocated into a fourth instar host spent all its larval developmental time in a host which would be vulnerable to rain. It may be that a wasp has to balance her potential gain in fitness by ovipositing in a high quality host against potential higher mortality risks of her offspring. However, even though Aphidiinae show preference for certain instars of their host they also display extensive relaxation of this morph preference which enables them to react flexibly to changing situations in the field (e.g. Liu et al., 1984).

A. rosae parasitized younger host instars preferentially which were killed before they reached reproductive age, a quality which certainly enhanced the biological control capacity of the wasp.

The role of predators. The number of predators on shoots were low (Table 10.6). Maelzer (1977) stated that predators have the potential to reduce aphid numbers, at least in autumn, and Tomiuk & Wöhrmann (1981) concluded that predation caused the decline of aphids on roses.

In the present study it was unlikely that the observed numbers of predators alone had any significant impact on aphid numbers.

The rarely found larvae of the syrphid species *Melangyna viridiceps* (Macq.) and *Simosyrphus grandicornis* (Macq.) were heavily parasitized by *Diplazon laetatorious* F.. These findings on numbers of syrphids agreed with data from Ebrahim Soleyman (pers. communication) who studied the impact of syrphids on rose aphids in Adelaide for three successive years from 1992 to 1995.

The numbers of the lacewing *M. tasmaniae* were low as well. This may have had a positive effect on *A. rosae* since chrysopid larvae were frequently observed feeding on mummified aphids (Frazer & Van den Bosch, 1973). In the present study, the lady beetle *H. conformis* was observed to feed on mummies during times of low aphid densities. This impact of predation on mummies was minor (Table 10.5)

The common earwig, *Forficula auricularia* L., was commonly found underneath the sepals of faded flowers. It used the buds as shelter during the day. *F. auricularia* can be an effective predator of aphids (Mueller et al., 1988). The impact of most polyphagous predators such as earwigs, beetles and spiders on aphid colonies is still unknown, but they may play an important role in aphid control, especially in situations where aphid densities are low (Sunderland, 1988).

The role of diapause. Quiescence and diapause are strategies of aphid parasitoids to overcome unfavourable conditions (Stary, 1970). At the end of the season large numbers of parasitoids enter diapause somewhat earlier than their hosts, before aphids numbers decline (Stary, 1988). Diapause in aphidiine wasps may be induced by a combined effect of temperature and photoperiod (Brodeur & McNeil, 1994), or solely by larval feeding in oviparous hosts (Polgar et al., 1991, 1995).

Some *A. rosae* entered facultative diapause throughout the year with a progressive increase towards the end of the season. Similar observations were made during the rearing of *A. rosae* in the laboratory (Chapter 3). Liu & Carver (1985) found that *Aphidius sonchi* Marshall produced a constant rate of 6% of individuals entering diapause per generation. A small percentage of individuals entering diapause at all times may be seen as an adaptive strategy of the parasitoid to survive unpredictable unfavourable conditions in the field. Parasitoids can overcome sudden host declines by escaping into time or space, but dispersal into space is costly and does not guarantee success. Therefore, investing in diapausing individuals even at favourable times of the year could mean that the parasitoid plays safe by spreading the risk on cost of optimal short term reproductive capacity.

Throughout winter, individuals of *A. rosae* were observed in the field. These individuals reproduced on the small numbers of rose aphids that were present. In contrast, no recoveries of adult *A. rosae* were made during mid summer. It is not clear if any parasitoids emerged and simply died because of the lack of hosts, or if *A. rosae* stayed in diapause until autumn. However, in 1994, the first recorded appearance of a

female of *A. rosae* after summer in Adelaide was on 15 March, and in 1995 first mummies were found on 29 March. Both incidences were recorded at the Urrbrae rose garden.

Seasonal synchronisation with the host is a major attribute of successful parasitic control agents. Generalist parasitoids failed to control rose aphids in California because diapause prevented synchrony between the enemies and the pest (Schlinger, 1960).

It appears that diapause in *A. rosae* affected its differential performance in spring and autumn in two ways. Firstly, *A. rosae* was better synchronised with its host in spring than in autumn. Because *A. rosae* was active throughout winter in Adelaide, wasps were already present in the field in early spring when conditions first favoured aphid increase. Parasitoids normally emerge somewhat later from diapause than pests, which favours an increase in the densities of pests. In the case of *A. rosae,* small numbers of parasitoids continued to reproduce over winter and the individuals present in the early spring may have been able to counterbalance the delay of individuals emerging from diapause. Much depends upon the number of aphids killed during the initial period of colonisation by alatae (Carter et al., 1980). Wellings (1986) showed that parasitism of *Aphidius ervi* Haliday had little effect on caged populations of *Acyrthosiphon kondoi* Shinji, except when the aphid colonies started with low numbers. Therefore, the early impact of *A. rosae* on host populations could be one of the key factors responsible for low aphid populations in spring. In contrast, the build up of parasitoid numbers in autumn appeared to be entirely due to individuals that emerged from diapause. Under these circumstances, synchronisation and early impact may have been difficult.

The second effect of diapause on differential performance in spring and autumn arose because there were fewer diapausing mummies in spring than in autumn. Because numbers of aphids were lower in spring than in autumn, parasitoids were able to build up higher numbers in autumn. In addition, the frequency of diapausing mummies was low until late spring when aphid and parasitoid numbers were declining.

In autumn a high frequency of diapause mummies was observed already at the peak of abundance of *A. rosae*. Therefore, it may be assumed that much higher numbers of parasitoids entered diapause in autumn than in spring. As a result, the initial densities of parasitoids that emerged from diapause might have been higher at the start of spring than in autumn, enabling *A. rosae* to build up faster in spring.

A similar effect was suggested to explain high densities of the black bean aphid *A. fabae* in one year, followed by low densities the next year (Dixon, 1985). Coccinellids rapidly increased when there was an outbreak of *A. fabae* and large numbers of surviving predators were able to suppress aphids in the following year. When aphid populations were small, there were fewer predators which were unable to prevent an aphid outbreak in the alternate year. Similarly, after the introduction of the Iranian ecotype of *Trioxys pallidus* (Haliday) into California, this parasitoid kept densities of the walnut aphid low during spring time (Frazer & Van den Bosch, 1973). However, initially the parasitoid was not able to inhibit high aphid abundance in autumn.

Role of density-dependent parasitism. The spatial distribution of %-parasitism on single shoots was inverse density-dependent at times of high aphid densities (Fig. 10.10).

Experimental trials with *A. rosae* parasitizing *Sitobion fragariae* Walker on rose bushes in Germany also showed an inverse density-dependent parasitism (Völkl, 1994). In glasshouses, female *Ephedrus cerasicola* Stary gathered around moderately to heavily infested plants whereas clean shoots were free from parasitoids (Hågvar & Hofsvang, 1987). This aggregation in high density patches did not result in density-dependent parasitism. *Aphidius ervi* Haliday was randomly distributed in experimental fields whereas its host *Sitobion avenae* (F.) showed a more clumped distribution in a three-year field survey in the USA (Feng et al., 1993). In another example, *Diadegma sp.* exhibited a clear aggregative response in the field, spending more total time on

higher density patches of its host *Plutella xylostella* (L.). Despite this aggregation, positive density-dependent parasitism was not found (Waage, 1983).

Lessells (1985) reviewed the pattern of parasitism for 45 parasitoid species. 15 species displayed direct density dependence and 17 cases showed inverse density dependence. The remaining 13 species displayed no clear relationship between host density and parasitism. These fundamentally different responses of parasitoids to host density were explained by the effects of host density on the searching behaviour and host handling time of parasitoids. E.g., an inverse density-dependent response was explained by increased host handling time in dense patches even though the search time was reduced (Waage, 1983; Hassell, 1986; Murdoch, 1990).

The size of rose aphid colonies affects the ability of *A. rosae* to parasitize its host. Female *A. rosae* concentrate attacks on the edges of a colony (Chapter 7). A greater proportion of aphids is protected from attacks in a large colony. Aphids in the middle of a dense colony on roses with long bud shoots like Tea hybrid 'McGredy's sunset' are virtually out of reach of *A. rosae*. With increasing colony size, the proportion of inaccessible aphids in the middle increases disproportionately to the numbers of accessible aphids at the edges. As a result, bigger colonies may help the aphid to minimise parasitoid pressure.

However, larger aphid colonies do not suddenly appear. They need considerable developmental time which strongly depends upon the number of foundresses (Dixon, 1985). In the initial phase of growth a small colony will not be able to achieve significant protection against parasitoid attack. If parasitoids are abundant, the encounter and parasitism of aphids in a small colony is likely. Nevertheless, the effect of parasitism on population growth is also delayed in time. Therefore, the fate of a colony may be determined by the initial reproductive success of *M. rosae*. As soon as colonies reach a sufficient size, aphid numbers increase disproportionately more than they suffer parasitism, until the bud becomes unfavourable. At times when aphids are under control, most colonies may not reach this critical size, whereas when heavy infestations occur most colonies may do so.

If the size of rose aphid colonies is crucial for the avoidance of parasitism, then there may be even further behavioural consequences. This hypothesis could partly explain why most individuals of *M. rosae* do not seek shelter underneath leaves in rain, even though they are vulnerable to rain drops. The colony may have a protective function as a whole which outweighs the protection of leaves in rain.

Extensive monitoring. The phenology presented so far, focused on one rose variety out of several hundred commonly grown in Adelaide. Rose varieties display a variety of different growth patterns and different plant management can alter the abundance of favourable bud shoots for *M. rosae* as well (Beales, 1992). Environmental factors, e.g. the presence of wind breaks, or simply coincidence, may favour outbreaks of rose aphids in one garden but not in another. This influences the seasonal phenology of aphids and their parasitoids in a particular garden. It is not surprising then that a survey in five different rose gardens indicated considerably different impressions on aphid abundance on four sample dates (Fig. 10.11). During a survey in Germany, relative numbers of three *Aphidius* species were dissimilar between two comparable cereal fields (Borgemeister et al., 1991). The differences were the result mainly of initial population size, temperature and hyperparasitism, which all acted on a local scale.

On an annual basis, peaks of abundance in spring and autumn, no aphids during summer and only low numbers in winter can be considered the general pattern of rose aphid occurrence on roses in the Adelaide region (Maelzer, 1981).

It is known that *M. rosae* occasionally alternates between roses and summer hosts in South-eastern Australia (Maelzer, 1977, Wöhrmann, 1991). However, no infestations of *M. rosae* on suspected hosts were observed in summer. This may be attributable more to hot maximum temperatures than to non-acceptance of host plants. The survey in the present study was only undertaken at a few locations and certainly can not exclude the possibility that *M. rosae* used summer hosts, but if so, this was probably insignificant (Maelzer, 1977).

Summary. Before the release of *A. rosae*, spring time was the season of main abundance of *M. rosae*. After the release, *M. rosae* was not able to reach high infestations levels in the surveyed rose plot in the spring. High rates of parasitism in spring suggested that *A. rosae* may have been a key factor in reducing aphid numbers. At this time of the year the parasitoid was well synchronised with its host since parasitoids were active in the field when aphid numbers started to build up. Additionally, high aphid numbers in autumn resulted in high numbers of diapausing mummies. These diapausing individuals could have increased the rate of parasitism in the following spring. Hyperparasitoid pressure was low in spring and predation was insignificant in spring. The effect of temperature and rainfall could not explain the decline of rose aphid populations in spring

During summer *M. rosae* and *A. rosae* virtually disappeared from roses in Adelaide.

In autumn, the numbers of aphids increased until the carrying capacity of rose plants was reached. Self-regulatory density-dependent production of alate *M. rosae* took place and towards the end of the season a decrease in available rose shoots reduced the numbers of aphids. *A. rosae* did not affect the growth of the population of rose aphids in autumn. Relatively low numbers of diapausing individuals that carry over from spring to autumn and/or poor synchronisation of *A. rosae* with its host could have delayed the response of *A. rosae* to aphid increase in autumn. By the time parasitoid numbers had built up, aphid colonies were already dense. Because of avoidance of aphid defence, *A. rosae* parasitized aphids at the edges of the colonies and had only little, if any impact, on population growth of *M. rosae*. Higher proportions of *A. rosae* entered diapause throughout the autumn, compared to spring. Towards the end of the season, increasing hyperparasitism may have contributed to the ineffectiveness of *A. rosae* as well.

Aphid infestations in different rose plots varied considerable. It is not known how well the result of the present study reflects the population dynamics of *M. rosae*

and *A. rosae* in the Adelaide region, but the patterns were consistent with the known phenology of *M. rosae*.

Experimental approach to assess the impact of *Aphidius rosae* in the field

11.1 Introduction

The establishment of a biological control agent does not guarantee that control of a target pest will occur. Only 44 to 60% of established control agents provide some degree of control (Hall et al., 1980; Waage, 1990). Even though introduced natural enemies may become abundant and widespread, the resulting mortality of the target pest may be compensated by density-dependent changes in natality (Hughes, 1989).

The comparison of pest densities *before* and *after* introduction can indicate the impact of a control agent (e.g. Hughes et al., 1987) and is judged by some as the most reliable method (Legner, 1969). In contrast, DeBach et al. (1976) argued that the clearest and most reliable means of evaluation of natural enemies is through use of experimental or comparative methods, which can be classified as 1) addition, 2) inclusion 3) exclusion (or subtraction), and 4) interference (DeBach & Huffaker, 1971). They emphasised the advantages of these experimental field approaches because the paired plots furnish a reliable measurement of the effectiveness of natural enemies.

In the addition method, comparisons are made between plots in which natural enemies are added and others which do not receive any. Differences between pest densities are then assumed to be caused by the natural enemy.

Inclusion experiments use selected natural enemies together with the pest in closed cages that exclude other enemies (e.g. Wellings, 1986).

The exclusion or subtraction technique is a method in which natural enemies are excluded from the pest, usually by cages. The experimental design must consider significant behavioural characteristics of the species being studied and ecological

characteristics of the crop - pest system. Target species with low dispersive power, such as scale insects are easiest to study with this method. DeBach & Rosen (1991) considered the use of paired open sleeve cages as the best exclusion method for sedentary pests and active natural enemies. One of the cages is impregnated with a long-lasting insecticide so natural enemies eventually come in contact with the insecticide in the treated cages whereas the sedentary pest does not.

In the interference method, the effectiveness of natural enemies is greatly reduced in one group of plots whereas in other plots natural enemies are not disturbed. This can be achieved by regular hand removal, or by the use of an insecticide that kills sensitive natural enemies but has little effect on the pest. If applied rigorously, this method might serve as an exclusion method. However, this goal normally can not be achieved so the overall impact of the enemy can only be estimated.

Periodic census and life-table analyses are tools used to reveal the impact of natural enemies (e.g. Southwood, 1978a). However, quantitative methods, including regression analysis and modelling techniques (e.g. Gutierrez et al., 1990) may not take into account the impact of all interacting factors which easily can mask the true impact of control agents (DeBach et al., 1976). DeBach & Rosen (1991) pointed out that a major weakness of such methods is that they generally can not distinguish between cause and effect.

Every pest/natural enemy system has its own characteristics and a method for determination the impact of an enemy in one system might not be suitable in another. The different techniques were examined for their practicality in assessing the impact of *A. rosae* on *M. rosae*.

The use of a mathematical model to evaluate the impact of *A. rosae* was never seriously considered, because of insufficient data on the biology of the parasitoid and the pest. Much more emphasis on the estimation of biological parameters would have been needed in this thesis to obtain the relevant data than was possible.

Cages of different mesh sizes have been used to select the exclusion of natural enemies of aphids, based on their size (e.g. Campbell, 1978). This approach is promising when distinctive differences in size of the pest and natural enemies exist. The smaller individuals of the lacewing *Micromus tasmaniae* (Walker) are the same size as large *A. rosae*. Additionally rose aphids are often larger than *A. rosae*, and a mesh size just big enough to exclude the parasitoid would have restricted migration of adult aphids. Furthermore, cages may change the behaviour of insects (Luck et al., 1988). As the mesh width becomes smaller it becomes more likely that the natural enemy will avoid entering the cage.

Experimental methods to investigate the impact of *A. rosae* in the field could have been inclusion and exclusion experiments. For example, Wellings (1986) demonstrated the impact of *Aphidius ervi* Haliday on blue-green aphid *Acyrthosiphon kondoi* Shinji in an inclusion experiment. However, aphid populations display density-dependent regulatory mechanisms that can be affected by cages. For example, the development of alatiforms is increased when there are more than 50 *M. rosae* per bud (Maelzer, 1977). Under natural conditions most of those alatae would disperse but completely closed cages force them to stay on the plant. Additionally, aphids dislodged from roses can easily return to the plant in a closed cage, whereas under field conditions mortality of dislodged rose aphids is high (Maelzer, 1977). A reduction of the pest might not only result from parasitism but also from disturbance of the aphid colony (Tamaki et al., 1970; Gowling & van Emden, 1994). This effect would not be detected in a closed cage. Furthermore, parasitoids are likely to disperse when rates of parasitism increase. For example, Way (1966) showed that aphid populations of *Brevicoryne brassicae* L. were quickly eliminated by the parasitoid *Diaeretiella rapae* McIntosh when held together in closed cages, whereas an oscillating host population was maintained for more than ten months when dispersing parasitoids and aphids were removed. Ruth et al. (1975) and Sinha & Singh (1980) found in laboratory experiments that repeated interactions between aphidiine wasps in overpopulated patches resulted in an increased

tendency to disperse. Therefore, the use of closed cages to evaluate the impact of natural enemies on *M. rosae* was considered an inappropriate method.

Maelzer (1977) excluded enemies with completely closed cages to demonstrate their impact on rose aphid colonies. Similar experiments were made in various other aphid systems (e.g. Chambers et al., 1983; Hopper et al., 1995). These experiments are meaningful when used together with population sampling (e.g. Way, 1968). But again, using a completely closed cage creates artificial behaviour, inhibits density-dependent self-regulatory dispersal as well as immigration, and creates artificial weather protection resulting in microclimatic differences (Hand & Keaster, 1967; Tamaki et al., 1981; Luck, 1988).

The addition method was considered as unsuitable since most rose plots consist of a mix of different varieties. As seen in Chapter 10, aphid infestations can vary considerably between gardens and to achieve comparable conditions would have been difficult. This was also a problem with the interference method.

As it would be difficult, if not impossible, to measure the net impact of *A. rosae* alone, an exclusion method to measure the total impact of all natural enemies was developed using paired open cages, one of them treated with an insecticide. This method allows insects inside the cage to disperse and those outside to enter the control cages but not the treated cages. Effects on the microclimate are minimised. Young aphid colonies can be considered sedentary and therefore are unlikely to contact the insecticide. This method has only been used with sedentary scale insects that have persistent colonies (DeBach & Rosen, 1991). Therefore, the method had to be adjusted to the special needs of the rose plant / *M. rosae* / *A. rosae* and predator system.

This chapter deals with the development of this cage exclusion method to estimate the net impact of natural enemies on rose aphids in the field. The outcome of the experiment is discussed in regard to aphid infestation in the field at the time of the experiment.

11.2 Methods

Roses. The roses used in this experiment consisted of potted plants (pot diameter 21 cm) *Rosa sp.*, var. 'Spirit of peace', that had new autumn growth. Plants had between three and five main suitable shoots. All plants were fertilised with Osmocote® Controlled Release Fertiliser 'Outdoors, trees and shrubs' (Grace Sierra Australia Pty Ltd, 89 Cevil Avenue, Castle Hills, NSW 2154). Ten days before the experiment, pots were sprayed with Pyrethrum (CRG LTD, 25 ml insecticide [4 g/L Pyrethrins & 16 g/L Piperonyl Butoxide] : 1 l water) to kill predators. Plants were then kept indoors at 18°C, 16L:8D under fluorescent True Light®.

Insects. Six days after the application of the insecticide, apterae of *M. rosae* were collected from uncrowded small colonies in the field to reduce the possibility of the production of alate offspring. These were placed on 2nd stage buds on the plants used in the experiment. After 24 hours adults were removed. Three days later the number of 'initial aphids' on each plant were reduced to 50 and the pots were placed in the field. This procedure had the advantage that aphids had a defined age, were not disturbed or injured by handling, and were settled on the plant and likely to stay. Rose aphids are easily disturbed but 3 - 4 day old second instars rarely drop from the plant, an important consideration for the transportation to the experimental site. At this stage aphids had undergone approximately one third of their immature development.

Cages. Field cages made of two cylinders of chicken wire were placed over the plants. The inner cylinder prevented the plant from touching the insecticide-treated outer cage and had a diameter of 28 cm whereas the outer cylinder measured 38 cm in diameter. Both cylinders had a height of 45 cm (Fig. 11.1a). The outer cylinder was covered with 2 layers of green polypropylene net (type 44 R, Sarlon-Industries, 51 Moxan Rd, Punchball, Sydney)(Fig. 11.1b). The mesh size was 0.5 cm x 0.5 cm. Even though *A. rosae* could fly straight through the net, it was never observed to do so in preliminary

tests. The parasitoid always landed on the net before it passed through. In contrast, in a preliminary trial of a cylindrical sleeve cage with an open top, *A. rosae* was regularly observed to land on the plant without touching the gauze

The cage was fitted with a clear polyethylene square roof, 106 cm x 106 cm (Fig. 11.1a). To achieve stability the plastic roof was overlayed with chicken wire. Each corner of the chicken wire in the roof was turned down and connected to four wooden poles. Additional stability was achieved by four guy lines from the ground to the edge of the roof and through to the top of the outer cylinder.

Treated and untreated cages were used in the experiment. The outer cylinders of treated cages were dipped in an insecticide solution.

Insecticide. The insecticide used in this experiment had to fulfil a range of requirements. It had to be a persistent contact insecticide which still kills immediately after 20 days in the field. Pyrethroid based insecticides such as Permethrin or Deltamethrin seemed promising, but all were vulnerable to rain or dust. Since the first prototypes of cages had no roof, the weather resistance of the insecticide was an important factor. Cages treated with Maverick® (Sandoz Ltd.) did not kill wasps that landed on them fast enough. Borer, a wood protection product, (Rentokil Ltd., 554 Pacific Highway, Chatswood, N.S.W. 2067, Australia) (Active constituent :100 g /L Permethrin [25/75], solvent 668 g/L liquid hydrocarbons) was selected for use in experiments because it is formulated for dilution in diesel or oil. This combination is meant to soak into wood, but it also produces an oily film on non-porous surfaces and lighter compounds of the diesel can penetrate the surface of polypropylene products (P. Philp, Rentokil, pers. communication). The treatment mixture consisted of Borer : Diesel : Mineral oil 20W/40 in the ratio 1 : 7 : 2. This was equivalent to 10 times the recommended concentration of insecticide. To apply the solution, cages were rolled in a flat container filled with one litre of solution. After one day, excess fluid was shaken off and cages stored for six days before use. This allowed the solution to penetrate the net and the most volatile components of the diesel to evaporate.

In the first pilot experiment, cages were constructed without roofs but only netting on top. Most aphids inside the treated cages were dead after the first heavy rain, presumably because insecticide from the top dripped onto the roses. However, the performance of the Borer mixture before rain was good. Therefore, roofs that overlapped each cage about 10 cm were added to stop insecticide dripping on to the plants. However, most aphids inside the cages were dead after the first heavy rain, presumably because the rain caused splashing from the sides. This failure led to the larger roofs on the cages that were used in the experiment.

Set up. The experiment was conducted in a rose plot at the Waite Campus, Adelaide. The plot consisted of 101 rose bushes *Rosa sp.,* var. Tea Hybrid 'McGredy sunset' and 27 bushes of *Rosa sp.,* var. 'Indigo major' (Fig. 11.2)(for details see Chapter 10). Pots were placed into pre-dug holes in the ground, their edges levelled to the surface. This was thought to be important in cases where aphids dropped from the plant as it allowed movement away from the plant. Plants were surrounded by the inner cage cylinder which was dug slightly into the ground. Finally the outer cage cylinder with roof was placed over the inner cage and secured to the ground.

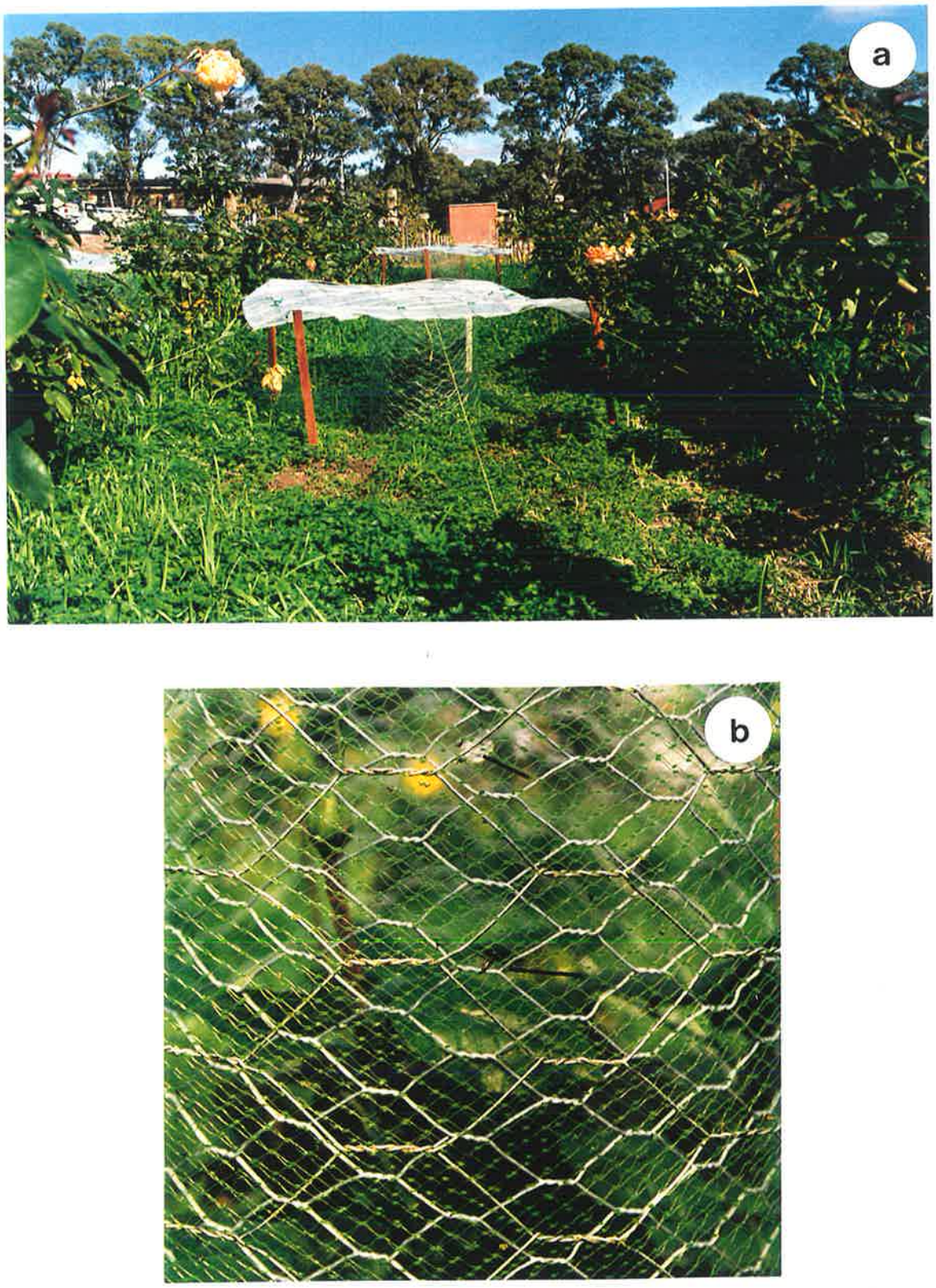


Fig. 11.1 Insect exclusion cage. **a)** Two cylinders with polypropylene net were placed around plants, height of cylinder 45 cm. The roof protected the insecticide film on the net from rain. **b)** The barrier consisted of insecticide-treated polypropylene net. Arrows show dead insects sticking to the net.

Fig. 11.2 The experimental rose plot at the Waite Campus, Adelaide. The plot consisted of 101 rose bushes *Rosa sp.,* var. Tea Hybrid 'McGredy's sunset' and 27 bushes of *Rosa sp.,* var. 'Indigo major'. 27 potted roses were placed into pre-dug holes in the ground and their edges were levelled to the surface. 18 plants were surrounded by cage cylinders, of which nine of them had been treated with insecticide. Potted plants were set up in six rows and were at least four meters apart.

Pots were checked and watered daily. The weeds in a 50 cm circle around the cages were cut back once.

The experiment was conducted in a completely randomised block design. Three uncaged rose plants, three rose plants each surrounded by untreated control cages and three rose plants each inside an insecticide treated cage, made up one block. Three blocks were set up in the field between the 25 April and 20 May 1995.

Conceptual model of experiment. The conceptual model of the experiment is shown in Fig. 11.3. Throughout the text, adults per plant plus the number of mummies per plant are referred to as 'potentially reproducing aphids'. Aphids which were initially set up on the plants are referred to as 'initial aphids'.

Collection of data. One day after the first offspring had moulted to the fourth instar in one of the treated cages only, the cages were taken down and aphids collected. Hence, only the initial aphids were responsible for reproduction in the treated cages (Fig. 11.3). Since plants without protection from insecticide were subject to aphid immigration, offspring from early immigrants on these plants could have reached adulthood much earlier than offspring of initial aphids. Thus the aphids on these plants could not be used to determine an equivalent ending time for the experiment.

The samples were taken in plastic bags to the laboratory and stored in a refrigerator at 4°C until the numbers of 1/2 instar, 3/4 instar, apterae, alatae and mummies per plant were counted. Adults only were reared for five days at 18°C, 16L:8D on rose shoots in cylindrical gauze cages (Fig. 3.1b), kept fresh by immersion in water supplemented with fertiliser for cut flowers (Flower Fresh™, Flower Fresh Products, Glengowrie, South Australia). After five days, mummies were counted and surviving adults dissected to search for parasitoid larvae (see Chapter 10 for details). Immature aphids were not reared since they did not contribute to reproduction and their rate of parasitism had no influence on the number of aphids on the plant at the time of sampling (Fig. 11.3)

The number of parasitized aphids in the group of potentially reproducing aphids per plant was then calculated as the sum of mummies found on the plant, the number of mummies formed during the rearing process and the corrected number of aphids containing larvae of *A. rosae* which was corrected for mortality (Chapter 10). The numbers of mummies on plants at the time of collection had to be added as well since the time requirement of *A. rosae* to form mummies is comparable to time requirements of *M. rosae* to reach adulthood (Chapter 4 and 5). Hence, mummies represented aphids which would have reached adulthood if they would not had been parasitized. This implies also that mummies on the plant did not represent any aphids of the F1 since under given circumstances the time from oviposition to mummification of *A. rosae* would have been longer than the developmental time of oldest F1 from birth to fourth instar (Fig. 11.3).

Outer cages were washed in soapy water to recover insects sticking on to the net. Specimens were counted only when clearly recognisable.

Analysis. The statistical analysis of results were undertaken with the SAS ANOVA procedure and the Student-Newman-Keuls test. Data were transformed ($\log_{10}[1+x]$) to stabilise variances.

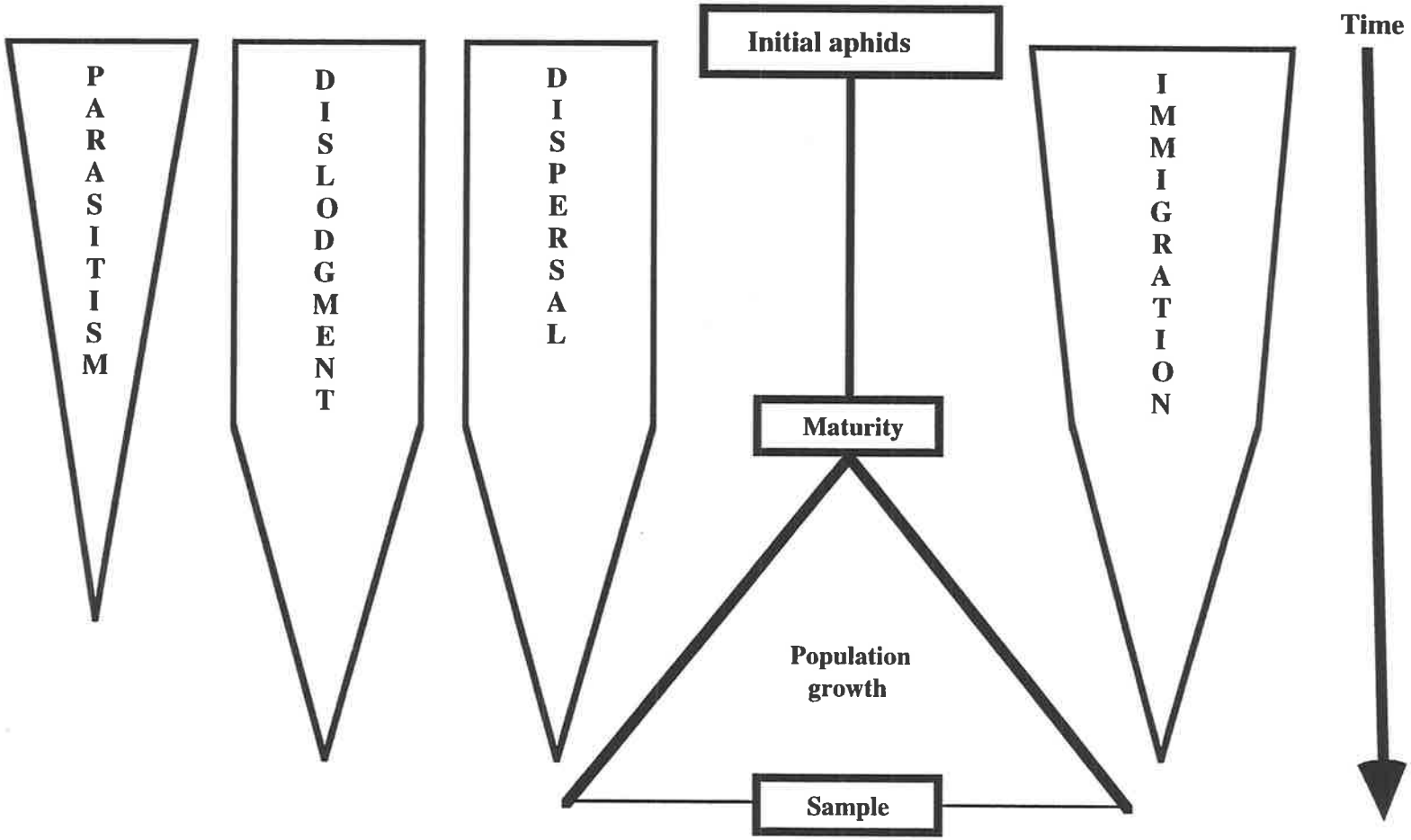
Correlation analysis of data was performed with the SAS CORR procedure.

Weather. Temperature data were obtained by taking the mean of intermittent recordings of daily minimum and maximum temperatures in °C from the Waite meteorological station.

Fig. 11.3 Conceptual model of the effects of positive and negative factors that influenced the numbers of *Macrosiphum rosae* in the field experiment. 50 second instars of *M. rosae* were set up on a rose plant at the beginning of the experiment. Without disturbance or mortality the numbers should have remained constant until aphids would have reached maturity and started to reproduce. From then on the population in each cage should have grown until the sample day, just before the F_1 would have reached maturity. Therefore, the experiment is meant to measure the survival and reproductive success of initial aphids.

During the time in the field, various factors could have affected the growth of a colony. In this figure the width of a column is proportional to the possible impact of a factor at any particular time. The width is *not* meant to be related to the frequency of occurrence of these factors. An immigrating adult aphid at the time when initial aphids would reach maturity, would contribute as many offspring as those already established. However, the earlier an aphid would have immigrated to the plant the greater would have been its contribution to population growth. Compared to initial aphids it would have produced offspring earlier. Before initial aphids would have reached maturity any event of dispersal or dislodgment (e.g. by rain) would have resulted in an instant decrease of reproductive females. The impact of such an event would not have changed over time. After initial aphids would have reached maturity any loss of a reproductive female would have been proportional to the time it would have had to reproduce. In contrast, the effect of parasitism would have been time delayed. Only very early parasitization would have prevented reproduction. Aphids parasitized later would have suffered reduced reproductive success but still would have been able to contribute to an increase in numbers. Direct impact would have been caused by parasitoids that would have induced dislodgment of attacked aphids. Parasitization of initial aphids three or four days before sample date would have had no effect on the number of total aphids.

The influences of predation and diseases are not shown since hardly any predators and no deseased aphids were found during the experiment.


PARASITISM
DISLODGMENT
DISPERSAL
Initial aphids
IMMIGRATION
Time
Maturity
Population growth
Sample
negative factors
positive factors

11.3 Results

No statistically significant differences were found in total numbers of aphids when the three different treatments were compared, ANOVA, $F_{2, 22} = 2.56$; $p = 0.1$ (Table 11.1 and Fig. 11.4). According to Maelzer's economic threshold of 50 aphids per shoot (pers. communication), pots could have tolerated between 150 and 250 aphids. The infestations in most pots exceeded this value three to six times.

Table 11.1 Influence of cage treatments on **total numbers of aphids per plant.** Treatments were plants in insecticide-treated cages, plants in untreated cages and uncaged plants. Treatments were replicated three times. Data were transformed ($\log_{10}[1+x]$) before analysis.

Source	DF	Anova SS	Mean Square	F-value	Pr < F
Treatment	2	0.21595	0.10798	2.56	0.1
Block (starting date)	2	0.02726	0.01363	0.32	0.7271
Error	22	0.9275	0.0422		

The insecticide treated poly net was an effective barrier for insects. Only one case of parasitism occurred in aphid colonies reared in nine treated cages compared to 516 parasitized adult aphids on the 18 unprotected plants. Significantly more cases of parasitism occurred on uncaged plants than on plants in control cages, ANOVA, $F_{2,22} = 98.2$, $p < 0.0001$ (Table 11.2)(Fig. 11.5).

Table 11.2 Influence of cage treatments on **total numbers of parasitized adult aphids and mummies per plant.** Treatments were plants in insecticide-treated cages, plants in untreated cages and uncaged plants. Treatments were replicated three times. Data were transformed ($\log_{10}[1+x]$) before analysis.

Source	DF	Anova SS	Mean Square	F-value	Pr < F
Treatment	2	11.40725	5.70362	98.2	0.0001
Block (starting date)		0.19269	0.09635	1.66	0.2133
Error	22	1.2778	0.058		

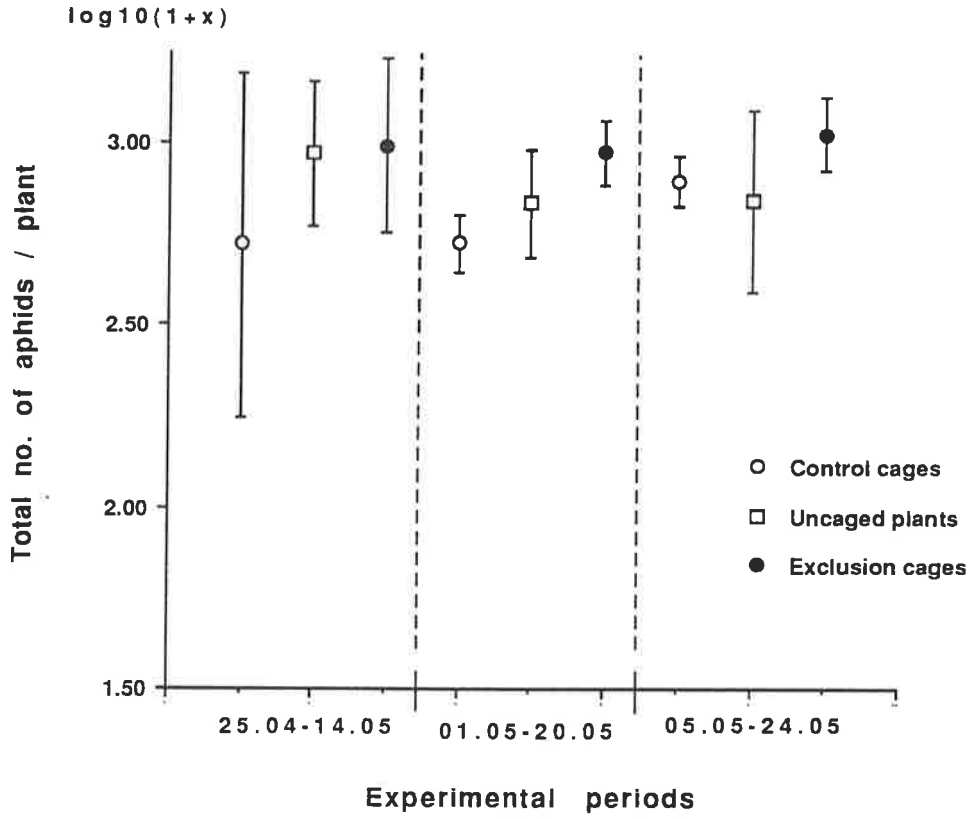


Fig. 11.4 Total numbers of *Macrosiphum rosae* per rose plant in a cage exclusion experiment. Exclusion of natural enemies was achieved by using insecticide-treated net cages. N for each data point = 3. No significant differences were found among treatments (ANOVA, $F_{2,22} = 2.56$; $p = 0.1$). Error bars show SD.

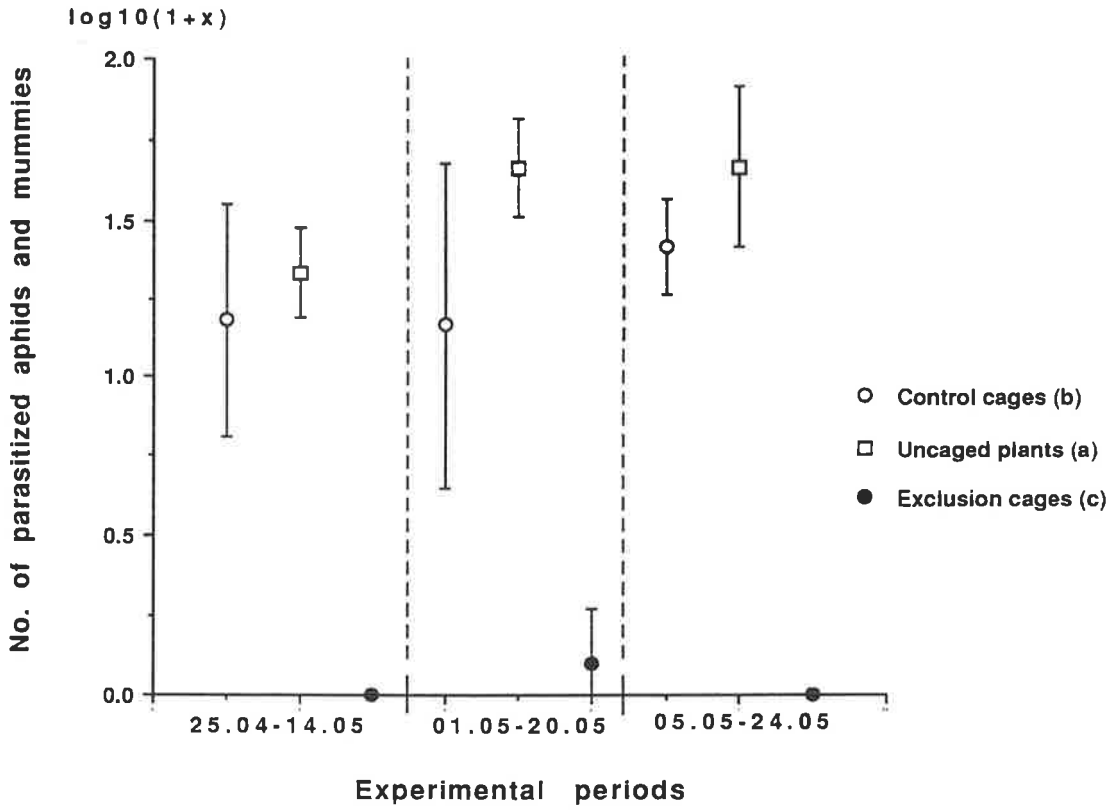


Fig. 11.5 Numbers of parasitized adult *Macrosiphum rosae* and mummies of *Aphidius rosae* in a cage exclusion experiment. Exclusion of *A. rosae* was achieved by using insecticide-treated net cages. N for each data point = 3. Treatments followed by different letters are significant different (Student-Newman-Keuls test, $p < 0.05$). Error bars show SD.

Significantly more potentially reproductive aphids were found on uncaged roses than on roses in control cages and treated cages, in that order ANOVA, $F_{2,22} = 11.28$, $p = 0.0004$ (Table 11.3) (Fig 11.6a).

Table 11.3 Influence of cage treatments on **total number of adults and mummies per plant**. Treatments were plants in insecticide-treated cages, plants in untreated cages and uncaged plants. Treatments were replicated three times. Data were transformed ($log_{10}[1+x]$) before analysis.

Source	DF	Anova SS	Mean Square	F-value	Pr < F
Treatment	2	1.21807	0.60903	11.28	0.0004
Block (starting date)	2	0.31406	0.15703	2.91	0.0757
Error	22	1.1879	0.054		

Significant differences were found in the number of alatae per plant, ANOVA, $F_{2,22} = 9.3$, $p = 0.0012$ (Table 11.4). On uncaged plants, more alatae rose aphids were found than on caged plants (Fig. 11.6b).

Table 11.4 Influence of cage treatments on **number of alatae per plant.** Treatments were plants in insecticide-treated cages, plants in untreated cages and uncaged plants. Treatments were replicated three times. Data were transformed ($log_{10}[1+x]$) before analysis.

Source	DF	Anova SS	Mean Square	F-value	Pr < F
Treatment	2	1.57834	0.78918	9.3	0.0012
Block (starting date)	2	0.32166	0.16083	1.9	0.174
Error	22	1.8665	0.0848		

Significant differences occurred in the number of immature aphids per potentially reproductive female, ANOVA, $F_{2,22} = 26.87$, $p = 0.0001$ (Table 11.5). The numbers in treated cages were significantly different from the numbers in control cages and uncaged roses, which showed no significant differences (Fig. 11.7). In this comparison, Block 2 showed significant differences from Blocks 1 and 3 (Student-Newman-Keuls test, $p < 0.05$).

Table 11.5 Influence of cage treatments on **total number of immature aphids per adult (including mummies)**. Treatments were plants in insecticide-treated cages, plants in untreated cages and uncaged plants. Treatments were replicated three times. Data were transformed ($\log_{10}[1+x]$) before analysis.

Source	DF	Anova SS	Mean Square	F-value	Pr > F
Treatment	2	2.01884	1.00942	26.87	0.0001
Block (starting date)	2	0.51021	0.25511	6.79	0.005
Error	22	0.8264	0.0376		

No correlation was found between rate of parasitism and number of aphids per plant when treated cages were excluded from the analysis, $r = 0.068$, $p = 0.788$.

Appendix 8 shows the complete data set.

147 males and 48 females of *A. rosae* were recovered from the insecticide-treated net. Also, 15 apterae and 49 alatae of *M. rosae* as well as one lacewing, *M. tasmaniae*, stuck on the net. The majority of insects were Diptera. One individual of *Harmonia conformis* (Boisd.) and 104 millipedes of *Ommatoiulus moreletii* Lucas, were found on the ground near the treated cages.

The weather conditions varied during the exposure of plants in the field but due to the long, overlapping experimental periods among blocks, average temperatures were similar (Appendix 9). Pots were exposed to an average temperature of 16.2 °C in the first block, 15.9 °C in the second and 15.7 °C in the third block.

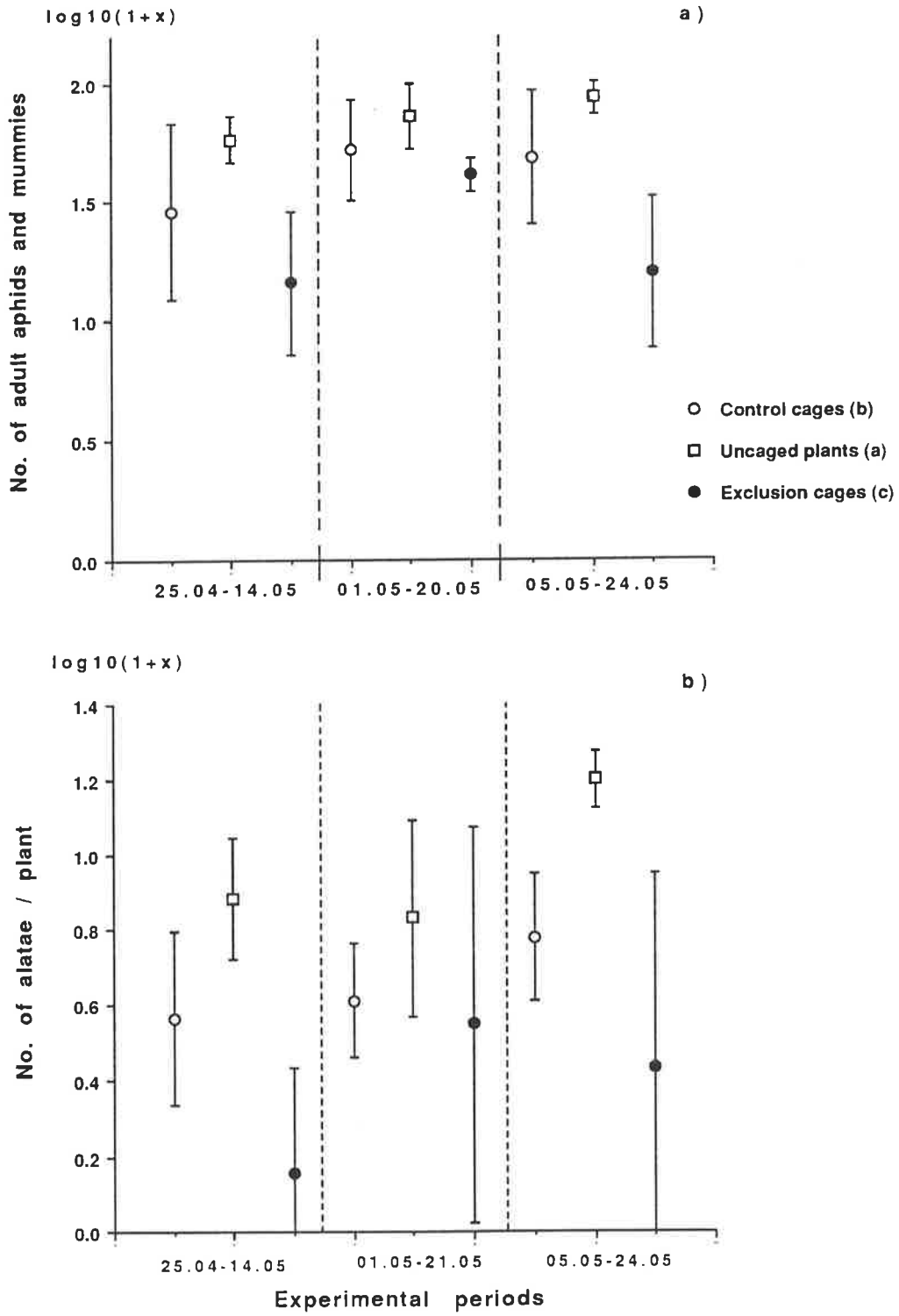


Fig. 11.6 **a)** Numbers of adult *Macrosiphum rosae* and mummies of *Aphidius rosae* that would have become adult per rose plant in a cage exclusion experiment. Exclusion of *A. rosae* was achieved by using insecticide-treated wide meshed net cages. N for each data point = 3. Treatments followed by different letters are significant different (Student-Newman-Keuls test, $p < 0.05$). **b)** Numbers of alatae rose aphids on plants (Student-Newman-Keuls test, $p < 0.05$). Error bars show SD.

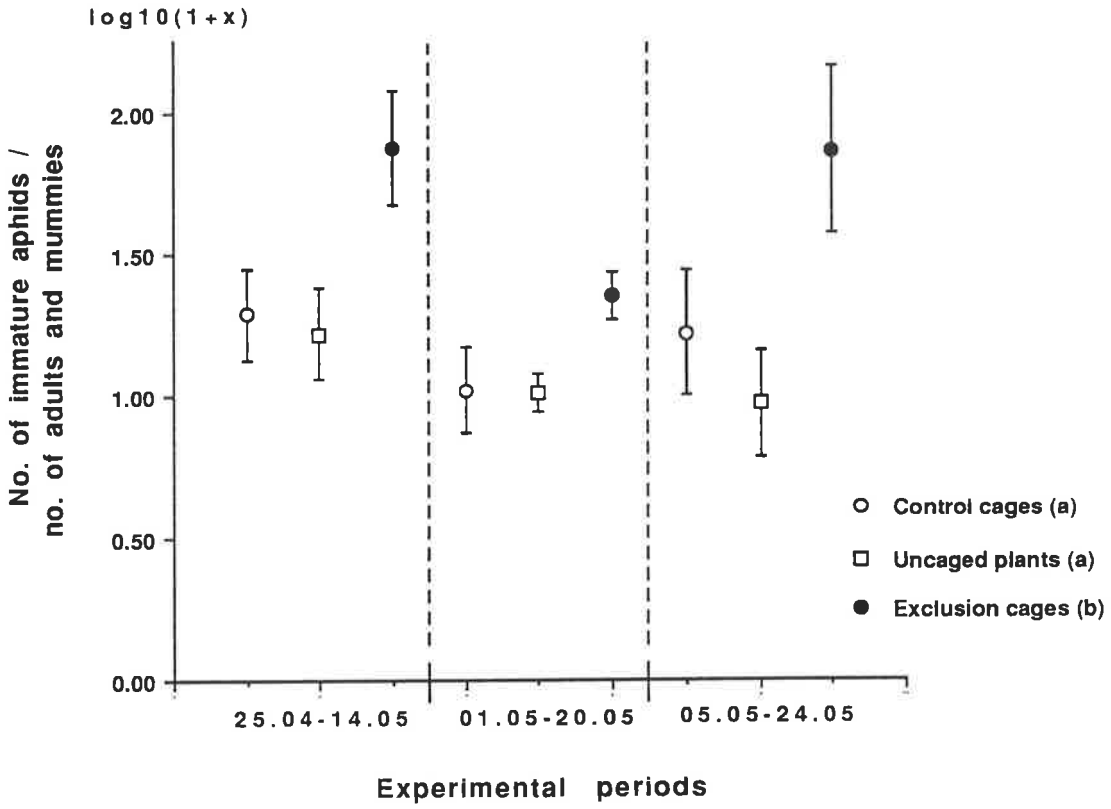


Fig. 11.7 Numbers of offspring of *Macrosiphum rosae* per adult (including mummies that would have become adults) in a cage exclusion experiment. Exclusion of *Aphidius rosae* was achieved by using insecticide-treated net cages. N for each data point = 3. Treatments followed by different letters are significantly different (Student-Newman-Keuls test, $p < 0.05$). Error bars show SD.

11.4 Discussion

Suitability of cages. The insecticide treated net proved to be an effective barrier for *A. rosae*, immigrating aphids and presumably also for predators. Inside nine treated cages only one case of parasitism occurred, compared to 516 in adult aphids on 18 potted plants that were not in treated cages, not taking into account parasitism of younger instars and the undetected numbers of parasitized adult aphids which might have left the plant to mummify. It is unlikely that a single mummy, found in a treated cage resulted from a wasp crossing the barrier. If a parasitoid successfully would have crossed the net during the experiment, more parasitism would be expected. Even though great care was taken during the set up of the experiment, it is possible that oviposition occurred in the short time when pots were set up in the field and lacked cage protection.

The finding of 195 *A. rosae* sticking to the net revealed that the parasitoids regularly tried to cross the net. This number is expected to under-represent the real number of landing parasitoids. Not every wasp stuck to the net. Some might have fallen off and died on the ground as observed in one instance. Furthermore, the washing procedure of cages did not remove all insects and many others were destroyed.

During the exposure of potted plants in the field, predator activity presumably was very low. Only one dead lacewing of *M. tasmaniae* was found sticking to the net and one individual of *H. conformis* was recovered on the ground. These results confirm the findings in Chapter 10 that predator activities in the experimental plot were low. 104 dead millipedes demonstrated the effectiveness of the nets around their base.

In comparison, in an earlier pilot experiment undertaken at a different location in spring, 34 dead individuals of *H. conformis* were found around nine cages after three days.

Three times more males than females of *A. rosae* were found on the cages but at the same time the sex-ratio of *A. rosae* was around 0.4 (Chapter 10). It is possible that cages had a more repellent effect on females, but it is more likely that males were more

mobile in their search for mates than females which tended to stay for prolonged periods close to suitable colonies (Chapter 7).

The number of 64 individuals of *M. rosae* recovered on the net was low, compared to the numbers of *A. rosae*. However, it is difficult to interpret this figure for the reasons mentioned above.

Cages had a negative effect on immigrating rose aphids. Consequently, more alatae, the principal migrating morph of aphids, were found on uncaged plants than on plants in untreated cages and on roses in treated cages, in that order (Fig. 11.6b). The roofs of cages were on a level 45 cm above the ground whereas the average level of rosebuds in the field was about 65 cm (Fig. 11.1a). This structural hurdle could have restricted approaches of flying aphids to caged plants. The number of potentially reproducing aphids was lowest on plants in treated cages followed by caged controls and uncaged plants (Fig. 11.6a). In treated cages the number of adults never exceeded fifty, the number of initial aphids. The adult cohorts suffered losses only (e.g. possible emigration or losses through interaction of wind shaken shoots or leaves with the inner cage) but had no possibility of recruitment. In untreated cages and on uncaged roses the number of adults exceeded more often than not the initial number (Appendix 8), showing that immigration of aphids took place. Not necessarily all the additional potentially reproducing aphids must have been immigrants. Matured offspring of early migrants would have been counted as well (Fig. 11.3).

Uncaged plants also carried more mummies and parasitized aphids than caged controls, suggesting that wasps were more frequently on uncaged plants (Fig. 11.5). They also may have been restricted by the cage structure.

Assessment of impact of A. rosae. The total number of aphids per plant was highest on plants inside treated cages but the small sample size compared to standard deviation of data did not allow detection of significant differences (Fig. 11.4). However, when the number of first to fourth instar aphids were divided by the numbers of potentially reproducing aphids, aphids in treated cages revealed the highest ratio (Fig. 11.7). These

findings suggest that *A. rosae* had an impact on aphid colonies in the field, but this effect was masked by immigrating aphids. Immigrating aphids and their offspring added to the total number of aphids on the plants but because of the activity of *A. rosae,* aphids were not able to display their fully reproductive capacity. In contrast, aphids in exclusion cages were not parasitized and were able to produce more offspring per individual.

At time of the experiments, the rose plot was heavily infested by *M. rosae* with infestation levels of around 90 aphids per infested shoot (Chapter 10, sample dates 26 April and 11 May 1995) and around 90 % of all buds infested. 72 - 90 % of fourth instars were the apterous form and dispersing aphids were dominant. Small colonies of rose aphids were even found on young shoots of river red gum *Eucalyptus camaldulensis* Dehn. (Chapter 10). Under these circumstances, migrants to the unprotected rose plants might have become more important for the increase in numbers of aphids than the initial aphids (Fig. 11.3). The relatively long exposure of young initial aphids before the start of their reproductive activities makes the experimental analysis vulnerable to early immigration.

Under the circumstances in the field, the findings showed that the parasitoids were not able to prevent an increase of aphid numbers to damaging levels. Nevertheless, the results suggested also that *A. rosae* helped to suppress the reproductive capacity of aphid colonies.

Method. It is most desirable to investigate the impact of natural enemies when pest densities are low in the field, rather than when pest levels are high and the impact of natural enemies is obviously low and hard to detect. However, because of time limitations of the project, the main aim of this part of the study was to demonstrate an appropriate method for assessing the impact of *A. rosae* and predators on *M. rosae*.

The proposed method produced meaningful results when used together with a sample program for changes of numbers of pest and enemies in the surrounding rose plot. Both approaches support each other whereas the use of only one of them gives only limited data for interpretation. E.g., the experimental approach suggested that wasps

were not responsible for the decline of aphid numbers in the plot observed in the sample program. Additionally, the observed high number of dispersing aphids observed in the sample program gave insight into the results of the cage experiment.

The density-dependent production of dispersing rose aphids is seasonally restricted. Therefore, this factor will have no masking effect on the outcome of the experiment under most circumstances in the field. It is assumed that the proposed method can reveal the impact of natural enemies on *M. rosae* better when non-immigratory aphids are dominant in the field.

The proposed method only measures the impact of natural enemies on one parental generation and their reproductive success. Therefore, results are valuable only for a short defined period in time. Consequently, the proposed method has to be applied in regular intervals during the periods of main abundance of *M. rosae* in the field.

Improvements. The cage method was not perfect and some improvements are suggested for future trials.

The cage cylinders should be wider (≈ 60 cm diameter) so that mechanical interaction between plant and cage cylinder is not possible. This would allow ellimination of inner cages, but would require an increased roof area.

The height of roofs should be better adapted to the height of rose shoots in the surrounding plot. The plastic roof could be made of greenhouse films (e.g. Klerk's© Greenhouse Films) to ensure optimal growth of the plants. Since the poly net is protected from rain, other surface insecticide formulas could be tested, e.g. PERIGEN 500 or CISLIN from Coopex. The mixture that was used is very unpleasant to work with and environmentally hazardous. So far it was used only at cooler temperatures up to a maximum of 24°C. An effect on insects inside the cage could be possible on hotter days.

Washing cages to sample trapped insects turned out to be very inadequate. The collection of insects by sub sampling with forceps straight from the net could be a better method.

The experimental design could be improved by adding additional aphid free plants in the field to get a better indication of net immigration. The high standard deviation of data demands an increase of n. However, for one person it is not practical to set up more than 18 cages per day.

The experiment was carried out at a time of high aphid and parasitoid abundance. At times of low aphid abundance, when the impact of parasitoids would be most interesting, parasitoids are supposed to fly more actively between bushes to search for hosts whereas aphids do not. Therefore, the rose plot should be big enough to avoid significant interference of deadly treated cage 'traps' with a presumably small density of searching parasitoids in the field.

Conclusion

The main aims of this study were to establish *Aphidius rosae* Haliday in South-Eastern Australia, to monitor the performance of the control agent for the first two years after initial release and to assess its impact on the target species *Macrosiphum rosae* (L.)(Chapter 1). Investigations on life-history parameters and searching behaviour were undertaken to obtain a basic understanding of the interaction of these two species in the field.

Host-specificity. A. rosae can be considered specific to *M. rosae* under given circumstances in Australia and negative environmental impacts are considered as almost non-existent (Chapter 5). Investigations on discrete steps in the process of host selection by *A. rosae* suggest that the parasitoid is attracted to roses over longer distances but that aphid-related volatiles involved in host location are detectable by the wasp only over a short distance. These results indicate that it is very unlikely that *A. rosae* will encounter most aphid species occurring in Australia. This specificity of *A. rosae* is desirable since it is expected that the parasitoid concentrates its activity on the target pest and does not waste large numbers of eggs by ovipositing in unsuitable hosts.

Suitability of host instars. A. rosae is able to exploit the whole range of instars and adults of *M. rosae* but growth and rate of increase of the parasitoid is greatly susceptible to changes in nutritional quality of its host (Chapter 4 and 5). The ability of *A. rosae* to parasitize all host stages allows the parasitoid to react flexibly to changing host situations in the field. Most mummies found in the field reveal that oviposition must have taken place in younger instars of the host (Chapter 10). These host stages are killed

before they reach reproductive age, a fact which enhances the biological control capacity of the wasp.

Establishment. A. rosae established in most places released, even though release numbers were relatively low (Chapter 8 and 9). This may be explained in several ways. Firstly, the pattern of distribution observed in Adelaide suggest that most individuals of *A. rosae* must have moved in a quite controlled manner in their garden habitats, exploiting abundant hosts and avoiding unwanted displacement. This is also indicated in Chapter 7 where long patch residential-times were observed, resulting in many ovipositions per aphid colony. The generally clumped distribution of aphids resulted in an aggregation of parasitoids. At times of low parasitoid density this may help to overcome the crucial aspect of mate finding. A high reproductive rate (Chapter 4 and 5) and a female-biased sex-ratio of approximately 0.4 (Chapter 3 and 10) enables *A. rosae* to increase rapidly in numbers when host colonies are encountered. The results from the release of *A. rosae* as well as examples from the literature give further indication that parasitoids of homopteran pests in general do not necessarily need to be released in high numbers to achieve establishment (Chapter 8).

Assessment of impact. Before the release of *A. rosae*, spring time was the season of main abundance of *M. rosae*. After the release, *M. rosae* was not able to reach high infestations levels in the intensively surveyed rose plot during spring. High rates of parasitism in spring suggest that *A. rosae* may have been a key factor in reducing aphid numbers. At this time of the year the parasitoid was well synchronised with its host since parasitoids were active in the field when aphid numbers started to build up. Additionally, high aphid numbers in autumn resulted in high numbers of diapausing mummies. These diapausing individuals could have increased the rate of parasitism in the following spring.

During summer *M. rosae* and *A. rosae* virtually disappeared from roses in Adelaide.

In autumn, *A. rosae* did not affect population growth of rose aphids (Chapter 10 and 11). The numbers of aphids increased until the carrying capacity of rose plants was reached. Self-regulatory density-dependent mechanisms of *M. rosae* took place and towards the end of the season a decrease in available rose shoots reduced the numbers of aphids. Relatively low numbers of diapausing individuals which carried over from spring to autumn and/or poor synchronisation of *A. rosae* with its host could have delayed the response of *A. rosae* to aphid increase in autumn. By the time parasitoid numbers had built up, aphid colonies were already dense. Because of avoidance of aphid defence (Chapter 5 and 7) *A. rosae* parasitized aphids at the edges of the colonies and had only low, if any impact, on *M. rosae*. Higher proportions of *A. rosae* entered diapause throughout the autumn, compared to spring. Towards the end of the season, increasing hyperparasitism may have contributed to the ineffectiveness of *A. rosae* as well.

Aphid infestations in different rose plots varied considerable. However the patterns of population dynamics of *M. rosae* and *A. rosae* in the Adelaide region, observed in this study were consistent with the known phenology of *M. rosae*.

Since release, the wasp has displayed qualities of an effective classical biological control agent : 1) despite small release numbers it became established easily and is abundant in enormous numbers in wide areas around the release points. 2) It has spread over more than 200 km^2 in the Adelaide region in less than seven months. 3) It is present as adult in late winter, a short time before the build up of host aphid populations. 4) Its potential for increase is at least comparable with that of its host.

The initial establishment of *A. rosae* in South-Eastern Australia is confirmed and chances for permanent establishment are high.

Future investigations. The population dynamics of introduced biological control agents and their target pests should be monitored for several years after initial release. Hence, it would be desirable to monitor the performance of *A. rosae* and *M. rosae* in future years to see if the observed pattern of population dynamics will be consistent. It also would be desirable to undertake a series of experiments (as demonstrated in Chapter 11) to assess the impact of *A. rosae* on *M. rosae* at different times of the season. Since *A. rosae* did not control aphid numbers in autumn, experiments should be undertaken to address the causes, e.g. lacking synchronisation with host. It would be highly desirable to investigate the impact of augmentative releases of *A. rosae* in early autumn. Finally, a survey in South-Eastern Australia should be undertaken to monitor the progress in spread.

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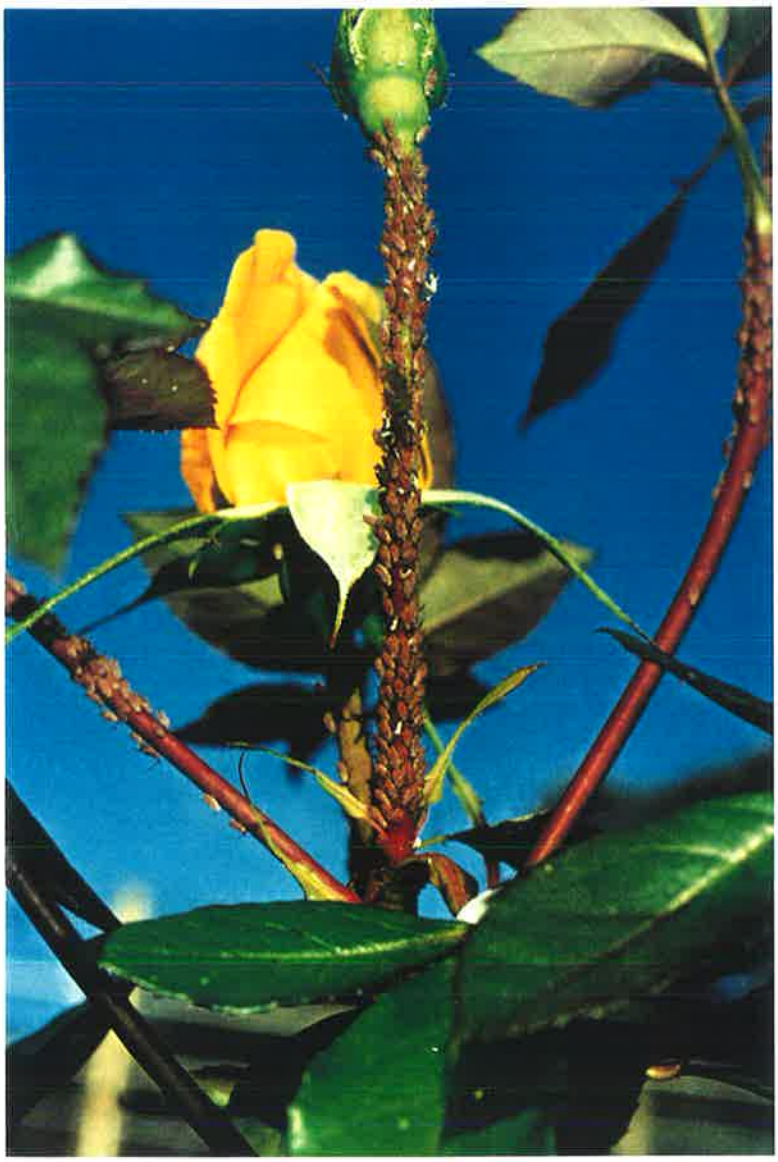


Appendix 1 *Rosa spec.*, Tea Hybrid 'McGredy's sunset'. The bud in the front represents a favourable bud stage for growth of a colony of *Macrosiphum rose* (bud stage 4, Maelzer, 1977). The bud in the back turned just unfavourable for aphids, indicated by the open bloom. On this bud only few aphids are left from the previously existing colony.

Appendix 2 Individual net reproductive rates (R_O) and intrinsic rates of increase (r) of *Aphidius rosae* depending upon female size, measured by hind tibia length. Wasps parasitized *Macrosiphum rosae* in the laboratory at 18 ± 2°C (80 third instars offered per day, results are based on gross fecundity, sex-ratio for calculation of r was 0.4).

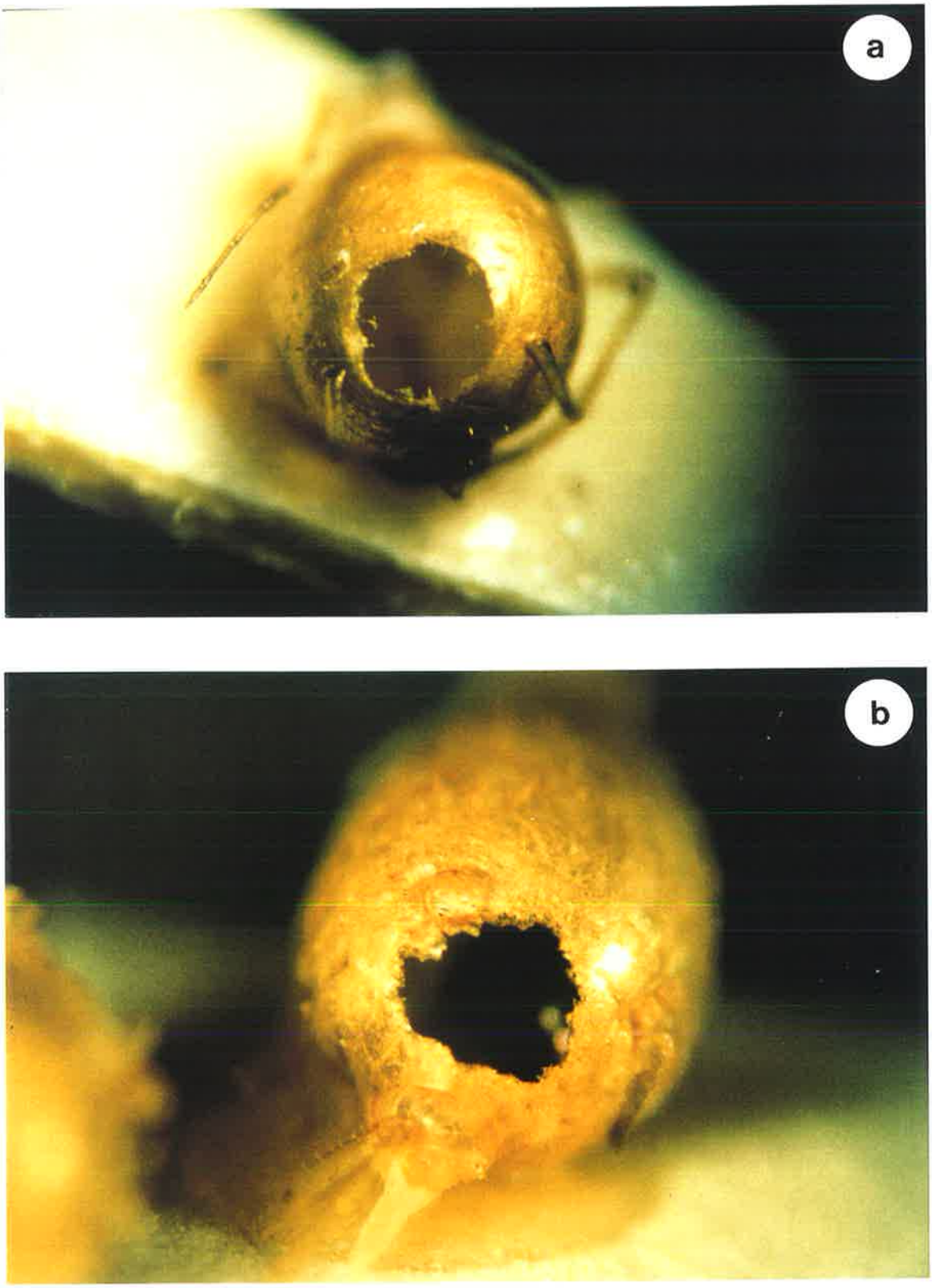
length of hind tibia (mm)	R_O 3rd day	r 3rd day	R_O 6th day	r 6th day	R_O life-time	r life-time
0.49	56	0.227	110	0.249	205	0.258
0.52	62	0.236	138	0.262	144	0.263
0.54	116	0.273	206	0.290	320	0.294
0.55	78	0.251	166	0.275	322	0.280
0.55	76	0.253	142	0.258	306	0.277
0.56	72	0.244	168	0.271	296	0.277
0.6	98	0.263	222	0.287	402	0.293
0.6	118	0.271	206	0.287	348	0.293
0.62	98	0.255	240	0.284	426	0.291
0.62	78	0.253	139	0.271	343	0.278
0.62	134	0.280	256	0.299	524	0.304
0.63	102	0.265	167	0.276	301	0.283
0.68	106	0.271	276	0.298	652	0.305
0.69	148	0.282	316	0.307	524	0.312
0.69	162	0.292	282	0.307	536	0.313
0.74	160	0.289	321	0.309	573	0.313
0.76	188	0.300	344	0.317	790	0.323
0.79	112	0.274	174	0.285	-*	-*
0.79	180	0.298	312	0.313	-*	-*
0.81	130	0.276	-*	-*	-*	-*

* Individuals were affected by cornicle wax of *M. rosae* and suffered early mortality or lost ability to oviposit.

Appendix 3 Population sizes and data on the spring climate of cities used for the release of *Aphidius rosae* in Victoria.

Town (Population)		Temperature, Max. (°C)				Temperature, Min. (°C)				Monthly Rainfall (mm)				Raindays			
		Sep	Oct	Nov	Jan	Sep	Oct	Nov	Jan	Sep	Oct	Nov	Jan	Sep	Oct	Nov	Jan
Hamilton	(10000)	16	18	21	26	6	8	9	12	72	66	51	33	17	15	12	8
Colac	(9500)	16	19	21	26	6	7	9	12	83	91	62	41	19	18	15	10
Seymour	(11400)	16	21	24	30	5	7	9	13	57	55	45	34	10	9	6	4
Stawell	(6200)	16	19	22	28	6	8	9	13	61	53	49	36	11	9	6	6
Horsham	(12200)	17	21	24	30	6	8	10	14	46	44	34	22	12	10	7	4
Ararat	(8200)	14	18	21	26	5	6	8	11	67	67	47	42	16	12	11	7
Maryborough	(7700)	15	20	21	29	5	7	8	13	58	58	44	33	13	10	9	6
Castlemaine	(6600)	16	20	23	28	5	7	9	13	60	62	46	40	14	11	9	6

Data were obtained from Hugo (1986) and the Bureau of Meteorology (1988).



Appendix 4 The emergence holes in mummies of *Macrosiphum rosae*, cut out by **a)** *Aphidius rosae* and **b)** a hyperparasitoid. *A. rosae* leaves always smooth more or less circular edges whereas the edges produced by hyperparasitoids appear jagged.

Appendix 5 Total numbers of *Macrosiphum rosae* and detected total parasitism by *Aphidius rosae* during a field survey on *Rosa sp.*, variety Tea hybrid 'McGredy's sunset' in Adelaide, South Australia.

a) October 1993 to May 1994

Sample date	Sample size (shoots)	L1/L2 instars of *M. rosae*	para-sitized	% para-sitism	L3/L4 instars of *M. rosae*	para-sitized	% para-sitism	Adults of *M. rosae*	para-sitized	% para-sitism
7 October 1993	20	137	16	**11.7**	27	6	**22.2**	42	0	**0**
14 October	20	344	75	**21.8**	155	80	**51.6**	65	15	**23.1**
21 October	20	132	35	**26.5**	44	30	**68.2**	21	7	**33.3**
2 November	20	198	76	**38.4**	29	19	**65.5**	35	20	**57.1**
10 November	20	135	27	**20.0**	12	6	**50.0**	26	7	**26.9**
18 November	14	95	6	**6.3**	9	0	**0**	7	0	**0**
7 April 1994	9	244	21	**8.6**	129	42	**35.6**	114	18	**15.8**
13 April	17	884	75	**8.5**	272	128	**47.1**	104	20	**19.2**
24 April	18	306	10	**3.3**	440	118	**26.8**	128	29	**22.7**
3 May	20	1025	122	**11.9**	1101	218	**19.8**	148	26	**17.6**
13 May	20	881	104	**11.8**	638	267	**41.8**	143	47	**32.9**
23 May	20	485	56	**11.5**	1602	272	**17.0**	101	19	**18.8**

b) August 1994 to June 1995

Sample date	Sample size (shoots)	L1/L2 instars of *M. rosae*	para-sitized	% para-sitism	L3/L4 instars of *M. rosae*	para-sitized	% para-sitism	Adults of *M. rosae*	para-sitized	% para-sitism
8 August 1994	8	42	0	**0**	22	0	**0**	4	0	**0**
24 August	10	20	12	**60.0**	16	10	**62.5**	4	2	**50.0**
6 September	20	271	91	**33.6**	658	332	**50.5**	29	19	**65.5**
20 September	5	99	31	**31.3**	125	70	**56.0**	11	2	**18.2**
4 October	20	92	45	**48.9**	146	107	**73.3**	5	2	**40.0**
20 October	20	278	81	**29.1**	229	81	**35.4**	54	9	**16.7**
29 March 1995	3	12	0	**0**	5	0	**0**	4	0	**0**
12 April	19	414	36	**8.7**	401	44	**11.0**	103	11	**10.7**
26 April	20	1238	92	**7.4**	1900	291	**15.3**	277	40	**14.4**
11 May	20	564	69	**12.2**	1663	230	**13.8**	262	21	**8.0**
15 June	20	96	29	**30.2**	253	127	**50.2**	43	14	**32.6**

Appendix 6 Selected data from a field survey on population dynamics of *Aphidius rosae* and *Macrosiphum rosae* in a rose plot consisting of 86 bushes of *Rosa spec.*, Tea Hybrid 'McGredy's sunset'. All data shown are discussed in Chapter 10 and of relevance for the sample procedure and some figures in Chapter 10. Data represent means ± SE of the mean, except of data for favourable shoots and infested shoots which show the means ± SD.

a) 1993/94

Sample date	Cumulative days from 1 August	Favourable shoots /plant n = 20	Infested shoots /plant n = 20	Sample size of shoots	No. of aphids per shoot $\log_{10}(1+x)$	Sample accuracy for aphids	No. of shoots with 3rd /4th aphid instars	No. of 3rd/4th aphid instar per shoot $\log_{10}(1+x)$	No. of parasitoid larvae in 3rd/4th aphid instar per shoot $\log_{10}(1+x)$	Survival rate of 3rd /4th aphid instars after a 5 days rearing period	Sample accuracy for parasitoids
7 Oct	68	18.3 ± 4.3	4.8 ± 2.0	20	0.96 ± 0.09	0.12	7	0.48 ± 0.1	0.11 ± 0.07	0.62 ± 0.17	0.65
14 Oct	75	23.9 ± 2.8	6.6 ± 1.3	20	1.16 ± 0.15	0.11	10	0.84 ± 0.15	0.60 ± 0.15	0.88 ± 0.05	0.24
21 Oct	82	24.9 ± 2.2	6.3 ± 1.1	20	0.85 ± 0.09	0.11	11	0.60 ± 0.09	0.46 ± 0.09	0.95 ± 0.03	0.18
2 Nov	94	35.7 ± 5.6	4.7 ± 1.2	20	0.91 ± 0.14	0.13	6	0.57 ± 0.07	0.45 ± 0.13	0.89 ± 0.05	0.42
10 Nov	102	38.8 ± 6.1	5.1 ± 1.4	20	0.83 ± 0.09	0.1	6	0.39 ± 0.09	0.22 ± 0.11	0.97 ± 0.03	0.75
18 Nov	110	21.6 ± 4.8	1.6 ± 0.7	14	0.53 ± 0.27	0.24	3	0.48 ± 0.0	0	-	-
29 Nov	121	3.5 ± 1.4	0.2 ± 0.1	0	-	-	0	-	-	-	-
27 Dec	149	24.3 ± 3.9	0	0	-	-	0	-	-	-	-
17 Jan	170	20.7 ± 2.7	0	0	-	-	0	-	-	-	-
1 Feb	185	5.3 ± 2.2	0	0	-	-	0	-	-	-	-
11 Feb	195	0.2 ± 0.2	0	0	-	-	0	-	-	-	-
23 Feb	207	1.9 ± 1.3	0	0	-	-	0	-	-	-	-
3 Mar	215	0.8 ± 0.7	0	0	-	-	0	-	-	-	-
9 Mar	221	6.4 ± 2.1	0.1 ± 0.1	0	-	-	0	-	-	-	-
23 Mar	235	3.2 ± 1.0	0	0	-	-	0	-	-	-	-
7 Apr	250	4.2 ± 1.4	1.1 ± 0.6	9	1.68 ± 0.1	0.05	7	1.07 ± 0.13	0.73 ± 0.07	0.76 ± 0.07	0.09
13 Apr	256	5.4 ± 1.2	2.0 ± 0.6	17	1.75 ± 0.13	0.05	6	1.22 ± 0.04	0.91 ± 0.05	0.84 ± 0.13	0.08
24 Apr	267	7.5 ± 1.8	2.1 ± 0.5	18	1.45 ± 0.11	0.07	12	1.21 ± 0.11	0.85 ± 0.07	0.81 ± 0.05	0.08
3 May	276	6.4 ± 2.3	4.9 ± 2.1	20	1.82 ± 0.13	0.07	18	1.48 ± 0.07	0.91 ± 0.11	0.78 ± 0.05	0.12
13 May	286	7.6 ± 2.1	7.5 ± 0.9	20	1.59 ± 0.15	0.09	15	1.36 ± 0.13	1.06 ± 0.08	0.84 ± 0.05	0.08
23 May	296	2.7 ± 0.9	2.3 ± 2.1	20	0.13 ± 0.13	0.07	18	1.69 ± 0.14	1.10 ± 0.06	0.81 ± 0.05	0.06

b) 1994/95

Sample date	Cumulative days from 1 August	Favourable shoots /plant n = 20	Infested shoots /plant n = 20	Sample size of shoots	No. of aphids per shoot $\log_{10}$ (1+x)	Sample accuracy for aphids	No. of shoots with 3rd /4th aphid instars	No. of 3rd/4th aphid instar per shoot $\log_{10}$ (1+x)	No. of parasitoid larvae in 3rd/4th aphid instar per shoot $\log_{10}$ (1+x)	Survival rate of 3rd /4th aphid instars after a 5 days rearing period	Sample accuracy for parasitoids
8 Aug	8	8.1 ± 1.6	0.6 ± 0.2	8	0.65 ± 0.20	0.32	2	0.63 ± 0.21	0	-	-
24 Aug	24	7.9 ± 1.4	1.2 ± 0.3	10	0.33 ± 0.20	0.21	2	0.78 ± 0.18	0.63 ± 0.15	0.71 ± 0.04	0.24
6 Sep	37	7.9 ± 1.8	4.5 ± 0.7	20	0.60 ± 0.23	0.12	4	1.6 ± 0.39	1.36 ± 0.36	0.80 ± 0.04	0.26
20 Sep	52	10.3 ± 2.0	0.6 ± 0.2	5	1.10 ± 0.16	0.33	4	1.24 ± 0.17	1.15 ± 0.14	0.87 ± 0.04	0.13
4 Oct	65	19.2 ± 2.0	6.2 ± 1.2	20	0.87 ± 0.10	0.14	20	0.87 ± 0.08	0.76 ± 0.07	0.86 ± 0.04	0.13
20 Oct	82	9.1 ± 1.4	2.7 ± 0.7	20	1.09 ± 0.15	0.12	13	0.94 ± 0.13	0.66 ± 0.11	0.71 ± 0.07	0.1
16 Nov	109	13.4 ± 1.5	0	-	-	-	-	-	-	-	-
25 Nov	118	23.1 ± 2.3	0	-	-	-	-	-	-	-	-
8 Dec	131	18.2 ± 2.6	0	-	-	-	-	-	-	-	-
22 Dec	145	4.6 ± 1.6	0	-	-	-	-	-	-	-	-
3 Jan	157	5.0 ± 1.4	0	-	-	-	-	-	-	-	-
3 Feb	188	0	0	-	-	-	-	-	-	-	-
22 Feb	207	4.4 ± 1.1	0	-	-	-	-	-	-	-	-
12 Mar	225	6.4 ± 1.8	0	-	-	-	-	-	-	-	-
29 Mar	242	7.0 ± 1.9	0.3 ± 0.1	3	0.84 ± 0.16	0.19	2	0.5 ± 0.2	0	0.88 ± 0.13	-
12 Apr	256	5.0 ± 1.0	2.2 ± 0.5	19	1.50 ± 0.10	0.06	17	1.18 ± 0.1	0.4 ± 0.08	0.65 ± 0.05	0.22
26 Apr	270	3.7 ± 0.8	2.9 ± 0.7	20	1.94 ± 0.62	0.07	17	1.79 ± 0.12	1.03 ± 0.11	0.78 ± 0.04	0.11
11 May	286	6.9 ± 1.1	6.1 ± 0.3	20	1.85 ± 0.10	0.07	16	1.65 ± 0.16	1.0 ± 0.09	0.75 ± 0.03	0.10
15 Jun	319	5.7 ± 1.1	5.1 ± 1.0	20	1.08 ± 0.10	0.10	18	0.987 ± 1	0.76 ± 0.09	0.83 ± 0.04	0.12

Appendix 7 The world first record of *M. rosae* feeding and reproducing on river red gum *Eucalyptus camaldulensis* Dehn. a) Two adults of M. rosae are colonising a young shoot b) The same shoot five days later. Even though colonies were able to survive for several generations in a glasshouse on river red gum, they soon vanished in the field.


a


b

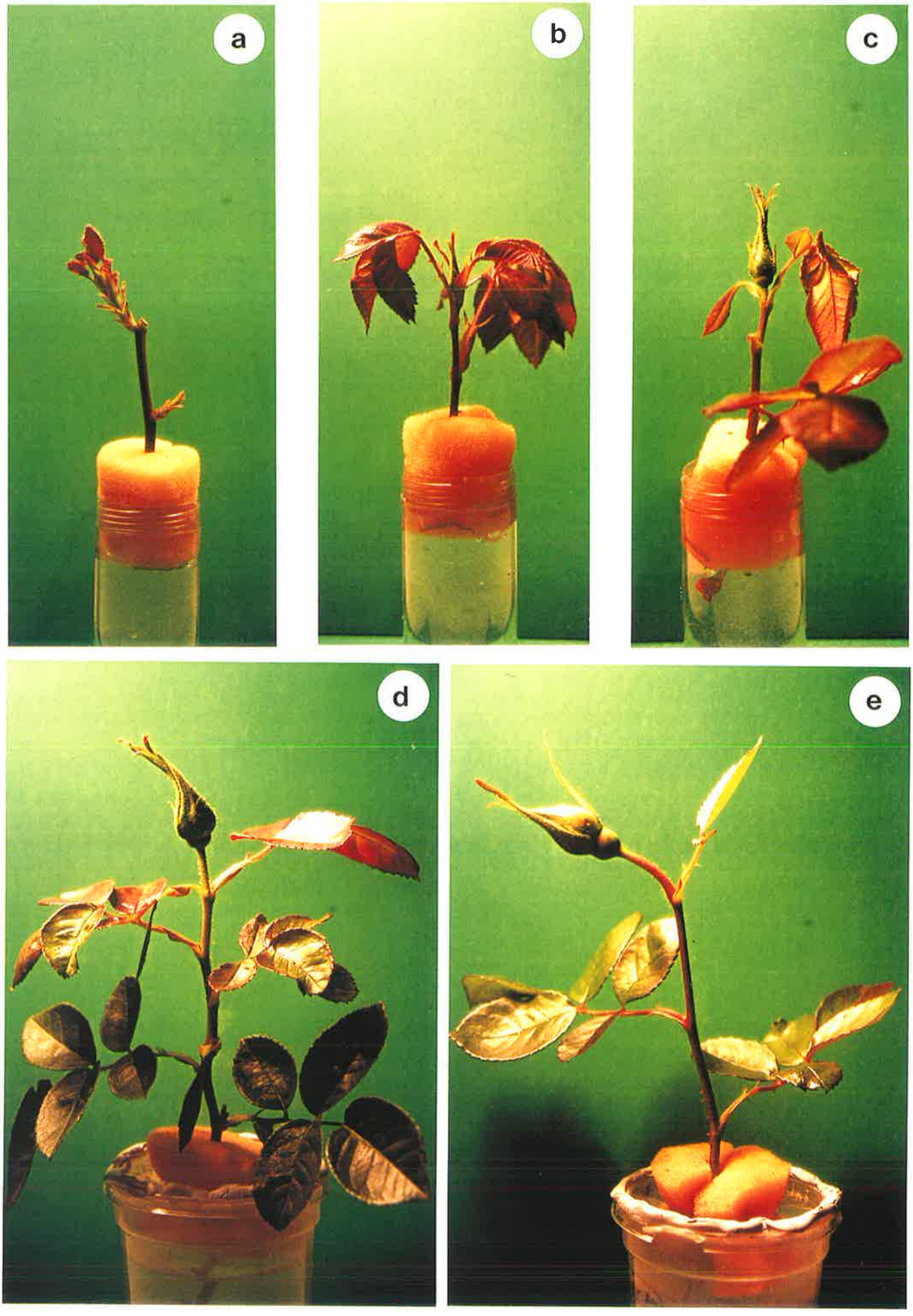
Appendix 8 Data from cage exclusion experiment for evaluation of impact of *A. rosae* on *M. rosae* in the field.

	Block no.	1st/ 2nd instar	3rd/ 4th instar	Apterae	Alatae	Mummies formed	Parasitoid larvae/ disected aphids	Mummies on plant	Overall *A. rosae* (*M. rosae* adults + mummies)	Proportion of *M. rosae* adults parasitized	No. of *A. rosae* on net male/fem.	No. of *M.rosae* on net apt./ala.
Untreated Cages	1.1	269	792	19	1	4	0(12)	17	21(37)	0.57		
	1.2	62	81	3	4	1	0(5)	3	4(10)	0.36		
	1.3	298	537	46	1	11	2(36)	8	21(55)	0.38		
	2.1	155	274	40	4	2	3(36)	6	11(50)	0.22		
	2.2	176	414	39	5	9	2(24)	40	52(84)	0.62		
	2.3	234	210	27	2	0	2(25)	2	4(31)	0.13		
	3.1	264	366	8	3	4	1(4)	12	18(23)	0.78		
	3.2	516	271	53	8	12	0(40)	24	36(85)	0.42		
	3.3	222	571	31	5	5	1(23)	16	22(52)	0.42		
Uncaged plants	1.1	436	1040	53	9	12	2(41)	0	14(62)	0.18		
	1.2	285	314	15	8	6	0(9)	21	27(44)	0.61		
	1.3	271	505	56	4	11	3(39)	8	23(68)	0.32		
	2.1	342	365	37	5	9	1(31)	48	58(90)	0.64		
	2.2	97	335	27	3	8	3(18)	18	29(48)	0.6		
	2.3	333	521	23	12	4	3(24)	41	50(76)	0.66		
	3.1	225	161	60	12	13	6(34)	3	32(75)	0.43		
	3.2	502	565	30	16	24	6(12)	31	65(77)	0.84		
	3.3	443	338	47	17	20	4(36)	36	61(100)	0.61		
Treated cages	1.1	158	371	6	0	0	0(5)	0	0(6)	0	7/2	0/0
	1.2	427	1118	13	2	0	0(12)	0	0(15)	0	3/3	0/4
	1.3	245	864	26	0	0	0(18)	0	0(26)	0	12/3	5/0
	2.1	534	412	33	3	0	0(27)	0	0(36)	0	4/2	1/5
	2.2	424	262	27	10	0	0(28)	0	0(37)	0	17/7	2/4
	2.3	575	392	47	0	0	0(41)	1	0(48)	0.02	15/3	2/2
	3.1	315	622	6	0	0	0(6)	0	0(6)	0	20/3	0/9
	3.2	682	616	18	9	0	0(23)	0	0(27	0	47/16	4/17
	3.3	317	670	19	1	0	0(14)	0	0(20)	0	22/9	1/8

The column 'mummies formed' refers to the number of mummies formed during the 5 day rearing process of adult aphids. The column 'parasitoid larvae / disected aphids' refers to the number of parasitoid larvae found in surviving aphids after the 5 day rearing process.

Appendix 9 Weather data (25 April to 24 May 1995) obtained from the Waite meteorological station. Temperatures represent the mean of intermittent recordings.

Date	Temperature	Daily Rainfall	Averages for experimental periods		
25.4	14.4	0			
26.4	15.5	1.6			
27.4	15.2	0			
28.4	19.0	0	**Block 1**		
29.4	19.3	0			
30.4	14.7	13.6	Average		
01.5	16.3	4.4	temperature		
02.5	15.5	0			
03.5	15.6	13.8	16.2°C		
04.5	15.4	0.2		**Block 2**	
05.5	14.5	0.8	Total		
06.5	15.2	0	rainfall	Average	
07.5	15.8	0	(MMS)	temperature	
08.5	15.7	0			**Block 3**
09.5	16.8	0	41.0	15.9°C	
10.5	16.4	0			Average
11.5	17.2	0	9 Raindays	Total	temperature
12.5	16.9	0.6		rainfall	
13.5	17.2	1.4		(MMS)	15.7°C
14.5	16.8	4.6			
15.5	16.1	0.2		26.4	Total
16.5	15.4	0.2			rainfall
17.5	14.3	0		10 Raindays	(MMS)
18.5	14.2	0			
19.5	14.2	0			8.0
20.5	15.8	0.2			
21.5	14.9	0			7 Raindays
22.5	14.1	0			
23.5	15.1	0			
24.5	16.7	0			


a
b
c
d
e